Sterile Insect Technique

Principles and Practice in Area-Wide Integrated Pest Management

Editors

V. A. Dyck, J. Hendrichs and A. S. Robinson

Springer

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PREFACE

It is a challenge to bring together all relevant information about the sterile insect technique (SIT) and its application in area-wide integrated pest management (AW-IPM) programmes; this book is the first attempt to do this in a thematic way. Since SIT practitioners tend to operate in the context of only one insect pest species, it was also a challenge for authors to develop and write their chapters from generic and global points of view, stressing the principles of the technology, and including examples from a range of pest species. We appreciate the understanding shown by the authors in accepting our many suggestions to emphasize the principles of the technology and to minimize details of field programmes. We also thank them for their patience with the prolonged editing process of the book.

We are especially grateful to the authors for writing the chapters without financial compensation. Authors who are retired, or worked on their own time, deserve special commendation.

Each chapter was peer-reviewed, and we thank the reviewers for helping to make the book accurate, complete, up-to-date, and generic in content.

The need for this book has been evident for many years, and now that it has finally been published, it is expected to serve the scientific community for many years to come. We are pleased to have been able to participate in its development.

The Editors
March 2005

DISCLAIMER

FOREWORD

For several major insect pests, the environment-friendly sterile insect technique (SIT) is being applied as a component of area-wide integrated pest management (AW-IPM) programmes. This technology, using radiation to sterilize insects, was first developed in the USA, and is currently applied on six continents. For four decades it has been a major subject for research and development in the Joint FAO/IAEA Programme on Nuclear Techniques in Food and Agriculture, involving both research and the transfer of this technology to Member States so that they can benefit from improved plant, animal and human health, cleaner environments, increased production of plants and animals in agricultural systems, and accelerated economic development. The socio-economic impacts of AW-IPM programmes that integrate the SIT have confirmed the usefulness of this technology.

Numerous publications related to the integration of the SIT in pest management programmes, arising from research, coordinated research projects, field projects, symposia, meetings, and training activities have already provided much information to researchers, pest-control practitioners, programme managers, plant protection and animal health officers, and policy makers. However, by bringing together and presenting in a generic fashion the principles, practice, and global application of the SIT, this book will be a major reference source for all current and future users of the technology. The book will also serve as a textbook for academic courses on integrated pest management. Fifty subject experts from 19 countries contributed to the chapters, which were all peer reviewed before final editing.

INTRODUCTORY REMARKS

As evidenced by the successful area-wide insect pest control programmes described in this book, the sterile insect technique (SIT), a component of these programmes, has come of age. The technology has expanded rapidly — additional target species, new rearing techniques, studies on genetics and insect behaviour, and especially integration into operational area-wide integrated pest management (AW-IPM) programmes. The SIT has matured to the point where a critical overview of its principles and practice will greatly facilitate further research, development, and application in the field.

The SIT was among the first biological insect control methods designed for area-wide application. While the SIT gained its reputation in insect eradication programmes, it is essential that the scientific community now recognizes its potential as a part of IPM strategies for the area-wide suppression, containment, prevention and, where advisable, eradication of pests.

Insect control methods in the first 70 years of the 20th century were based largely on chemical insecticides; this was especially so after the Second World War with the introduction of synthetic insecticides. The concept of IPM became popular after 1970, and a more selective use of insecticides was emphasized. Attempts to significantly reduce insecticide applications have only gradually become more prominent. Biological control of pest insects, together with the breeding of insect-tolerant or resistant plants, is probably now receiving the major emphasis in IPM programmes. According to an international standard under the International Plant Protection Convention (IPPC), the SIT is now officially considered as one type of biological control, and it is ideally suited for incorporation into AW-IPM programmes.

The scientific underpinning of SIT programmes has broadened as new areas of science have developed, e.g. insect mass production and quality, geographic information systems and data management systems, genetics and molecular biology, insect behaviour, aerial release of sterile insects, and modelling of AW-IPM. The practical success of a programme incorporating the SIT requires a holistic and multidisciplinary approach, and effective management, since in the last analysis programmes must produce substantial economic benefits. This is clearly evident in the major successes using the SIT against screwworms, fruit flies, and moths.

In spite of documented successes, many colleagues in the scientific community are partially or inadequately informed on the application and importance of this powerful addition to the biological weapons that can be used against insect pests that are economically important or a threat to human health. The credibility and impact of the technology needs to be described in an objective, comprehensive, and balanced fashion, and in an accessible format. New insect pest problems, new restrictive legislation, as well as older problems such as insecticide resistance and minimum residue levels, require new solutions. There is a real need, and an

increasing demand, for information on the SIT so that its potential for addressing some of these problems can be assessed.

The chapters have been written by well-known experts on the SIT and other technologies that are integrated into IPM systems. A "first" in its field and worldwide in scope, this book will provide an in-depth resource for the whole range of documented scientific information about the SIT. The target audience of the book is the scientific community worldwide. It will assist animal health and plant protection practitioners, as well as students, teachers, and researchers, in understanding and applying the SIT. It is anticipated that the book will have a considerable impact on the science and practice of pest control systems.

Research workers new to this field have difficulty accessing the literature — it tends to be widely scattered in multiple publications (some with very limited distribution), in conference proceedings, and in unpublished programme reports. To further the science and application of the SIT, the accumulated knowledge and experience needs to be integrated and synthesized from a generic standpoint. The consolidation of comprehensive information into one volume, with references to the large amount of previous work, is long overdue. Such a consolidation will facilitate the application of the SIT to those pest problems for which it is appropriate. It will also lay the groundwork for future applications. The present book is uniquely designed to fill this gap. The strengths and weaknesses, and successes and failures, of the SIT have rarely been evaluated openly and fairly from a scientific perspective.

This is just the beginning. This book will help develop further the use of the SIT for pest suppression, and where advisable, eradication. It will be a gold mine for graduate students who want to learn about the history, accomplishments, problems, and promises of the SIT. As an "autocidal" biological control method, it fits into present-day concerns regarding human health and the environment. There is great potential for significant advances that will make the SIT more effective and economically viable, such as commercializing the different components, developing genetic sexing strains that permit the release of only males, treating sterile insects hormonally and semiochemically to increase their quality and competitiveness, releasing insects from improved aerial systems, and using modern biotechnology.

It is an honour to have been asked to write these introductory remarks. The developments in this technology are exciting, and I will always remain a part of them.

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PART I

INTRODUCTION

CHAPTER 1.1.

HISTORY OF THE STERILE INSECT TECHNIQUE

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SUMMARY

During the 1930s and 1940s the idea of releasing insects of pest species to introduce sterility (sterile insect technique or SIT) into wild populations, and thus control them, was independently conceived in three extremely diverse intellectual environments. The key researchers were A. S. Serebrovskii at Moscow State University, F. L. Vanderplank at a tsetse field research station in rural Tanganyika (now Tanzania), and E. F. Knipling of the United States Department of Agriculture. Serebrovskii's work on chromosomal translocations for pest population suppression could not succeed in the catastrophic conditions in the USSR during World War II, after which he died. Vanderplank used hybrid sterility to suppress a tsetse population in a large field experiment, but lacked the resources to develop this method further. Knipling and his team exploited H. J. Muller's discovery that ionizing radiation can induce dominant lethal mutations, and after World War II this approach was applied on an area-wide basis to eradicate the New World screwworm *Cochliomyia hominivorax* (Coquerel) in the USA, Mexico, and Central America. Since then very effective programmes integrating the SIT have been mounted against tropical fruit flies, some species of tsetse flies *Glossina* spp., the pink bollworm *Pectinophora gossypiella* (Saunders), and the codling moth *Cydia pomonella* (L.). In non-isolated onion fields in the Netherlands, the onion maggot *Delia antiqua* (Meigen) has since 1981 been suppressed by the SIT. In the 1970s there was much research conducted on mosquito SIT, which then went into "eclipse", but now appears to be reviving. Development of the SIT for use against the boll weevil *Anthonomus grandis grandis* Boheman and the gypsy moth *Lymantria dispar* (L.) has ended, but it is in progress for two sweetpotato weevil species, *Cylas formicarius* (F.) and *Euscepes postfasciatus* (Fairmaire), the false codling moth *Cryptophlebia leucotreta* (Meyrick), the carob moth *Ectomyelois ceratoniae* (Zeller), the cactus moth *Cactoblastis cactorum* (Berg), the Old World screwworm *Chrysomya bezziana* (Villeneuve), additional *Glossina* spp., other *Anastrepha* spp. and *Bactrocera* spp. fruit flies, and other pest insects.

1. PROLOGUE

When using the sterile insect technique (SIT), it is applied usually as a component of area-wide integrated pest management (AW-IPM) (Klassen, this volume). The density of the target insect pest population is reduced, eliminating already mated females, with auxiliary control methods (Mangan, this volume). Then the SIT imposes birth control on the population to further reduce its numbers (Klassen, this volume). The SIT involves rearing large numbers of the target species, exposing them to gamma rays to induce sexual sterility (Robinson, this volume), and then releasing them into the target population. The released sterile males mate with wild females to prevent them from reproducing.

Runner (1916) found that large doses of X-rays applied to the cigarette beetle *Lasioderma serricorne* (F.) rendered it incapable of reproduction. Soon afterwards H. J. Muller (1927) showed that ionizing radiation induced visible mutations in *Drosophila*, and also a much larger number of dominant lethal mutations, which were expressed through a reduction in the hatch of eggs laid by treated females or fathered by treated males. However, only after 1950, when Muller made a special effort to publicize the biological effects of radiation, did economic entomologists

become aware that, through irradiation, sexual sterility in male insects was quite easily achieved (Bakri et al., this volume).

Nevertheless, already in the 1930s and 1940s, the idea of releasing pest insects to introduce sterility into wild populations, and thus control them, had been conceived independently by A. S. Serebrovskii at Moscow State University, F. L. Vanderplank at a tsetse field research station in rural Tanganyika (now Tanzania), and E. F. Knipling of the United States Department of Agriculture (USDA). Serebrovskii and Vanderplank both sought to achieve pest control through the sterility that arises when different species or genetic strains are hybridized (Robinson, this volume).

The debut of the most successful AW-IPM programme integrating the SIT to date occurred in the 1950s. It was started to rid the south-eastern USA of the New World screwworm *Cochliomyia hominivorax* (Coquerel), a deadly parasite of livestock. During the next 43 years the technique was used to eradicate this screwworm from the USA, Mexico, and Central America to Panama (Vargas-Terán et al., this volume).

Currently, the SIT is most widely applied against tephritid fruit flies (Enkerlin, this volume). Following extensive Research and Development since the late 1950s (Klassen et al. 1994), the first large-scale programme, established in the 1970s, stopped the invasion of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) from Central America into southern Mexico (Hendrichs et al. 1983). In Japan, the SIT was employed in the 1980s and 1990s to eradicate the melon fly *Bactrocera cucurbitae* (Coquillett) in Okinawa and all of Japan's south-western islands, permitting access for fruits and vegetables produced in these islands to the main markets in the Japanese mainland (Kuba et al. 1996). In Chile, the SIT was used to rid the country of the Mediterranean fruit fly. By 1995 the entire country had become a fly-free zone, and a joint programme with Peru operates in northern Chile and southern Peru. Since then Chilean fruits in huge volumes have entered the US market without the need for any quarantine treatment, providing a major benefit to the Chilean economy (Enkerlin, this volume). Argentina also has developed significant SIT Mediterranean fruit fly programmes in several fruit-producing provinces, some of which have recently succeeded in establishing pest free areas. Mexico has also applied the SIT to get rid of various *Anastrepha* species from northern Mexico (Enkerlin, this volume). The SIT is increasingly applied with the objective to reduce losses and pesticide use rather than fruit fly eradication, with effective suppression programmes ongoing in Israel, South Africa, and Thailand, and in preparation in Brazil, Portugal, Spain, and Tunisia. To prevent Mediterranean fruit fly establishment in the continental USA through infested imported (smuggled) fruit, sterile males are being released regularly in the Los Angeles Basin, Tampa, and Miami. Consequently, there is no longer a need to spray these urban areas with malathion insecticide to suppress pest outbreaks.

Since 1967, sterile pink bollworm moths *Pectinophora gossypiella* (Saunders) have been released over cotton fields in the San Joaquin Valley of California to prevent the establishment of this pest by moths immigrating from southern California. The SIT is also being used to suppress the codling moth *Cydia pomonella* (L.), an economic pest of apples and pears, in the Okanagan region of British Columbia, Canada (Bloem et al., this volume).

Tsetse flies, which in sub-Saharan Africa transmit the disease trypanosomosis [trypanosomiasis] to humans (sleeping sickness) and livestock (nagana), are regarded as a major cause of rural poverty because they prevent mixed farming. Crops are produced with hoes because nagana kills draught animals. The existing cattle produce little milk, and manure is not available to fertilize the worn-out soils. The conquest of sleeping sickness and nagana would be of immense benefit to rural development in sub-Saharan Africa (Feldmann et al., this volume). The eradication in 1997 of the tsetse fly *Glossina austeni* Newstead in Zanzibar, Tanzania, confirmed the feasibility of integrating releases of sterile males with other suppression methods to create sustainable tsetse-free areas (Vreysen et al. 2000). As a result, in 2001, the African Heads of State and Government committed their countries to rid Africa of this disease (Feldmann and Jannin 2001). However, the dream to conquer nagana and sleeping sickness will require many decades of concerted effort. Currently there is a debate about the desirability of using the SIT to eradicate major tsetse populations from large areas of the African mainland (DFID 2002, Hargrove 2003), and the perceived high cost of applying the technique.

2. SEREBROVSKII AND POSSIBLE USE OF CHROMOSOMAL TRANSLOCATIONS TO CAUSE INHERITED PARTIAL STERILITY

Beginning in 1922, Muller encouraged and assisted Serebrovskii's genetic studies on *Drosophila*. In 1933, Muller became the director of a genetics laboratory, a position created for him by N. I. Vavilov, head of the Lenin All-Union Academy of Agricultural Sciences. Serebrovskii became embroiled in the fierce controversy with T. D. Lysenko about the validity and usefulness of Mendelian genetics in advancing Soviet agriculture (Medvedev 1969), whether genes exist, and whether the Lamarckian concept of inheritance of acquired traits is correct. Lysenko had gained the support of Stalin, and he attempted to force Vavilov, Serebrovskii, Muller, and other geneticists to recant their adherence to Mendelian genetics. In December 1936, exponents of the two trends in Soviet biology confronted each other at a special session of the Lenin All-Union Academy of Agricultural Sciences, and the geneticists vigorously defended their science. Subsequently several prominent geneticists were arrested. Probably Serebrovskii was motivated to develop the concept of using chromosomal translocations for pest population suppression as a means to deflect Lysenko's strident criticism that research in genetics was devoid of promise to benefit Soviet agriculture (Carlson 1981).

Serebrovskii (1940) noted that it was already well known in 1940 that a translocation of segments between two chromosomes caused an abnormal association of four chromosomes during meiosis in heterozygotes, resulting in the formation of gametes with lethal genetic duplications and deficiencies. These abnormalities manifested themselves as partial sterility in the translocation heterozygote. Such partial sterility tended to be passed on from one generation to the next. Those translocations that were viable in the homozygous state had normal meiotic pairing, and were fully fertile. Serebrovskii appreciated that, in such conditions of negative heterosis (or underdominance as it has more recently been called (Davis et al. 2001)), natural selection would favour whichever chromosome

type was initially in the majority, with a point of unstable equilibrium, which would be at a frequency of 50% if the viability of the two homozygous karyotypes were equal. At a frequency of 50%, the proportion of heterozygotes, and hence of sterility in the population, would be maximal.

On the basis of Mendelian principles, Serebrovskii worked out: (1) the extent to which sterility would continue to appear in a population in the generations after a single release of translocation homozygotes, (2) ways of enhancing levels of sterility by using several different translocations, and (3) the effects of releasing only males to avoid a temporary increase in the breeding population. Years later the alternative possibility was proposed — the deliberate release of a majority of insects with translocations as a means of "driving" into a vector population a gene that would render it harmless to man, e.g. a gene for inability to transmit disease (Curtis 1968).

Serebrovskii (1940) started practical work on translocations in *Musca domestica* L. and *Calandra granaria* L., but presumably it was impossible to continue it in the catastrophic conditions in the USSR during World War II. Unlike some other opponents of Lysenko, Serebrovskii was not arrested, but he died of natural causes in 1948. Before his death he expanded his ideas in a book (Serebrovskii 1971), but which could not be published until after the fall from power of N. S. Khruschev and of Lysenko (whom Khruschev supported).

3. VANDERPLANK AND USE OF HYBRID STERILITY TO COMBAT TSETSE FLIES

In the 1930s and 1940s, Vanderplank and his colleagues developed and field-tested an entirely different system of insect control, based on sterility from species crosses and in the hybrids from such crosses. Based on field studies on the *Glossina morsitans* Westwood group of tsetse flies in East Africa, they had discovered the subtle but unequivocal differences between *G. morsitans sensu stricto* and *G. swynnertoni* Austen. Laboratory crosses between *G. morsitans* and *G. swynnertoni* were made by Corson (1932), Potts (1944), and Vanderplank (1944, 1947, 1948), but the cross-matings had low fertility. Vanderplank (1947) reported that the genitalia of the hybrids were distinguishable from both parent species, the hybrid males were sterile, and the female hybrids partially sterile. Hybrid sterility in tsetse flies has been studied further by Curtis (1971), and extensively by Gooding (1985, 1993).

Vanderplank (1944) proposed that sterility from crosses could be used for tsetse control, and Jackson (1945) showed that there was random mating between the two species in the field. On this basis, Vanderplank organized the mass collection of *G. morsitans* pupae, and released emerging flies in a 26-km² area occupied only by *G. swynnertoni*. This habitat was separated by at least 19 km from other tsetse populations, and was considered too arid for *G. morsitans* to establish itself permanently.

Vanderplank (1947) briefly described the success of this experiment, noting that the initial effects were as theoretically expected. Surprisingly, he never published the detailed results, but he kindly gave them to C. F. Curtis. After F. L. Vanderplank's death, his son, R. J. R. Vanderplank, gave permission that these remarkable data be

published (Table 1). The releases of *G. morsitans* did indeed virtually eliminate the less numerous *G. swynnertoni*, and there was a period in which hybrids could be identified, before they also declined in numbers. Finally, the predicted decline of *G. morsitans* also occurred, presumably because of its lower tolerance of aridity than that of *G. swynnertoni*. When the density of tsetse flies had been reduced to a low level, local people moved into the area, and apparently completed tsetse eradication by bush clearance. It is unfortunate that the details of this remarkable trial have remained almost unknown for so long, and were not followed up.

Table 1. *Effect of releasing* G. morsitans *pupae into* G. swynnertoni *habitat on the density of these two species and of the interspecific hybrids (*G. morsitans *released into a 26-km^2 habitat in Tanzania separated from other tsetse habitats by at least 19 km) (data from F. L. Vanderplank and C. H. N. Jackson, 1944–1946, reproduced with permission)*

Date	*G. morsitans* released (number)	Average catch of old males (per 5 hours of catching)		
		G. morsitans	*G. swynnertoni*	Hybrids
June 1944	0	0	54	0
July	0	0	69	0
August	27000	64	50	0
September	25000	138	25	0
October	26000	169	16	5[1]
November	11000	54	15	7[1]
December	4500	39	12	11[1]
January 1945	5200	49	9	19
February	2300	68	5	21
March	0	40	4	28
April	0	22	4	20
May	0	17	3	10
June	0	16	1.2	9
July	0	13	1.1	7
August	0	15	0	4
September	0	11	0.2	2.4
October	0	7	0.1	2.1
November 1945 – March 1946	No surveys: local inhabitants now grazing cattle in the area			
April 1946	0	0.6	0.4	0.8
After April 1946	Area given over to local inhabitants who cut down most of the bush			

[1]In this period, not all males were examined under the microscope, so the numbers recorded as hybrids were possibly inaccurate.

4. KNIPLING AND USE OF STERILITY INDUCED BY IONIZING RADIATION

4.1. New World Screwworm

4.1.1. Early Attempts at Control, and Importance of Correct Identification

Since ancient times, the tropical and semi-tropical New World screwworm has been a serious enemy of warm-blooded animals, including humans, in an area extending from Argentina to the southern USA. Descendants of European settlers managed their herds and flocks so that the birth of most calves and lambs, as well as castration, branding, and dehorning operations, occurred only during months when screwworms were scarce. Each animal was checked for wounds at least twice per week, and each wound was treated with an insecticidal "smear" (Knipling 1985).

The correct identity of the insect concerned was established in 1858, by French entomologist C. Coquerel, who published an accurate description of the New World screwworm in the Annals of the Entomological Society of France. Coquerel assigned the name *Lucilia hominivorax* Coquerel to this parasite. "Hominivorax" literally means "man eater".

North American entomologists were, unfortunately, unaware of Coquerel's paper. Indeed, until 1933, North Americans confused the identity of the New World screwworm with the abundant scavenger of dead carcasses, *Cochliomyia macellaria* (F.). Due to this inability to recognize that a different species was involved, livestock producers wasted much energy in burying or burning carcasses, and trapping adult flies, in the vain hope of reducing the population of what was believed to be the myiasis-causing screwworm.

E. C. Cushing, under the guidance of W. S. Patton of the Liverpool School of Tropical Medicine, discovered that the genitalia of adult flies that had developed in carrion were different from those of most flies collected from wound-reared specimens, and named the latter species *Cochliomyia americana* (Cushing and Patton 1933). Later this species was found to be the *C. hominivorax* described 75 years earlier by Coquerel (Laake et al. 1936). *C. hominivorax* is now referred to as the New World screwworm, and the scavenger *C. macellaria* as the secondary screwworm. As soon as the true identity of *C. hominivorax* had been clarified, Knipling and his colleagues made a concerted effort to elucidate its biology and ecology. They concluded that the number of screwworm flies that survives the winter as pupae in the soil was very low, perhaps only 40–80 per km^2 (Lindquist 1955, Meyer and Simpson 1995).

4.1.2. Studies on Reared Screwworms in 1930s, and Conception of SIT

C. hominivorax was the first obligate insect parasite to be reared on an artificial diet (Melvin and Bushland 1936), and this enabled very large numbers of screwworms to be available for study. Knipling observed the extreme sexual aggressiveness of male screwworms, as well as the refusal of females to mate more than once, and he realized that, if sexual sterility could be induced in males, and if vast numbers could be sterilized and released in the field, then the screwworm population would be

suppressed. He also realized that, if releases continued for several successive generations, and the wild population density decreased, the ratio of the number of sterile males to that of fertile wild males would increase sharply. Provided that the wild population was isolated, the sterile:fertile ratio would become so great that probably not even a single fertile mating would occur, and thus the wild population would be eradicated (Knipling 1955, 1985). Knipling introduced simple mathematical models to assess the effects of the SIT and of insecticides on the dynamics of screwworm populations (Barclay, this volume; Klassen, this volume).

The idea of the SIT may well have been triggered in part by the observation of monogamy in female screwworms. However, Bushland (1960) asserted that, in instances in which irradiation induces dominant lethal mutations in sperm which can still penetrate eggs, female monogamy is not a requirement of the SIT, and this view was accepted by Knipling (1959, 1979) (Lance and McInnis, this volume; Whitten and Mahon, this volume).

In the 1930s, mass-rearing was not developed, and no method to induce sexual sterility was known. For a decade, the paramount urgency of World War II prevented Knipling from pursuing this sterile-male concept (Klassen 2003), but R. C. Bushland made a few attempts to induce sterility using chemicals.

4.1.3. Sterility Based on Radiation-Induced Dominant Lethal Mutations
In 1946, H. J. Muller was awarded the Nobel Prize in Medicine for his discovery of induced mutagenesis, and this gave him the prestige to lead a vigorous campaign against the atmospheric testing of atomic weapons. He wrote a popular article in the American Scientist in which he used tombstones as symbols to depict graphically the dead progeny from matings of irradiated *Drosophila* (Muller 1950). A. W. Lindquist recognized that Muller had developed a means of sexually sterilizing insects, and drew Knipling's attention to this paper.

Knipling wrote to Muller, asking if ionizing radiation could be used to induce sexual sterility in the New World screwworm. Upon receiving Muller's confident assurance, Bushland and D. E. Hopkins used the X-Ray Therapy Section of Brooke Army Hospital to conduct the first screwworm irradiations. They found that, when 6-day-old pupae were exposed to 50 Gy, the adults that emerged appeared to be normal. However, when irradiated males were mated with untreated females, none of the eggs hatched. Females that had been irradiated and mated to untreated males produced almost no eggs, and none hatched. When untreated and irradiated males were caged together with untreated females, the irradiated males competed about equally with untreated males (in accordance with Knipling's model) (Bushland and Hopkins 1953).

4.1.4. Sanibel Island Field Evaluation Pilot Test
Sanibel Island (47 km^2), 4 km from the coast of Florida, was selected for a release-recapture experiment (Bushland 1960; Itô and Yamamura, this volume) using ^{32}P-labelled flies. In addition, the ratio of radioactive egg masses to non-radioactive masses was assessed. The release of approximately 39 sterile male flies per km^2 per week for several weeks resulted in up to 100% sterility of the egg masses from

wounded goats, and it greatly reduced the wild population. However, eradication was not achieved, apparently because wild fertile flies were flying to the island from the mainland (Baumhover 2002).

4.1.5. Curaçao Eradication Trial — Proof of Concept

In 1954, Knipling was informed that screwworms were causing severe damage to the dairy industry on the island of Curaçao, 65 km from Venezuela, with an area of only 435 km^2. Flies were reared in Orlando, Florida, and irradiated pupae were packaged in paper bags, air freighted to Curaçao, and released by air twice per week. On Sanibel, the release of 39 sterile males per km^2 per week had been effective, but on Curaçao this rate caused only 15% sterility of egg masses, and it had little effect on the incidence of myiasis cases due to the presence of thousands of unattended goats and sheep. Since wounds on these animals were not treated, they supported a high screwworm population. The release rate was increased to about 155 sterile males per km^2 per week, whereupon egg sterility increased to 69%, and then to 100% by the time two generations had elapsed. Subsequently two more fertile egg masses were found, and so sterile-fly releases were continued for another 8 weeks. Evidently eradication had been accomplished within 14 weeks, and the releases were halted after 22 weeks (Baumhover et al. 1955).

4.1.6. Florida Eradication Programme

At a meeting of the Florida Livestock Association in 1956, A. H. Baumhover suggested that eradication of the screwworm in Florida might eventually be possible, and he outlined a plan that called for the release of 50 million sterile flies per week. However, Knipling was reluctant to implement a high-risk USD 10-million programme on the mainland — there were too many unknown factors, with problems in mass-rearing and distribution requiring several more years of research (which might reduce the eventual cost of the programme by USD 2 million). However, the governor T. L. Collins noted that the agricultural economy of Florida was losing more than USD 20 million per year due to the screwworm, and he pressed for immediate implementation. Nevertheless, to upgrade the rearing and release methodology, a further trial was conducted in 5000 km^2 along the Atlantic coast (Baumhover et al. 1959, Graham and Dudley 1959). Meanwhile, in July 1957, the Florida Legislature appropriated USD 3 million to match federal funds for an operational programme.

A rearing facility was constructed at an Air Force Base at Sebring, Florida, with the production capacity of 60 million flies per week, and the programme was scheduled to begin in July 1958 (Scruggs 1975, Meyer and Simpson 1995). However, this schedule could be accelerated following the unusually cold winter of 1957–1958, which eliminated all screwworms in the south-eastern states, except for the southern one-third of Florida. To contain the surviving screwworm population, the production was rapidly increased in the research facilities at Orlando and Bithlo, from 2 to 14 million sterile flies per week, and by May 1958 sterile flies were being distributed north of the infestation to the border with Georgia using 10 aircraft. The programme established a quarantine line across central Florida to prevent the

shipment of any infested livestock out of southern Florida, and any localized concentrations of screwworm cases in northern Florida were quickly eliminated by treating infested wounds, spraying herds, and releasing large numbers of sterile flies.

The mass-rearing facility at Sebring reached full production in August 1958, and 20 aircraft were used to distribute sterile flies throughout Florida and parts of neighbouring states. The Florida Cooperative Extension Service conducted a public information programme, and trained county agents to educate producers. Field inspectors assisted and trained producers in treating cases and submitting larvae from wounds for identification at eradication headquarters in Sebring. Cases of myiasis in each county were plotted each day. The number of sterile flies released per km^2 per week was increased at persistent "hot spots" from about 155 to 1160. The last autochthonous case occurred on 19 February 1959 (Baumhover 2002). All sterile fly releases were terminated in November 1959. The total cost of the programme was USD 11 million, about 50% of the annual losses in Florida (Meadows 1985).

4.1.7. Eradication and Area-Wide Population Management in South-Western USA

The Florida programme aroused the interest of cattle producers in Texas, the western states, and Mexico. In 1959 the presidents of the USA (D. D. Eisenhower) and Mexico (A. Lopez Mateos) agreed to a feasibility study to eradicate the New World screwworm in Mexico.

The strategy for dealing with the south-west was a by-product of the use of the 160-km-wide sterile-fly barrier across Florida, with the release of sterile flies only in the overwintering area, and to let the cold weather destroy the screwworms to the north of this area. Following eradication from that area, sterile flies would be deployed to create a barrier zone along the US-Mexico border to protect against reinvasion (Bushland 1985).

A mass-rearing facility was built in Mission, TX, and releases began in 1962. By 1964 no screwworms were found in Texas or New Mexico for a period of two or three generations, and USDA officials declared the screwworm eradicated from these states. In 1965, the programme was extended to the Pacific, and in 1966 the entire USA was declared free of screwworms; the federal government took full responsibility to maintain the barrier zone from the Gulf of Mexico to the Pacific. However, no agreement with Mexico to proceed southward had been reached, and the USA remained highly vulnerable to the influx of screwworms from Mexico. At this point in time, the goal of the programme was no longer eradication in the true sense, but had become population containment (Klassen 1989, 2000; Hendrichs et al., this volume).

4.1.8. Managing Screwworm Population Along US-Mexico Border

Both US and Mexican cattle producers were anxious to push the screwworm population south to the Isthmus of Tehuantepec, where a barrier of only 360 km would be needed. In 1972 the Mexico-United States Screwworm Eradication Agreement was signed, with the aim of eradicating the screwworm to the north of the Isthmus of Tehuantepec, and to establish a sterile-fly barrier there.

In the meantime, many difficulties arose. Screwworm cases occurred as much as 480 km north of the US-Mexico border. In 1968 almost 10 000 cases were recorded in the USA, and in 1972 such cases rose to 95 000. Knipling (1979) noted that, before the programme began, the maximum flight range of screwworm adults was estimated at 80 km. It was planned that the width of the sterile-fly barrier be twice this figure. However, Hightower et al. (1965) demonstrated that natural fly movement can occur up to at least 290 km, and the pattern of screwworm movement during the spring indicated that dispersal in a single generation was up to 480 km. In addition, Knipling (1979) concluded that the main reasons for the "breakdown" of the AW-IPM programme in 1972 were the unusually favourable conditions for winter survival of screwworms, the abandonment of animal husbandry practices needed to counter screwworm infestations (including the twice-weekly inspection and treatment of animals), and the explosion of the population of white-tailed deer as a result of almost no screwworm-induced mortality during the previous decade (Nagel and Peveling, this volume). Critics of the programme postulated changes in the behaviour of the native population through genetic selection, making wild adults prone to avoid matings with the released strain, and the existence of cryptic species (Richardson et al. 1982). However no data were generated to support these views (Krafsur 1998; Krafsur, this volume), and they were strongly rebutted (LaChance et al. 1982). Another important factor was the unwise attempt to reduce sterile fly distribution costs by releasing flies on parallel flight lanes spaced 8 or 16 km apart, and this failed to deliver adequate numbers of sterile flies to all locations where wild virgin females were present (Krafsur 1978, Hofmann 1985).

The Mexico-United States Screwworm Eradication Commission began field operations in Mexico in 1974. A mass-rearing facility, with a capacity of 500 million sterile flies per week, was built at Tuxtla Gutiérrez, and it reached full production in January 1977. Nevertheless, as late as 1976, almost 30 000 cases occurred in the USA. Major relief came when fly production at Mission, TX, was supplemented from the new factory in Mexico (Meyer and Simpson 1995). Subsequently the need for the Mission facility diminished rapidly, and in 1981 it was closed. The last autochthonous screwworm case in the USA occurred in August 1982.

Knipling (1979) stated:

> Had scientists known of the long flight range of the insect, they would not have recommended a sterile fly release programme in the south-west. This would have been unfortunate. By taking this gamble, up to a billion [1000 million] dollars [USD] have been saved. We have learned that despite the long-range movement of the insect, a high degree of pest population suppression can be achieved even against non-isolated populations.

4.1.9. Programmes in Central America: the Drive to Panama

By 1984 the Commission had achieved the goal of eradicating the screwworm to the Isthmus of Tehuantepec (Peneda-Vargas 1985). In 1986, operations were extended to the Yucatán Peninsula and the countries bordering Mexico (Irastorza et al. 1993). Eradication was declared as follows: Mexico 1991, Belize and Guatemala 1994, El Salvador 1995, Honduras 1996, Nicaragua 1999, Costa Rica 2000, and Panama 2001, where a permanent sterile-fly barrier is being maintained in the Darien Gap

along the border with Colombia (Wyss 2000; Vargas-Terán et al., this volume). A rearing facility is being constructed at Pacora, Panama, and eventually the facility at Tuxtla Gutiérrez will be closed.

4.1.10. Screwworm Eradication Programme in North Africa

In 1988, the New World screwworm was discovered at Tripoli, Libya, where it rapidly spread over $28\,000$ km^2. Many feared that the insect would spread throughout North Africa, the Middle East and southern Europe, and migrate up the Nile River to sub-Saharan Africa, with serious consequences for the African people, livestock, and the already endangered large mammals.

In 1989, the Government of Libya asked the Food and Agriculture Organization of the United Nations (FAO) for assistance in eradicating the screwworm. The operational programme was planned in detail by consultants assembled by the Joint FAO/IAEA Division, and Libya and various donor countries provided the funding (FAO 1992; Vargas-Terán et al., this volume).

The infested area was partially isolated by the Mediterranean Sea, desert to the south, and barren areas with few livestock to the east and west. On the other hand, all of the conditions for successful overwintering and dispersal existed in a 15–25-km-wide zone along the Mediterranean coast (Krafsur and Lindquist 1996). One hundred teams, each consisting of two individuals equipped with a jeep, inspected all livestock every 21–28 days, applied insecticide to every wound, and sprayed many of the animals. About 80 swormlure-baited wing traps were deployed across the lines of flight of the aircraft from which the sterile screwworms were dropped.

Sterile screwworms were supplied from Mexico — mating studies showed that the factory-strain flies were sexually compatible with the Libyan strain. Some differences in the mitochondrial DNA were observed, but they were not considered to indicate a barrier to applying the SIT (FAO 1992). Each weekly flight from Tuxtla Gutiérrez to Tripoli carried 40 million sterile screwworm pupae. In Tripoli, adult emergence was controlled to allow two early morning releases per week.

The attack on the pest population was planned for the early winter of 1990, since by then cool weather would have greatly reduced the density and the reproductive capacity of this insect. Also cool weather would synchronize the life stages, and eliminate generation overlap. The number of sterile flies released was quickly increased to the maximum to saturate all suitable niches with sterile males. Thus, from the time that indigenous females emerged from under the soil, they would be in the company of sexually sterile males.

The impact of this strategy was dramatic. Only six instances of wounds infested with screwworm larvae were found in 1991, compared with more than $12\,000$ cases in 1990. Releases of sterile flies were continued until October 1991, and surveillance of all livestock until June 1992 (Lindquist et al. 1993). Eradication was declared in June 1992 (FAO 1992).

4.1.11. Screwworm Programmes in the Caribbean

By 1975 the screwworm had been eradicated from Puerto Rico and the Virgin Islands. In 1999, the Government of Jamaica initiated a programme to eradicate the

screwworm (Dyck, Reyes Flores et al., this volume; Vargas-Terán et al., this volume). No eradication programmes have been initiated on Cuba and Hispaniola, even though *C. hominivorax* could easily be reintroduced into areas that have been cleared of this pest (Hendrichs 2000; Vargas-Terán et al., this volume).

4.2. Tephritid Fruit Flies

Several species of tropical fruit flies are extremely destructive pests of fruits and vegetables. Tephritid fruit flies are major economic pests because they have:
- A multivoltine life cycle with an explosive reproductive capacity
- The capacity to exploit a large number of host plants
- The ability to disperse widely as adults or to be moved in fruit as larvae
- The ability (adults) to survive several months of inclement weather

Tropical fruit flies not only cause great losses in fruit and vegetable production, but they also seriously impede international trade because of quarantine regulations designed to avoid cross-border introductions. Consequently, efforts to remove, suppress, or exclude these pests have been made in at least 32 countries (Hendrichs 1996, 2001; Klassen et al. 1994; Enkerlin, this volume).

The mating behaviours of tropical fruit flies are very different from the aggressive behaviour of male screwworms and involve complex courtship behaviours including female mate choice (Robinson et al. 2002). Thus, close attention must be given to the effects of colony-holding conditions, artificial diets, irradiation, and handling procedures on the acceptability to wild females of released sterile males (Cayol 2000, Hendrichs et al. 2002).

Investigations into the possibility of using the SIT to eradicate populations of the Mediterranean fruit fly, melon fly, and oriental fruit fly *Bactrocera dorsalis* Hendel were initiated in 1955 in Hawaii (Steiner and Christenson 1956). Also, prior to 1960, pioneering investigations were underway on the Queensland fruit fly *Bactrocera tryoni* (Froggatt) in Australia, and on the Mexican fruit fly *Anastrepha ludens* (Loew) in Mexico and the USA (Klassen et al. 1994; Enkerlin, this volume).

4.2.1. Mexican and Queensland Fruit Flies

In 1964, the SIT was used to eradicate the Mexican fruit fly from outbreaks in southern California, and as a quarantine measure to prevent the pest from re-entering California from Baja California Norte in Mexico, and a decade later to exclude the pest from the Rio Grande Valley of Texas. Both SIT containment programmes have continued since then, but the programme on the California-Mexico border was terminated after the Mexican states of Baja California Norte, Baja California Sur, Chihuahua, and Sonora, following successful SIT projects in the 1990s against *A. ludens* and the West Indian fruit fly *Anastrepha obliqua* (Macquart), were converted into fruit fly-free zones from which citrus, stone fruits, apples, and vegetables are now being exported without any postharvest treatment (Reyes F. et al. 2000; Enkerlin, this volume).

Field trials of the SIT against the Queensland fruit fly began in 1962 in New South Wales, Australia. Although the population was suppressed strongly, it could

not be eradicated because of long-range immigrants. Nevertheless, since the mid-1990s, an SIT containment programme protects a "Fruit Fly Exclusion Zone" comprising major fruit production areas in New South Wales, Victoria, and South Australia. In 1990, use of the SIT resulted in the eradication of this pest from an incipient infestation in 125 km^2 at Perth, Western Australia (Fisher 1996). Also, the SIT was used to eradicate the Mediterranean fruit fly in Carnarvon in Western Australia (Fisher et al. 1985), and is now used to eradicate recurrent outbreaks of this pest in South Australia (Smallridge et al. 2002).

4.2.2. Moscamed

In 1955, the Mediterranean fruit fly was found in Costa Rica. After the pest had established a small foothold in Nicaragua, and a pilot programme conducted in 1967 (Rhode 1970), an operational programme to contain this pest was initiated to prevent it from invading countries to the north (Rhode et al. 1971). However, very unfortunately, a review team concluded that the Mediterranean fruit fly is not economically important to Central America, and recommended that the programme be terminated (Rhode 1976; Dyck, Reyes Flores et al., this volume). By 1976 the pest had expanded its range into Honduras, El Salvador, and Guatemala, and in a few years occupied 15 000 km^2 in southern Mexico. To meet this emergency, the Government of Mexico entered into cooperative agreements with Guatemala and the USA to establish the first large-scale fruit fly AW-IPM programme using the SIT. Construction of a rearing facility at Metapa, Mexico, to produce 500 million sterile flies per week, began in 1978. Pest eradication in the infested area of Mexico was achieved in 1982 (Hendrichs et al. 1983), and a barrier was created through Guatemala (Villaseñor et al. 2000; Enkerlin, this volume). For over 25 years, this programme has kept Mexico, the USA, and half of Guatemala free of the Mediterranean fruit fly, allowing Mexico over this period to significantly expand its fruit and vegetable exports to the USA. According to The Economist (2004), the Mexican horticulture export earnings since 1994 have tripled to over USD 3500 million per year, with exports of fresh vegetables rising by 80% and fresh fruit by 90%. In the meantime, the production capacity of the Moscamed programme has increased to over 3500 million sterile males per week, the majority of which are produced at the El Pino facility in Guatemala (Rendón et al. 2004).

4.2.3. Melon Fly Eradication in South-Western Islands of Japan

Between 1919 and 1970, the melon fly was discovered to have invaded various island groups in the south of Japan, including Okinawa. The shipment of fruits and vegetables to markets in mainland Japan was strictly forbidden. Consequently, the Japanese National Government assisted the Prefectural Governments of Kagoshima and Okinawa to conduct two separate programmes to eradicate the melon fly from all of the south-western islands.

A pilot eradication experiment on small Kume Island (60 km^2) began in 1972, and eradication was declared in 1978. In 1984, an operational programme was undertaken in the Miyako Islands. The capacity of the rearing facility was 30 million flies per week. Since the wild population was estimated at 34.4 million, male

annihilation (using cotton strings impregnated with cuelure and insecticide) was used to reduce it to 5% of its original level. The production of high-quality flies, and supplementary releases in high-density areas, were critically important (Yamagishi et al. 1993, Kakinohana 1994). By 1986 the production capacity had been expanded to almost 200 million sterile flies, and the programme gradually moved from island group to island group until eradication of the melon fly from all of Japan was achieved in 1993 (Kuba et al. 1996, Koyama et al. 2004).

4.2.4. Mediterranean Fruit Fly Genetic Sexing Strains

In the early 1960s, the Citrus Marketing Board of Israel developed an insecticide-based area-wide programme against the Mediterranean fruit fly that was able to meet the certified quarantine security requirements of fruit importing countries (Cohen and Cohen 1967, Hendrichs 1996). Evidently bisexual releases of sterile flies could not be used for this programme because sexually sterile female Mediterranean fruit flies sting fruit with their ovipositors. However, in work with the Australian sheep blow fly *Lucilia cuprina* (Wiedemann), Whitten (1969) found that male and female pupae of a strain, in which the segment of the autosome bearing a gene for black puparium is translocated to the Y chromosome, could be separated mechanically. Therefore, all males are brown, and all females black. This encouraged Rössler (1979) to construct a similar strain of the Mediterranean fruit fly in which male pupae (brown) could be separated from female pupae (white). This special strain was mass-reared and sorted at the FAO/IAEA Seibersdorf laboratory in Austria, and performed very well in large-scale tests in Israel (Nitzan et al. 1990; Franz, this volume). Subsequently the Seibersdorf laboratory developed a genetic sexing strain in which a segment of an autosome bearing the dominant wild type allele of a temperature-sensitive lethal (*tsl*) mutant was translocated to the Y chromosome (Franz and Kerremans 1994, Caceres et al. 2004). In addition, this laboratory developed a "filter rearing system" to maintain stability in the mass-rearing of genetic sexing strains (Fisher and Caceres 2000; Franz, this volume; Parker, this volume).

4.2.5. Sterile "Genetic Sexing Strain" Males Alleviate Mediterranean Fruit Fly Crises in California and Florida, USA

Until 1980, the Mediterranean fruit fly invaded California and Florida at only infrequent intervals. Outbreaks were eliminated mainly by applying malathion-bait sprays. It cost more than USD 100 million to eradicate the infestations in California detected in 1980 and 1982. In the decade 1987–1997, multiple new infestations were encountered annually in California and Florida. Since each outbreak was addressed independently, this non-area-wide approach resulted in continuous new satellite infestations, and there was a real threat that the pest would become established. Therefore, in 1994, an area-wide SIT eradication programme was initiated, with twice-weekly releases of sterile Mediterranean fruit flies over the entire Los Angeles Basin. This programme was so successful and cost-effective that, in view of the many introductions, in 1996 a permanent preventive release programme was established (Dowell et al. 2000, Barry et al. 2004). The same preventive approach

was also followed in 1998 in three high-risk Florida counties. Sterile males of the *tsl* sexing strain VIENNA 7, mainly produced by the Moscamed programme in Guatemala, are used for these preventive release programmes. Sexing strains are now used in most Mediterranean fruit fly suppression, containment, or eradication programmes (Caceres et al. 2004; Enkerlin, this volume; Franz, this volume; Hendrichs et al., this volume).

4.2.6. *Jordan-Israel-Palestine Mediterranean Fruit Fly Programme*

The signing of the Oslo Peace Accord created an opportunity for the international community to assist Middle East countries, notably the Hashemite Kingdom of Jordan, the Palestinian Authority, and Israel, to undertake joint projects that would foster cooperation. Consequently, the various governments and the Palestinian Authority met in Vienna, agreed to develop a cooperative area-wide programme with the support of the FAO/IAEA, and asked these organizations to conduct an economic analysis of three area-wide programme alternatives (Enkerlin and Mumford 1997). In the operational programme, initially focused on the Arava/Araba region between Israel and Jordan, the genetic sexing strain VIENNA 7 (Franz, this volume) has been used for population suppression rather than eradication (at present, there is no intention to establish disruptive quarantines along a major highway). As a result of this suppression programme, the export of fresh vegetables from the Arava region has reached almost USD 30 million per year (Cayol et al. 2004; Enkerlin, this volume). The sterile pupae for this programme have been shipped from the Moscamed facility in El Pino, Guatemala, although the largest producer of biological control agents in Israel is currently constructing a commercial mass-rearing facility, with the goal of expanding suppression to the area north of the West Bank, including northern Jordan.

4.2.7. *Trend is Suppression, not Eradication, of Fruit Flies*

For technical and economic reasons, including difficulties in maintaining effective quarantines (even with large capital investments in facilities), today most fruit fly AW-IPM programmes that integrate the SIT aim to suppress the pest populations (Mumford 2004; Enkerlin, this volume; Hendrichs et al., this volume; Mumford, this volume). Examples of suppression programmes are:

- Mediterranean fruit fly
  - Cap Bon, Tunisia (Ortiz Moreno 2001)
  - Costa Rica (Reyes Flores 2004)
  - Hex River Valley, South Africa (Barnes et al. 2004; Enkerlin, this volume)
  - Madeira, Portugal (Dantas et al. 2004)
  - San Francisco Valley, Bahia, Brazil
  - Valencia, Spain (Generalitat Valenciana 2003)
- Mexican fruit fly
  - North-east Mexico (SAGAR/IICA 2001)
- Oriental fruit fly
  - Ratchaburi Province, Thailand (Sutantawong et al. 2004)

4.3. Onion Maggot

Since 1981, the SIT has been applied by a private firm (De Groene Vlieg) in The Netherlands to control the onion maggot *Delia antiqua* (Meigen) on an aggregate area of 2600 hectares (Loosjes 2000). The flies are reared year-round, and stockpiled in diapause for release during the onion-growing season. Individual farmers contract for the SIT independently of their neighbours, many of whom use chemical control. Much efficiency is lost since the sterile flies are not applied on an area-wide basis (protected fields do not form a contiguous block). Some growers in the general area of sterile-fly releases benefit from them, but refuse to contribute to the programme (free-riders). The programme has not been able to expand beyond 16% of the onion production area.

4.4. Tsetse Flies

Tsetse flies are unique among pest insects in being larviparous, i.e. females do not lay eggs but gestate a larva in a uterus (one larva at a time), with a gestation period of about 9 days. Thus, these flies have extraordinarily low rates of reproduction. Therefore, relatively low release rates should be sufficient, compared with those required for highly fertile oviparous pests (Hendrichs et al., this volume). However, rearing tsetse flies is relatively laborious and expensive because both sexes require frequent blood feeding (Parker, this volume).

Table 2 summarizes the SIT trials and operations that have been conducted on tsetse flies. (Data from the trial by Vanderplank is shown in Table 1.) In a trial on *G. m. morsitans* Westwood in 1969 on an island (5 km^2) in Lake Kariba, Zimbabwe, pupae collected in the field were chemosterilized in the laboratory, and then returned to the field to permit adult flies to emerge. The sterile flies were fully competitive, but adult flies that were sterilized after emergence and held in captivity suffered an 80% loss in field competitiveness. These studies were followed in 1977–1978 by a larger-scale (195 km^2) trial in Tanzania using factory-reared *G. m. morsitans* fed on live animals, which demonstrated full sterile fly competitiveness following irradiation and release in the pupal stage.

Among the other releases, conducted in the 1970s and 1980s, were several that successfully integrated releasing sterile males with the recently developed attractant traps and insecticide-treated targets. Three tsetse species were eradicated simultaneously in 3000 km^2 in Burkina Faso (Politzar and Cuisance 1984), and one species in 1500 km^2 area in Nigeria (Takken et al. 1986). The technology was successfully applied, but unfortunately the programmes were not conducted area-wide and thus the pest free status of the areas was not sustainable.

Traps were also used on a small island (12 km^2) in Lake Kariba, Zimbabwe, to attract wild flies that were autosterilized by coming into contact with the traps, and then departed (Hargrove and Langley 1990). This, and another failed eradication trial, also in a small island in a Ugandan lake, are almost the only attempts to date to apply the autosterilization principle which avoids or minimizes the need for a rearing facility.

Table 2. Summary of SIT trials with tsetse flies Glossina *spp.*

Species, habitat, and location	Method	Outcome and objectives	References
Glossina swynnnertoni, savannah, north-western Tanzania	Release of *G. morsitans*, which mated with *G. swynnertoni*	99% suppression in 256 km^2, permitted development of the area for agricultural production	Vanderplank 1947, and hitherto unpublished data shown in Table 1
G. morsitans morsitans, savannah, Lake Kariba, Zimbabwe	Insecticidal suppression followed by release of chemically sterilized pupae	> 99% suppression on 5-km^2 island, feasibility study	Dame and Schmidt 1970, Dame et al. 1981
G. tachinoides Westwood, riverine, Chad	Radiation-sterilized, transport from France, ground release sterile ♂	Feasibility study, sterilization, transport, release	Cuisance and Itard 1973
G. palpalis gambiensis Vanderplank, riverine, Burkina Faso	Suppression by aerial insecticide treatment, ground release sterile ♂	Feasibility study (16 linear km) to control sleeping sickness	Van der Vloedt et al. 1980
G. palpalis palpalis Robineau-Desvoidy with *G. tachinoides* as a control, riverine, Lafia, Nigeria	Suppression with traps and targets followed by ground release of radiation-sterilized adults	Eradication of *G. p. palpalis* in 1500 km^2	Takken et al. 1986, Oladunmade et al. 1990
G. morsitans morsitans, savannah, Tanzania	Insecticidal suppression followed by ground release of radiation-sterilized pupae	90% suppression (195 km^2), feasibility study	Dame et al. 1975, Williamson et al. 1983
G. morsitans morsitans, and *G. pallidipes* Austen, savannah, Lake Kariba, Zimbabwe	Autosterilization of wild flies with pyriproxyfen	Suppression (12 km^2), feasibility study	Hargrove and Langley 1990
G. morsitans submorsitans Newstead, *G. palpalis gambiensis, G. palpalis palpalis, G. tachinoides*, riverine and savannah, Burkina Faso, Nigeria	Insecticide application and trapping suppression followed by ground release of radiation-sterilized adults	Eradication (3000 km^2 - Burkina Faso, 1500 km^2 - Nigeria)	Politzar and Cuisance 1984, Takken et al. 1986
G. austeni, bushland and forest, Unguja, Zanzibar, Tanzania	Suppression with insecticide on livestock and attractive devices followed by aerial release of radiation-sterilized adults	Eradication (1650 km^2), trypanosomosis transmission ceased	Msangi et al. 2000; Vreysen et al. 2000; Feldmann et al., this volume
G. fuscipes fuscipes Newstead, forest, Buvuma Islands, Uganda	Autosterilization of wild flies with triflumuron vs. insecticide-impregnated traps	Suppression (5 km^2), abandoned because of funding shortfall	Oloo et al. 2000

The first aerial releases of sterile males were carried out in the 1990s in Unguja Island, Zanzibar, Tanzania, culminating in the eradication of *G. austeni* (Vreysen et al. 2000). This successful programme freed the cattle from the burden of nagana (Msangi et al. 2000; Feldmann and Jannin 2001; Feldmann et al., this volume).

Vreysen and Van der Vloedt (1992) described a sensitive method to determine whether or not eradication has been achieved. This consists of the release of virgin sterile female flies, and their recapture and dissection, to determine if, while in the field, they became inseminated by wild males.

4.5. Mosquitoes

As shown in Table 3 and in Benedict and Robinson (2003), several release trials were conducted with sterile male mosquitoes in the 1960s and 1970s. For mosquito SIT, since females may transmit disease, it is essential that releases of female mosquitoes be reduced to an absolute minimum.

The largest-scale trials were conducted in El Salvador and India. Unfortunately, in both countries, political factors in the mid-1970s interrupted further work — civil war in El Salvador, and in India false accusations that the project was intended to collect data on biological warfare (Nature 1975, WHO 1976).

The work in India showed that two important vector species of culicine mosquitoes could be mass-reared, and the sexes separated (according to pupal size) to ensure that 99.8% of the released mosquitoes were males. Males were chemosterilized in the pupal stage, or by male-linked chromosome translocations combined either with cytoplasmic incompatibility or sex-ratio distortion due to meiotic drive. Field tests showed that the mating competitiveness of the males of both species was acceptable. However, the mass release of *Culex quinquefasciatus* Say males in villages achieved only limited levels of sterility in eggs laid by wild females. This was attributed to the influx of already-mated females from outside the target release area. A planned mass release of sterile male *Aedes aegypti* (L.), aimed at the eradication of this urban mosquito from a whole town, was prevented by the political problem mentioned above.

In El Salvador, the target was the malaria vector *Anopheles albimanus* Wiedemann. It was multi-resistant to insecticides (partly due to the agricultural use of insecticides), and therefore difficult to control by conventional means. In the initial study, releases during 5 months around Lake Apastapeque were successful in inducing 100% sterility in eggs laid by wild females (Lofgren et al. 1974, Weidhaas 1974). Sex separation in that trial was based on pupal size differentiation; however, it was very imperfect, yielding 15% females among the released males. In a second larger trial, a second separation was added; adults were offered malathion-laced blood through a membrane. This effectively eliminated most of the females, but the males were debilitated from being caged and handled. Therefore a genetic sexing strain was developed — a chromosome translocation was induced, linking a propoxur-resistance gene to the Y chromosome, and this was combined with a crossover-suppressing chromosome inversion. Propoxur treatment at the egg stage selectively eliminated all but 0.2% of females, thereby allowing a doubling of the male production for release (Seawright et al. 1978). By eliminating the handling

losses in the adult stage, the net release was increased from 200 000 males per day to over 1 million, and these males, when released as sterile pupae, were almost fully competitive in the field. Compared with the seasonal upward trend of the untreated population, the releases reduced the target field population by more than 97% (Dame et al. 1981, Benedict and Robinson 2003). However, complete control was thwarted by immigration, and political unrest and war caused the trials to be terminated.

Table 3. Summary of release trials with sterile or semi-sterile male mosquitoes

Target species	Location	Sterilization and sex-separation method	Outcome	References
Anopheles quadrimaculatus Say	Lakes in Florida, USA	Pupal irradiation, adult release, sex separation by pupal size	Poor competitiveness of colonized males for wild females, which may have been mismatched for sibling species	Weidhaas et al. 1962, Dame et al. 1964
Culex quinquefasciatus	Okpo, Myanmar	Cytoplasmic incompatibility	Eradication of small village population	Laven 1967
Anopheles gambiae s.s. Giles	Pala, Burkina Faso	*An. melas* Theobald x *An. arabiensis* Patton cross yielding sterile hybrid males and few females	Poor competitiveness of hybrid males	Davidson et al. 1970
Culex quinquefasciatus	Sea Horse Key, Florida, USA	Chemosterilization with thiotepa	Sterilization of small island population, moderate competitiveness	Patterson 1970
Culex pipiens L.	Village near Montpellier, France	Chromosome translocations	Persistent semi-sterility in wild population	Laven 1972, Cousserans and Guille 1974
Culex quinquefasciatus	Villages near Delhi, India	Thiotepa sterilization or cytoplasmic incompatibility plus translocations, sex separation by pupal size	300 000 released per day, 99.8% male, adequate competitiveness in the field for females of wild origin, but high egg sterility not achieved by mass release due to immigration	Singh et al. 1975, Sharma et al. 1972, Grover et al. 1976a, Yasuno et al. 1978, Curtis et al. 1982, Curtis 1976
Aedes aegypti	Urban areas in or near Delhi, India	Thiotepa sterilization or sex-ratio distorter plus translocations, sex separation by pupal size	Rearing and sex separation as for *Culex quinquefasciatus* above, high competitiveness in the field for females of wild origin	Ansari et al. 1977, Suguna et al. 1977, Grover et al. 1976b, Curtis et al. 1976

Table 3. Continued

Target species	Location	Sterilization and sex-separation method	Outcome	References
Aedes aegypti	Mombasa, Kenya	Chromosome translocations	Partial sterility detected in wild population	McDonald et al. 1977
Anopheles albimanus	Lake Apastapeque, El Salvador	Chemosterilization of pupae with bisazir, inaccurate sex separation based on pupal size	100% sterility induced in wild population, which fell below detection level after 5 months	Lofgren et al. 1974
Anopheles albimanus	Pacific coast of El Salvador	Bisazir sterilization, sex separation originally by pupal size + feeding on malathion-treated blood, later by a Y chromosome propoxur-resistance translocation inversion (MACHO strain)	Eventually 1 million MACHO released per day and found competitive, a natural population increase was suppressed, but eradication prevented by immigration in spite of a barrier zone	Seawright et al. 1978, Dame et al. 1981
Culex tritaeniorhynchus Giles	Village near Lahore, Pakistan	Pericentric inversion plus translocation, sex separation by temperature-sensitive lethal	Assortative mating — competed for females of colony origin but not for females of wild origin	Baker et al. 1978, 1979
Anopheles culicifacies Giles	Village near Lahore, Pakistan	Bisazir sterilization, sex separation by Y chromosome dieldrin translocation	Released males behaved normally in the field but showed subnormal mating competitiveness	Reisen et al. 1981, Baker et al. 1980, 1981
Culex tarsalis Coquillett	Kern County, California, USA	Adult irradiation after separation of males by hand	Partial assortative mating — reduced competitiveness for wild females, but supercompetitiveness for colony females	Reisen 1982

The idea of genetic control of mosquitoes is now undergoing a revival, based on the production of transgenic strains. Such strains may either have been rendered unable to transmit the pathogens that their species normally transmit (Ito et al. 2002), or carry a "release of insects carrying a dominant lethal" (RIDL) system (Thomas et al. 2000; Robinson and Hendrichs, this volume). The latter could provide both the elimination of biting females from batches being prepared for release, and elimination of females from the progeny of matings of the released males to wild females (Alphey and Andreasen 2002).

4.6. Coleoptera

4.6.1. Field Cockchafer
Cockchafers (Scarabaeidae) are important pests of root vegetables. The flight period can be accurately forecast, and is restricted to a few weeks every third year, with males emerging before females. In 1959 and 1962, Horber (1963) conducted two field trials on *Melolontha vulgaris* F. in 30 hectares of agricultural land in Switzerland. He collected adult males in light traps, and gave them a sterilizing dose of 33 Gy. In the two trials, 3109 and 8594 sterile males were released, and the wild populations were reduced by 80% and to eradication, respectively.

4.6.2. Boll Weevil
Since the boll weevil *Anthonomus grandis grandis* Boheman necessitated the use of one-third of the insecticides applied in US agriculture, and highly insecticide-resistant boll weevil populations had emerged, the National Cotton Council was determined to eradicate the boll weevil from the USA. Brazzel and Newsom (1959) showed that, in autumn, the boll weevil enters diapause in trash along the edges of cotton fields. If insecticides are applied just before diapause, the number of weevils that survives the winter is reduced by 90%. As predicted by Knipling's model, if insecticide sprays were targeted to kill also the generation producing individuals going into diapause, the number that overwinter is reduced by more than 99% (Knipling 1963, 1968a). This ignited interest in the possibility of eradicating the boll weevil. An effective pheromone-baited trap was developed for detection. Using the SIT was problematic, however, since the competitiveness of the weevils is reduced by irradiation. Nevertheless, this problem is largely avoided if they are sterilized with the anti-leukemia drug busulfan (Klassen and Earle 1970), or with low doses of fractionated irradiation (Haynes and Smith 1992).

In 1971–1973, a large pilot field experiment, centred in southern Mississippi, was conducted to assess the feasibility of eradication. The eradication zone was surrounded by three buffer zones. Very intensive suppression was implemented in the two inner zones, and farmers were expected to practice diligent control in the outer zone. Following a single integrated insecticide and SIT application, the boll weevil population in the eradication zone was suppressed below detectable levels in 203 out of 236 fields. All of the remaining 33 infested fields were located in one area less than 40 km from substantial uncontrolled populations. Knipling (1979) concluded that eradication could be achieved subsequently by further attacks on those portions of the population that had survived the first attack (Klassen 1989). However, other prominent entomologists disagreed, arguing that the method could be considered successful only if eradication was achieved throughout the area by one combined application (Perkins 1982); a report by the National Research Council (NRC 1975) concurred.

Since then, piecemeal AW-IPM programmes, that did not include the SIT, have removed the boll weevil from about 3 million hectares in the south-eastern and south-western states (USDA/APHIS 2004). Apparently, because of the disputed interpretation of the original trial, funding is being made available only piecemeal. Neither sterile males, nor other ecologically selective control tactics, are being used,

and broad-spectrum insecticides are the main eradicant employed (Cunningham and Grefenstette 2000, Dickerson et al. 2001). Pheromone traps are used to delimit infestations and to time insecticide applications, planting by all growers is synchronized and delayed, short-season varieties are grown, crops are harvested as soon as possible, and stalks are destroyed immediately after harvest. Eradication is usually achieved by the end of the third growing season. These area-wide programmes have led to significant reductions in the total amount of pesticide used on cotton in the USA. Eradication efforts are continuing in the remaining 40% of the original infested area in the USA, and have even expanded into cotton-growing regions of northern Mexico. The SIT might still be used in wildlife sanctuaries and parks where the application of insecticides is prohibited.

4.6.3. Sweetpotato Weevils

Two weevil species are found in Okinawa Prefecture in south-western Japan — sweetpotato weevil *Cylas formicarius* (F.) and West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire) (Yasuda 2000). These are invasive alien pests that threaten agricultural production, and have been designated as plant quarantine pests. Consequently, the transport of fresh sweet potatoes from Okinawa to mainland Japan is prohibited. Currently both species are the targets of pilot AW-IPM eradication programmes integrating the SIT on Kume Island.

From 1994 to 1999, the male annihilation technique (MAT), using synthetic sex pheromone and insecticide in wood fibreboard squares, was applied. The population of *C. formicarius* was suppressed by about 90%, and the plant infestation level dropped from 9.5 to less than 1%. Subsequently sterile weevils were aerially released, and "hot spots" were treated with additional ground releases. In 2002, no wild weevils were found, except at the south-eastern coast, and eradication appears to be imminent (Kohama et al. 2003).

Following a successful SIT trial in 1995, and using weevils reared on artificial diet (Shimoji and Miyatake 2002, Shimoji and Yamagishi 2004), an island-wide AW-IPM programme integrating the SIT against *E. postfasciatus* on Kume was initiated.

4.7. Lepidoptera

Throughout the world, lepidopteran larvae cause immense damage to food and forage crops, forests, and stored products. However, there are several problems when applying the SIT against moths (Bakri et al., this volume; Lance and McInnis, this volume):

- Resistance to dominant lethal induction by ionizing radiation, because of the holokinetic chromosomes of Lepidoptera
- Production of eupyrene and apyrene sperm
- Reductions in ability to mate
- Spermatophore formation
- Complex sperm transfer

For species, such as the codling moth, that undergo diapause, the potential exists to stockpile and activate them for release in synchrony with the wild population (Parker, this volume). However, on the whole, the large-scale rearing of moths is more difficult than that of most flies. For these reasons, the development of the SIT against moths has lagged behind work with flies. Nevertheless, the potential to use the SIT against lepidopteran pests appears to be very great (Carpenter et al., this volume).

M. D. Proverbs (1962, 1982) in British Columbia, Canada, developed a programme to suppress the codling moth using the SIT. He found that very high and debilitating doses of ionizing radiation (about 400 Gy) are required to induce 100% sterility. However, considerably lower and less debilitating doses induce inherited sterility, and the level of sterility of the F_1 progeny is greater than that of the irradiated parent (North 1975). Simulation models showed that released males with inherited sterility would suppress the wild population to a greater extent than the release of equal numbers of fully sterile males (Carpenter et al., this volume). In 1994, releases of irradiated codling moths were initiated in the Okanagan Valley of British Columbia, and continue with the objective of area-wide population suppression (Dyck et al. 1993; Bloem and Bloem 2000; Bloem et al., this volume).

The only other AW-IPM programme integrating the SIT, which has been implemented as a large-scale operational programme, is for the pink bollworm. Since 1968 sterile moths have been released in 0.4 million hectares of cotton fields in the San Joaquin Valley of California to prevent the establishment of the pink bollworm (it migrates from southern California) (Staten et al. 1993, Walters et al. 2000). This programme, largely funded by growers, has been key to the survival of cotton production in California. Recently, in conjunction with the use of *Bt*-cotton, the programme is being expanded with the aim of eliminating this pest from south-west USA and north-west Mexico (Bloem et al., this volume).

5. EPILOGUE

Krafsur (1998) noted that many scientists view the SIT and related genetic methods negatively (Whitten and Mahon, this volume). For example, Hargrove (2003) cast doubt on the potential value of the SIT for tsetse eradication. Krafsur (1998) identified the fundamental problem as follows:

> There is a paucity of published data that relate sterile male releases to population suppression. It would lend much credibility to the efficacy of SIT if sterile mating frequencies were estimated in challenged populations. Numerous models have been constructed that relate sterility and genetic deaths to population density but few were tested with field data. Studies in which target population dynamics are evaluated in terms of sterile mating rates and other covariates are badly needed. In this way, useful mathematical models could be made and tested with actual data.

Nevertheless, in the screwworm programmes, sterile and fertile egg masses were collected routinely in the Sanibel Island, Curaçao, and Florida programmes, and these data were related to target population decline (Baumhover 2002). Equivalent work on mosquitoes is summarized in Table 3. Vreysen et al. (2000) described a clear connection between the release of sterile tsetse males and induced sterility in

wild females, and between induced sterility and subsequent population decline in the field. Also Rendón et al. (2004) established this relationship on a large scale for the Mediterranean fruit fly. Regarding lepidopteran pests, North and Snow (1978) confirmed the interaction of released irradiated moths and the wild population by capturing wild females, allowing them to oviposit, dissecting them to determine whether or not the spermatophores were coloured with the dye from the rearing medium, and examining their progeny cytogenetically for chromosomal aberrations. Others have adopted this approach for other species (Bloem et al., this volume; Carpenter et al., this volume; Vreysen, this volume). All these data confirm the relationship between sterility and pest suppression, yet much remains to be done to further address the paucity of field data identified by Krafsur.

AW-IPM programmes that integrate the SIT serve a very useful role in focusing attention on the need for area-wide strategies in managing major pest problems (Klassen, this volume). AW-IPM programmes are difficult to organize and to manage, but those that exist have shown that the conventional IPM programmes tend to be conducted on too small a scale for optimum efficiency, and often employ insecticides in a way that destroys natural enemies.

Progress in developing and implementing the SIT for AW-IPM programmes has been slow in some cases, but it has been rapid for such groups as fruit fly pests. As noted by Hendrichs (2000), currently the SIT is practiced on an industrial and area-wide scale against the New World screwworm, Mediterranean fruit fly (and other fruit fly species, e.g. *Anastrepha ludens, A. obliqua, Bactrocera cucurbitae, B. dorsalis, B. tryoni*), pink bollworm, and codling moth. Mass-rearing facilities in several countries produce several thousand million sterile Mediterranean fruit flies per week, and the capacity still exists to produce 500 million New World screwworms per week. In 1994 the capacity to rear pink bollworms was increased, and the distribution of moths was extended to include the southern irrigated desert valleys of California (Walters et al. 2000). The codling moth programme in Canada has weathered major concerns (Myers et al. 1998), and has significantly reduced insecticide use. The small onion maggot programme releasing sterile flies in The Netherlands survives, even without authority for a mandatory area-wide application. The African Union is attempting to facilitate the integrated use of the SIT to establish tsetse-free zones (Feldmann et al., this volume). The SIT has been shown to be valuable in dealing with certain transboundary pest problems, and in eliminating outbreaks of invasive pests in urban settings. Finally, the SIT may benefit from the development of modern biotechnologies that may make disease vectors harmless by replacing genes for vectorial capacity in populations of mosquitoes and other vectors. The field evaluation of transgenic technology in vector control would be facilitated by studies on the effects of sexually active sterile transgenic vectors (Benedict and Robinson 2003; Robinson and Hendrichs, this volume).

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PART II

PRINCIPLES OF THE STERILE INSECT TECHNIQUE

CHAPTER 2.1.

AREA-WIDE INTEGRATED PEST MANAGEMENT AND THE STERILE INSECT TECHNIQUE

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SUMMARY

Area-wide integrated pest management (AW-IPM) focuses on the preventive management of pest populations throughout the ecosystem. It seeks to treat all habitats of the pest population so that none produces migrants to re-establish significant infestations in areas of concern. In contrast, the conventional strategy focuses narrowly on defending the valued entity (crop, livestock, people, buildings, etc.) from direct attack by pests. AW-IPM requires multiyear planning, and an organization dedicated exclusively to its implementation, whereas conventional pest management involves minimal forward planning, tends to be reactive, and is implemented independently by individual producers, businesses, or households. AW-IPM tends to utilize advanced technologies, whereas the conventional strategy tends to rely on traditional tactics and tools. The sterile insect technique (SIT) is a species-specific form of birth control imposed on the pest population. It is a powerful tool for "mopping up" sparse pest populations, and is most efficient when applied as a tactic in a system deployed on an area-wide basis. On environmental, economic and biological grounds, the case for the SIT is compelling.

1. AREA-WIDE INTEGRATED PEST MANAGEMENT STRATEGY

The area-wide concept is a modernizing strategy of providing services efficiently wherever needed. Area-wide management takes advantage of the power of organization intelligently applied. Police protection, distribution of potable water, and removal of garbage and sewage have been provided on an area-wide basis to varying degrees for several millennia, whereas mail service, retailing, ambulance, fire protection, public health, electrical supply, high-speed transport, telephone, and internet services have been provided relatively more recently (Lindquist 2001).

Area-wide integrated pest management (AW-IPM) also has ancient roots in coping with locust plagues and vector-borne diseases (Klassen 2000). By systematic use of quarantines, Venice and Milan in 1347–1350 contained bubonic plague transmitted by the oriental rat flea *Xenopsylla cheopis* (Rothschild), and this approach was gradually adopted throughout Europe. AW-IPM came into a degree of prominence in the late 19th and early 20th centuries with the development of classical biological control (1888), the use of pest-resistant plants (such as the grafting of all European grapes on phylloxera-resistant American rootstocks in the 1870s), campaigns to eradicate the gypsy moth *Lymantria dispar* (L.) in Massachusetts (1890–1901) and *Boophilus* spp. cattle ticks from the Southern USA (1906–1943), the suppression of yellow fever and malaria vectors in Havana (1898) and in the Panama Canal Zone (1904), and the organization of mosquito-abatement districts, first in West Africa and then in several continents (1900).

AW-IPM is one of several major strategies for coping with pest problems (Kogan 2000). Various pest management strategies have evolved in response to differences in the challenges presented by various pests (Hendrichs et al., this volume). The strategy for coping with a pest problem on an empirical basis gives only temporary alleviation. As methods of control, and guidelines for their use, are developed and refined, they form the basis of the conventional strategy of localized management of only fractions of populations. This is the most widely used strategy, and individual producers, businesses and households practice it independently and with no coordination. However, since individual producers or households are not capable of adequately meeting the challenge of certain very mobile and dangerous pests, the total population management or AW-IPM strategy developed (Rabb 1972). Mobile pests include not only those that fly long distances, but also those

transported passively on wind, water, or animal hosts, or in infested commodities traded locally or internationally. Table 1 lists examples of area-wide programmes not involving the sterile insect technique (SIT).

1.1. Defining Area-Wide Integrated Pest Management

For the purposes of this book, AW-IPM is defined as:

> IPM against an entire pest population within a delimited geographic area, with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area.

The common thread that runs through all AW-IPM programmes is the strong emphasis on the preventive control of foci of infestation from which recruits emerge to re-establish damaging densities of the pest population in areas of concern.

A few scientists have attempted to define the AW-IPM strategy. Knipling, as cited by Dickerson et al. (1999), stated:

> Area-wide pest management is the systematic reduction of a target key pest(s) to predetermined population levels through the use of uniformly applied control measures over large geographical areas clearly defined by biologically based criteria.

Lindquist (2000) wrote:

> An area-wide insect control programme is a long-term planned campaign against a pest insect population in a relatively large predefined area with the objective of reducing the insect population to a non-economic status.

Both of these definitions have considerable merit, and they fit the majority of area-wide programmes. However slightly different definitions may be needed to describe programmes on the conservation of natural enemies, and on classical biological control where the adaptation of the introduced biological agent to all new environments cannot be known in advance of release. Also, in using the SIT to contain a population, it may not be possible to clearly define the boundary of the pest population. In a similar vein, Showler (2002) stated:

> Locust swarms can be highly variable, influenced by many factors, including geography, vegetative conditions, land-use patterns, environmental sensitivity, availability of resources and tactics, prevailing winds, insecure areas, and rainfall patterns. Reliance on a single control strategy is therefore unrealistic [Showler 1997]. A more appropriate approach would be to develop specific strategies that will fit with projected scenarios, mostly by harmonizing them with national contingency plans.

1.2. Characteristics of Area-Wide Integrated Pest Management

It is intuitively obvious that the immigration of pests into a managed ecosystem prevents their effective suppression or eradication. Indeed, the immigration of pests, usually from small untreated foci into a managed area, has a tremendous impact. For example, very few codling moths *Cydia pomonella* (L.) develop in well-managed commercial orchards. Butt et al. (1973) found that the number of codling moths that

Table 1. Area-wide programmes to suppress or eradicate insect pests without SIT

Programme	References
Anti-locust programmes in Africa, southwest Asia and China — some are coordinated by the FAO	Showler 1997, 2002
Area-wide biocontrol of the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero with the parasitoid *Epidinocarsis lopezi* (De Santis) in 38 countries of sub-Saharan Africa	Herren and Neuenschwander 1991
Area-wide biocontrol of the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) with two parasitoid species in the Caribbean Basin, Florida and California	Meyerdirk 1999
Area-wide control of the brown planthopper *Nilaparvata lugens* (Stål) in Indonesia and the Philippines	Oka 1991
Dendroctonus pine bark beetles: long-term landscape level management aimed at age and species mosaics unfavourable for large outbreaks in western USA and Canada	Keen 1952
Global Malaria Eradication Campaign, initiated by the WHO in 1955, but disintegrated in 1969; malaria was eradicated in 37 countries, and in the end 74% of the people at risk were protected	Wright et al. 1972
Mosquito control districts first implemented in West Africa in the 1890s against malaria vectors; $\approx$ 260 districts in the USA, many in other countries	AMCA 2004
Onchocerciasis Control Programme in 1 000 000 km^2 in West Africa; insecticides used to kill *Simulium* spp. larvae in rivers, and ivermectin to treat infections	WHO 1994, Hougard 2000
Caribbean *Amblyomma* Programme to eradicate the tropical bont tick *Amblyomma variegatum* F., Bridgetown, Barbados; treatment of all ruminant livestock with pour-on Bayticol[R]	Pegram et al. 2000
Chagas' disease, reduviid vectors in Latin America; spray infested homes, eliminate habitat, screen blood banks for trypanosomes	Schofield 2000, Dias et al. 2002
Boll weevil *Anthonomus grandis grandis* Boheman eradication; pheromone trapping, insecticide treatment and cultural control	Cunningham and Grefensette 2000, Dickerson et al. 2001
Corn rootworm management programmes in Texas, Illinois, Indiana, Iowa, Kansas, and South Dakota; adult bait and kill system using cucurbitacins as attractants, carbaryl and non-toxic carrier applied to corn foliage	Chandler 2002
Codling moth suppression; area-wide use of pheromone-mediated mating disruption in Washington State, Oregon and California	Calkins et al. 2000, Coop et al. 2000
Area-wide use of encapsulated semiochemicals in lure and kill sprays against the olive fruit fly *Bactrocera oleae* (Gmelin) in Greece and Spain	Jones and Casagrande 2000
Eradication of *Bactrocera papayae* Drew and Hancock from North Queensland, Australia, by male annihilation and strategic foliage baiting	Hancock et al. 2000
Eradication of oriental fruit fly *Bactrocera dorsalis* Hendel from Mauritius using male annihilation and bait application technique	Seewooruthun et al. 2000
Eradication of the carambola fruit fly *Bactrocera carambolae* Drew and Hancock in Suriname, Brazil, and Guyana; Male annihilation	Malavasi et al. 2000
Pacific Islands Regional Fruit Fly Management Programme	Allwood 2000
Eradication of *Hypoderma* warbles from livestock in France using pour-on insecticides	Amouroux 2000

overwintered in the Wenas Valley of Washington State, USA, dropped by 96% when a few abandoned orchards and neglected non-commercial apple trees were either removed or sprayed with insecticide. This study indicated that most of the moths originated from untreated trees that in aggregate were less than 5% of the host resources of the moth. Similarly, experience in coping with the pink bollworm *Pectinophora gossypiella (*Saunders), and the boll weevil *Anthonomus grandis grandis* Boheman, have shown that the few growers who do not destroy crop residues immediately after harvest provide food for these pests to reproduce and to enter diapause. This lapse in field sanitation can be the direct cause of devastating levels of these pests appearing in the following season on neighbouring farms.

Knipling (1972) graphically pointed out the implications of allowing a small fraction of a population of a major pest to reproduce without control (Fig. 1). Knipling showed that more pest individuals would be produced if 1% of the total population were allowed to reproduce without control, while 100% control were applied to 99% of the population, than if only 90% control were imposed uniformly on the total population. Thus Knipling (1972) elaborated the basic principle of total population suppression:

> Uniform suppressive pressure applied against the total population of the pest over a period of generations will achieve greater suppression than a higher level of control on most, but not all, of the population, each generation.

The defining imperative, in all variations of the AW-IPM strategy, is the strong emphasis on preventing the existence of foci of infestation from which recruits can re-establish damaging densities of the pest population. AW-IPM is preventive and thoroughly inclusive.

Currently, for the most part, individual producers, who rely heavily on the use of insecticides, carry out defensively the control of highly mobile and very destructive pests. Although other control technologies are often incorporated into the producer's IPM system, these technologies are also usually applied by the individual producer independently of other producers. This conventional uncoordinated farm-by-farm strategy provides opportunities for the pest population to build-up and to infest well-managed fields. Consequently, on most farms, insect pest populations increase to damaging levels each year, and the farmer is forced to apply broad-spectrum fast-acting insecticides as a rescue treatment. This defeats the primary goal of the IPM system, which is to take maximum advantage of naturally occurring biological control agents (Rohwer and Knipling 1992). Moreover, the application of insecticides, when an insect pest population reaches the economic threshold, does not prevent the losses that occur before the threshold has been reached. For commodities that are planted on vast areas, such losses are immense. For example, the world production of maize (corn) is roughly 600 million metric tons (USDA/FAS 2002). Avoidance of a loss of only 3% would make available 18 million metric tons, which could be a major factor in alleviating hunger.

AW-IPM differs from conventional pest management of local pest populations in several important ways (Lindquist 2000): (1) it focuses on the preventive management of pest populations throughout the ecosystem, while the conventional strategy focuses narrowly on defending the valued entity (crop, livestock, people, buildings, etc.) from direct attack by pests, (2) it requires multi-year planning, and

IMPORTANCE OF TOTAL POPULATION SUPPRESSION
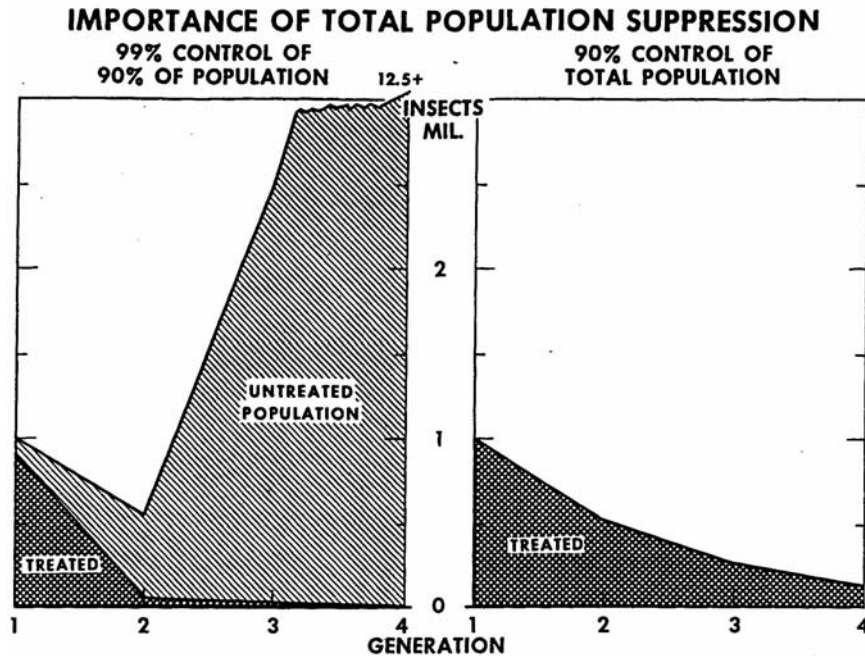
Figure 1. Results of model that shows the outcome of neglecting to suppress a small fraction of a pest population in an agroecosystem versus the effect of uniformly suppressing the entire pest population. Left: 10% of the population is untreated, and in four generations it produces a large number of individuals, while the 90% of the population that is treated declines. Right: Entire pest population in the agroecosystem is suppressed uniformly, and its numbers decline from generation to generation. (Figure from Knipling 1972, reproduced with permission.)

an organization dedicated exclusively to implementing the strategy; the conventional strategy involves only minimal forward planning, tends to be reactive, and is implemented independently by individual producers, businesses, or households, and (3) it tends to utilize advanced technologies, "high-tech", whereas the conventional strategy tends to rely on traditional tactics and tools that can be reliably implemented by non-specialists, "low-tech" (Lindquist 2000, 2001).

Indeed, it is the use of organizations dedicated to conduct area-wide programmes that provides the opportunities to employ sophisticated technologies and professional management. Computer-based models are utilized in planning and management, and satellite imagery is used to identify localities of alternate hosts that can be treated to reduce the number of migrants that cause the damage in commercial production areas. Area-wide programmes acquire or develop highly sensitive detection systems, and employ software for geographic information systems (GIS), global positioning system (GPS), and data management. They may implement approaches to prevent or retard the development of insecticide resistance

or the loss of host-plant resistance. Computer programmes and real-time environmental data to predict insect populations can be used effectively in an area-wide programme, but usually not on an individual farm basis. Thus pest immigration patterns, analysis of weather to predict increases or decreases of populations, genetic analysis to determine and forecast resistance levels, etc., are utilized in area-wide programmes.

Finally, area-wide programmes are able to take advantage of the power and selectivity of specialized methods of insect control that, for the most part, are not effective when used on a farm-by-farm basis. These include the SIT, certain programmes of inundative releases of parasitoids, semiochemicals, mating inhibitors, large-scale trap cropping, treatment of hosts on public lands and in private gardens, etc.

1.3. Benefits of Area-Wide Integrated Pest Management

Suppressing highly mobile pests on an area-wide basis is usually more benign environmentally, more effective, and more profitable, than on a farm-by-farm basis because of economies of scale (Carlson and Wetzstein 1993). Also AW-IPM is better able to capture the benefits of mobile natural enemies (Knipling 1992a).

Area-wide programmes enable many producers to pool resources to utilize technologies and expertise that are too expensive for individual producers. These may include mass-rearing facilities, aircraft, information technologies, and highly trained specialists. In addition a coordinated area-wide programme can avoid or internalize external costs. External costs (externalities) are the harmful effects arising from pest control operations, which affect parties other than the pest control decision-maker, but for which no compensation is paid to the persons harmed (Reichelderfer et al. 1984). Spray drift onto neighbouring properties frequently provokes disputes. Also pesticide use to protect agricultural crops has caused insecticide resistance to develop in insect vectors of disease. This has been an important factor in the resurgence of malaria.

Finally, economies of scale can be captured in area-wide programmes, although complex trade-offs may be involved. The more mobile the pest and the more uniform the damage caused by the pest, the larger the area under coordinated management can be. The total cost of pest detection, monitoring, and suppression per hectare of crop usually declines as the size of the managed area increases. However, as the project size increases, the per-hectare organizational cost usually increases because of the increased need for coordination and other communication costs. For these reasons, in very large programmes such as the effort to eradicate the boll weevil in the USA, the vast area was subdivided into several zones. Also considerable organizational cost savings may be realized in instances where towns, municipalities, or cooperatives already have structures in place for decision-making and communication.

1.4. Contingencies Often Dictate Changes in Strategy

Contingencies often arise that require replacement of one strategy with another (Geier 1970). For example, at various times during the 43-year campaign to remove the New World screwworm *Cochliomyia hominivorax* (Coquerel) from the United States, Mexico and Central America, different pest management strategies had to be selected. This programme began when an unusual series of frosts, beginning early in December 1957, killed all screwworms in the south-eastern USA north of a line from Tampa to Vero Beach in southern Florida. Sexually sterile flies, from a culture in a research laboratory, were released in a broad band north of this line to contain the pest population. Meanwhile a high-capacity rearing facility was being constructed. This exclusion or containment strategy was replaced by the strategy of eradication in the summer of 1958, when the mass-rearing facility was able to produce 50 million sterile flies per week. Eradication was accomplished in 1959. Subsequently, in 1966, the screwworm was declared eradicated in the USA. However, it was obvious that, unless the insect was removed from northern Mexico, it would reinvade the USA. Nevertheless the eradication of the screwworm from northern Mexico could not commence immediately because, until 1972, there was no agreement between the Governments of the USA and Mexico. Finally, in 1974, operations in Mexico began, and the last screwworm case occurred in the USA in 1982. However, in 1966, the programme strategy clearly had to change from eradication to exclusion in the form of a sterile insect barrier along the entire Mexico-USA border. Sterile flies were released in a zone that was about 130-km wide. Unfortunately this barrier proved to be too narrow to exclude female screwworms from entering the USA and causing many cases of screwworm myiases (Graham 1985).

1.5. Legal Authority for Area-Wide Integrated Pest Management

The legal authority to conduct area-wide and other regulatory programmes is absolutely essential, and still evolving (Dyck, Reyes Flores et al., this volume). Without explicit legal authority, the organisation conducting an area-wide programme would not be able to conduct operations on private property, nor operate quarantines.

In about 1860 the grape phylloxera *Phylloxera vitifoliae* (Fitch) was transported from the USA to France. Within 25 years of its arrival, this insect had utterly destroyed 1 million hectares of vineyards (fully one-third of the productive capacity). To protect the German wine industry, the Government of Germany in 1873 passed the first law that provided for quarantines and regulatory control of agricultural pests (NAS 1969). Other governments quickly followed the example, and in 1881 representatives of many European countries together developed a set of regulations governing the movement of grape-propagating material.

In about 1880 the establishment of the San Jose scale *Quadraspidiotus perniciosus* Comstock in California, its rapid spread on nursery stock throughout the country, and the failure of a programme to eradicate it, caused Canada, Germany and Austria-Hungary in 1898 to prohibit the importation of American fruit and

living plants (Klassen 1989). This crisis in the USA led to the passage in 1905 of federal legislation on quarantine and the regulation of interstate shipments. Indeed now many countries have legislation on: (1) prevention of the introduction of new pests from foreign countries, (2) prevention of the spread of established pests within the country or state, and (3) enforcement of the application of control measures against exotic pests to prevent their introduction and establishment, eradicate their outbreaks, retard their advance, or prevent damage by them. In some countries the law allows people, who wish to organize a programme against a pest, to hold a referendum. If the referendum passes by a certain margin (usually 67% in US counties), then all parties at interest must cooperate in the venture.

In Florida, the programme to eradicate the citrus canker disease has been hampered for 4 years as a result of insufficient legal authority. This pathogen is carried considerable distances on driving rain, and to achieve eradication the Division of Plant Industry has found it necessary to destroy all citrus trees within a radius of 578 m from an infected tree. Homeowners in urban areas, who do not understand the need for such drastic action, feel that their rights are violated by workers who, as part of the eradication programme, enter residential yards and destroy citrus trees. Thus, the Broward County Circuit Court has ruled that programme employees must have a separate court-issued warrant to enter each privately owned property. The need to apply for tens of thousands of warrants prompted the Florida Department of Agriculture and Consumer Services to appeal this ruling; in 2004 the Florida Supreme Court overturned it.

1.6. Apathy, Outrage, and Area-Wide Integrated Pest Management

The strategy of eradication emerged just over a century ago as the brainchild of C. H. Fernald of the University of Massachusetts, USA. Under Fernald's leadership, Massachusetts attempted to eradicate an introduced pest, the gypsy moth, in an 11-year campaign from 1890 to 1901. Initially, the primary eradicant was Paris green, but this insecticide, of only modest efficacy and phytotoxicity, had to be abandoned because of adverse public reaction.

Forbush and Fernald (1896) noted:

> Considerable opposition to the use of Paris green for spraying was manifested by many people living in the infested towns. A mass meeting of opponents of the spraying was held in Medford. One citizen, who attempted to cut the hose attached to one of the spraying tanks, and threatened with violence the employees of the Board who had entered upon his land, was arrested and fined. Others neutralized the effects of the spraying by turning the garden hose upon trees and shrubs that had been sprayed, and washing off the solution. The opposition to the spraying affected the results of the work unfavourably to a considerable extent.

Clearly apathy by many members of the public had turned into outrage.

If not concerned primarily with the economic dimension of the pest problem, stakeholders tend to be highly concerned with ecological, environmental, social, and human-health implications of area-wide programmes (Rabb 1972, Dreistadt 1983, Scribner 1983, Myers et al. 1998). Therefore leaders of AW-IPM programmes need to be very sensitive to the perceptions and attitudes of the public toward certain

programme operations (Dyck, Regidor Fernández et al., this volume). Often eradication efforts must be conducted by the ground rules of the urban, rather than rural, setting. In programmes to eradicate the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in California and Florida, members of the urban public strongly protested the aerial pesticide applications (even the same insecticide that was used without dissent for mosquito abatement). On the other hand, the same public has usually applauded the release of sexually sterile male flies.

As explained by Sandman (1987), the public is usually apathetic towards technological programmes. However certain factors inherent in programmes, and in the manner in which they are managed, can precipitate an almost irreversible shift of the public's attitude from apathy to outrage. A sense of outrage can be evoked by involuntary exposure to pesticide residues, imposed levies of fees, quarantines, right of trespass, unfair and inequitable sharing of risks, costs and benefits, temporary loss of control of one's property or field operations, the perception that endangered species may be harmed, etc. Starr (1985) asserted that:

> Public acceptance of risk is more dependent on public confidence in risk management than on the quantitative estimates of risk consequences, probabilities and magnitudes.

He noted that, when a zoo wishes to acquire a tiger, the public does not demand a refined assessment of the risk that the tiger might escape and kill someone. Instead the public wants assurance that the zookeeper can be trusted to prevent the tiger from escaping, and that, if the tiger should escape, the zoo is fully prepared to implement an emergency plan to meet this contingency. Similarly the public's confidence in the management of an area-wide programme is of paramount importance.

In each area-wide programme, a special effort must be made to anticipate and identify those factors that may be emotionally upsetting to various stakeholders, and take pre-emptive actions to avoid or mitigate adverse reactions. Public officials must be kept apprised, fora must be created for effective two-way communication with the public, surrogates of the public must be included in oversight and decision-making processes, and referenda may have to be conducted to secure support and funding for the programme (Batra and Klassen 1987; Klassen 1989; Dyck, Regidor Fernández et al., this volume).

1.7. Invasive Pests, Global Trade, and Area-Wide Integrated Pest Management

The rapid globalization of trade in agricultural products, and increasing tourism, have dramatically increased the spread of invasive harmful organisms (Klassen et al. 2002). We have entered an era of an unprecedented level of travel by exotic invasive organisms. Native flora and fauna on islands are sustaining great harm from non-indigenous invasive organisms, and major pests are becoming established with increasing frequency on all continents, except Antarctica.

For about a century many countries relied on the inspection of arriving cargo and passengers at the port of entry as the primary exclusion strategy. However the volume of arriving cargo is doubling every 5 or 6 years (Zadig 1999), and it is not possible to increase similarly the human and other resources devoted to inspection at

ports of entry. Clearly exclusion at the port of entry is no longer sufficient to protect plant resources, even though a number of emerging technologies are likely to facilitate safeguarding activities (Batkin 1999). Thus, to stem the influx of exotic pests into the USA, the National Plant Board asserted that the most important change needed in the US safeguarding system is to shift primary reliance from exclusion at the port of entry to off-shore actions, i.e. pest-risk mitigation in the areas of production, certification at the point of origin, and pre-clearance at the port of export (NPB 1999).

An important approach to offshore risk mitigation is the creation of pest free areas. Indeed, countries that export raw agricultural commodities can effectively remove the threat of exotic pests to the importing country by creating and maintaining pest free areas (Rohwer 1992; Malavasi et al. 1994; Enkerlin, this volume; Hendrichs et al., this volume). A pest free area is an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (FAO 2002). There are two kinds of pest free areas: (1) pest free zones — large geographic areas, including entire countries such as Chile, Japan or New Zealand — that are certified free of tropical fruit flies of economic importance, and (2) pest free production fields or areas that require demonstration of non-host-status or the demonstrated suppression of quarantine pests to non-detectable levels. In addition, there are low-prevalence areas that are established by means of a systems approach through the application of a series of pre-and post-harvest suppression and mitigating measures.

According to Griffin (2000), both the International Plant Protection Convention (IPPC) and the Sanitary and Phytosanitary Agreement:

> … are structured to accept and encourage area-wide pest management as a tool for promoting safe trade and contributing as much as possible to the complementary goals of food security and economic security for all countries.

Chile used the SIT to rid the entire country of the Mediterranean fruit fly (Klassen et al. 1994). By 1995 (SAG 1995) the entire country had become Mediterranean fruit fly-free, and since then huge volumes of Chilean fruits have entered the USA and other markets without the need for any quarantine treatments. This has dramatically strengthened the economy of Chile (Enkerlin, this volume). Argentina has created Mediterranean fruit fly-free areas within the province of Mendoza (El Valle de Uco) (Los Andes 2004), and the region of Patagonia. Now Peru and other countries have AW-IPM programmes integrating the SIT that should enable them to create fly-free zones and thereby obtain access to markets in southern Europe, Japan, and the USA (Enkerlin, this volume).

The first US-recognized pest free area was established in the early 1990s in Sonora, Mexico (CNCMF 2002), and since then Mexico has been ridding large additional sections of its territory of all fruit fly species of economic importance. The Mexican states of Baja California, Chihuahua, and Sonora have been freed of all economically important species of fruit flies. Citrus, stone fruits, apples, and vegetables are being exported from these states without any suppression or post-harvest treatment. In other parts of Mexico, low-prevalence fruit fly areas are being established by means of a systems approach (Reyes et al. 2000; Enkerlin, this volume; Hendrichs et al., this volume).

An area of low pest prevalence is an area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels, and which is subject to effective surveillance, control or eradication measures (FAO 2002; Hendrichs et al., this volume). Requirements to establish low-prevalence areas include a sensitive detection programme, suppression of the quarantine-significant pest to non-detectable-levels, strict control of the fields, and safeguards to prevent infestation during packing and transit to the port of export (Riherd 1993, Simpson 1993, Malavasi et al. 1994, Riherd et al. 1994).

Florida is able to export grapefruit to Japan by creating pest free grapefruit groves in about 22 counties. Regulatory experts from Japan inspect the entire process of production, packing and transit. Official protocols for pest free fields can be found in Gomez (1999). Similarly fruit groves free of the South American cucurbit fruit fly *Anastrepha grandis* (Macquart) have been created in Mossoro, Brazil, and Guayaquil, Ecuador through demonstration of non-host status under appropriate crop management (Malavasi et al. 1994).

The concept of low prevalence, using bait sprays and the SIT, was pioneered during the early 1960s against the Mexican fruit fly *Anastrepha ludens* (Loew) along the Mexico-USA border (Knipling 1979). Also, in the early 1960s, the Citrus Marketing Board of Israel developed a bait-spray-based area-wide programme against the Mediterranean fruit fly that has been able to meet the certified quarantine security requirements of fruit importing countries (Cohen and Cohen 1967, Hendrichs 1996).

A more recent and highly significant development has been the continuous area-wide release of sexually sterile male medflies in the Los Angeles Basin (Dowell et al. 2000; APHIS 2004; Enkerlin, this volume), and around high-risk ports in southern Florida, to prevent Mediterranean fruit fly establishment (APHIS 1998).

2. BASIC ELEMENTS OF SIT

The SIT is a form of birth control imposed on an insect pest population to reduce its numbers. The SIT harnesses the sex drive of insects. According to the International Plant Protection Convention (IPPC) (FAO 2005), the SIT is defined as:

> a method of pest control using area-wide inundative releases of sterile insects to reduce fertility of a field population of the same species.

Thus far the SIT has involved rearing large numbers of the target pest species, exposing them to gamma rays to induce sexual sterility, and releasing them into the target population of the pest on an area-wide basis. Another kind of SIT avoids the need to mass-rear the target insect pest species since individuals of the wild populations are attracted to a chemosterilant, e.g. Mediterranean fruit fly (Navarro-Llopis et al. 2004) and *Glossina* spp. tsetse flies (Hargrove and Langley 1990, Langley 1999). Thus treated insects are prevented from reproducing; they act as biological agents that nullify the biological potential of untreated individuals with which they mate. Both kinds of SIT are effective only against pest species that reproduce sexually.

2.1. Mechanisms of Sterility

The type and level of sterility induced in mass-reared and released insects is critically important. A sterile insect is defined (FAO 2005) as:

> an insect that, as a result of an appropriate treatment, is unable to produce viable offspring.

Fecundity is the number of progeny produced per female. Thus only females can be infecund. Sterility may be caused by: (1) the inability of females to lay eggs (infecundity), (2) the inability of males to produce sperm (aspermia), or inability of sperm to function (sperm inactivation), (3) the inability to mate, or (4) dominant lethal mutations in the reproductive cells of either the male or female (LaChance 1967; LaChance et al. 1967; Lance and McInnis, this volume). All of these mechanisms may be induced by exposing insects to gamma rays, X-rays, or certain chemicals (Bakri et al., this volume). In addition, sterility may be induced by insect growth regulators which can be transferred from a treated male to an untreated female during mating, subsequently disrupting the development of the embryo by interfering with endocrine mechanisms (Hargrove and Langley 1990).

Dominant lethal mutations are of foremost importance for the SIT (Robinson, this volume). However, in some instances, the induction of permanent infecundity in females is required. For example, in the use of the SIT against the New World screwworm, both males and females are irradiated with a dose of gamma rays in the late pupal stage that induces dominant lethal mutations in all of the sperm of the male, and infecundity in all females. In addition, this dose must destroy the spermatogonia of the male to prevent the recovery of fertility, and permanently destroy either the oogonia or the trophocytes (nurse cells) required for egg formation. Since the irradiated females cannot lay eggs, all egg masses collected from the wounds of sentinel animals are laid by wild females, and the percentage of sterile egg masses directly reflects the percentage of wild females that have mated with sterile males.

Dominant lethal mutations, which are manifested by the inability of a treated individual to reproduce, are almost always caused by chromosome breaks (Muller 1954) induced in the germ cells. Chromosome breaks do not interfere with the ability of the gametes to participate in fertilization. The breaks persist, but the affected sperm fertilizes the egg in the normal fashion, and the dominant lethal mutations kill most embryos during the first few cleavage divisions (LaChance and Riemann 1964; Robinson, this volume).

The Lepidoptera, which have diffuse centromeres, are an exception to this generalized concept. In Lepidoptera some dominant lethal mutations may be expressed just prior to hatch, but most of the broken chromosomes pass on to the F_1 generation. It is mainly in the F_1 generation of Lepidoptera that induced chromosomal abnormalities express themselves as dominant lethal mutations (Carpenter et al., this volume).

During the process of inducing dominant lethal mutations, however, damage to somatic cells must be avoided or kept to a minimum. In the case of the New World screwworm, this is accomplished by irradiating pupae that have been incubated at 27°C for 5 days. Males are sterilized by a dose of 25 Gy. However, to completely

prevent females from laying eggs, a dose of 75 Gy is required. This insect tolerates 75 Gy without any apparent debilitation (Bushland 1960).

The dose required to induce complete sterility in the boll weevil is very debilitating. The resulting damage to the midgut prevents the weevil from digesting food, and also makes the gut vulnerable to penetration by microorganisms (Reinecke et al. 1969, Klassen and Earle 1970). The capacity of male weevils to mate falls off sharply several days after exposure to ionizing radiation or most alkylating agents.

In the Lepidoptera, the large doses required to induce sterility in males may impair the transfer of sperm from a male to the spermatheca of a female (Carpenter et al., this volume). Consequently, the females from such pairings do not lay eggs, and they call for another mate (North 1975). In addition, lepidopterans have two types of sperm, eupyrene (nucleate) and apyrene (anucleate). Eupyrene sperm occur in bundles, and possess nuclei and large mitochrondrial derivatives. These nucleate sperm are needed to fertilize the egg. Apyrene sperm appear to be necessary for the transport of the eupyrene sperm from the spermatophore down the seminal duct into the spermatheca, and to prevent the inseminated female from calling for another mate (Cook and Wedell 1999). Eupyrene sperm are susceptible to damage by irradiation, and in the F_1 male progeny, the number of eupyrene sperm per sperm bundle appears to be reduced, and many sperm exhibit ultrastructural abnormalities. However only normal-appearing sperm are found in the spermathecae of females that mated with an F_1 male (Riemann 1973).

2.2. How Sterile Males Suppress Populations: Numbers Game

Knipling (1968) recognized that the level of suppression required to stabilize the density of a population depends on its intrinsic rate of increase (Table 2).

Table 2. Rates of mortality and survival required to maintain a stable pest population

Intrinsic rate of increase between generations	Number of progeny per female	Number (fraction) that must survive to prevent population from declining.	To prevent population increase		
			Number (fraction) that must die	Percentage that must die	Percentage that may survive
2-fold	4	2 (1/2)	2 (1/2)	50	50
3-fold	6	2 (1/3)	4 (2/3)	67	33
4-fold	8	2 (1/4)	6 (3/4)	75	25
5-fold	10	2 (1/5)	8 (4/5)	80	20
10-fold	20	2 (1/10)	18 (9/10)	90	10
20-fold	40	2 (1/20)	38 (19/20)	95	5

Knipling (1968) estimated that an overwintering screwworm population typically increases approximately five-fold for the next two or three generations. For example, if in an area of 100 000 km^2, 1 000 000 screwworms overwinter, this population will increase to 5 000 000, 25 000 000 and 125 000 000 in the F_1, F_2 and F_3 generations, respectively. To calculate the consequences of releasing sterile screwworms into a population of fertile screwworms, Knipling listed the various types of matings, calculated the frequency of each type of mating, and assigned ten progeny to each mating of a normal female (NF) with a normal male (NM). When both sterile males (SM) and sterile females (SF) are released, there are four types of matings possible. Thus the wild population has 500 000 normal males [NM] and 500 000 normal females [NF], and 4 500 000 sterile males [SM] and 4 500 000 sterile females [SF] are released. The frequencies of the various matings, and the number of progeny produced, are shown in Table 3. Using this method, Knipling calculated the trend of this hypothetical screwworm population (Table 4).

Table 3. Method of calculating frequencies of various types of matings and resultant progeny when sterile males and females are released into a population of normal males and females (text provides details)

Type of mating	Number of matings	Number of progeny/ mating	Number of progeny
NF X NM	$\dfrac{500\ 000\ X\ 500\ 000}{5\ 000\ 000} = 50\ 000$	10	500 000
NF X SM	$\dfrac{500\ 000\ X\ 4\ 500\ 000}{5\ 000\ 000} = 450\ 000$	0	0
SF X NM	$\dfrac{500\ 000\ X\ 4\ 500\ 000}{5\ 000\ 000} = 450\ 000$	0	0
SF X SM	$\dfrac{4\ 500\ 000\ X\ 4\ 500\ 000}{5\ 000\ 000} = 4\ 050\ 000$	0	0

Table 4. Trend of an insect population subjected to sterile insect releases when the normal increase rate is five-fold

Generation	Uncontrolled natural population (5 X increase rate)	Controlled population		Ratio of sterile to fertile
		Natural population	Sterile population	
1	1 000 000	1 000 000	9 000 000	9:1
2	5 000 000	500 000	9 000 000	18:1
3	25 000 000	131 625	9 000 000	68:1
4	125 000 000	9535	9 000 000	942:1
5	625 000 000	50	9 000 000	180 000:1

Knipling realized that, to obtain a reduction of the wild population, the degree of sterility introduced into the wild population by releasing sterile males must be sufficiently high to overcome the rate of increase (reproductive success) of the wild females. Knipling (1968) stated:

> If we assume that a given insect has a net capacity to increase five-fold each generation, the ratio of fully competitive sterile to fertile insects will have to be 4:1 to keep the natural population stable. Theoretically, an initial ratio as low as five sterile to one fertile will be adequate to start a downward trend in the natural population when the net increase rate is only five-fold. Actually, starting with this lower overflooding ratio, theoretical elimination of the population will be achieved with fewer insects than will be required with a 9:1 ratio. However, in actual practice, the density of insects will vary in different parts of the environment. Moreover the distribution of sterile insects will never be uniform. Therefore, in control operations, the initial ratio should be sufficiently high to be certain that an overall reduction in the population will occur in all parts of the environment from the start. In some instances, the sterility procedures might reduce the vigour and competitiveness of the organism. Allowance must be made for this factor.

As shown in Table 4, the ratio of sterile to fertile insects increases asymptotically as the density of the wild population declines to low levels. Thus, to take advantage of the tremendous power of the SIT against sparse populations of pests, Knipling advocated that the release of sterile insects should be initiated when the wild population was at a seasonal low or immediately after its decimation by adverse weather events, such as freezes and hurricanes. In addition, Knipling (1966, 1979, 1992a) designed pest management systems in which insecticides, biocontrol agents, etc., were used to reduce the density of the target population to a level at which the SIT could manifest its great suppressive power (Fig. 2).
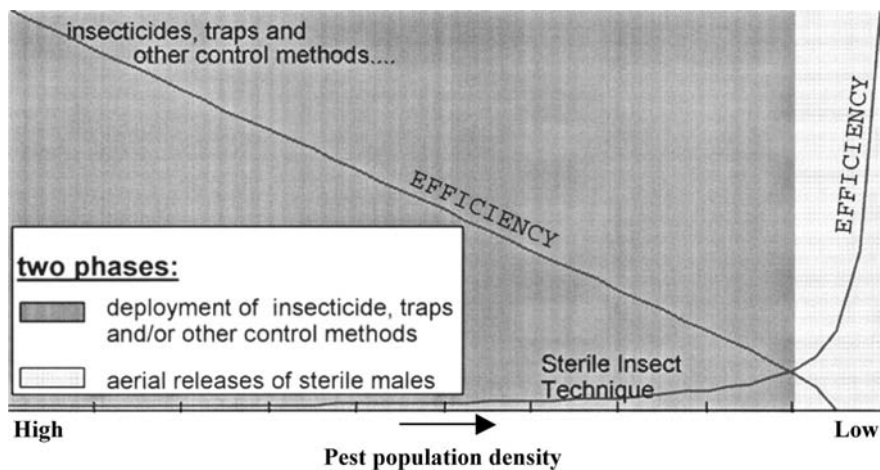

Figure 2. Schematic representation of the sequential use of methods of suppression that efficiently decimate a pest population followed by use of the SIT, which becomes progressively more suppressive as the population declines. The term "efficiency" refers to the ease in further suppressing the population, and not to the cost of eliminating an individual pest (cost per kill). (Figure adapted from Feldmann and Hendrichs 2001.)

3.   USE OF SIT IN IMPLEMENTING PEST MANAGEMENT STRATEGIES

The major pest management strategies are: (1) suppression of local populations, (2) suppression of total populations under an AW-IPM approach, (3) eradication of well-established pest populations, and (4) containment (exclusion) and prevention of invasion. The last three of these strategies are variants of AW-IPM (Hendrichs et al., this volume).

The SIT is a pest-specific tactic that can play a role in implementing all of these strategies. In this sense, a "tactic" is a method for detecting, monitoring or controlling a pest. A "system" is an assemblage of tactics that are applied simultaneously or sequentially so that the effects of individual tactics on the pest population are either additive or mutually potentiating, and counter-productive (negative) interactions are avoided or minimized. A pest management "strategy" is a broad overall plan. The merits of a pest management strategy may be judged by its short- and long-term ecological, economic, sociological and political impacts (Rabb 1972; Enkerlin 2003; Enkerlin et al. 2003; Hendrichs et al., this volume).

To implement a strategy effectively and efficiently, each control tactic has characteristics that need to be considered in the design of a system. The relationship of the density of the pest to the efficiency of the control tactic is of paramount importance. Some tactics are most useful only against dense and moderately dense populations, while others, such as the SIT and sex pheromones, are effective only against sparse populations. Also, ecological selectivity is important to avoid the destruction of natural enemies needed to prevent the resurgence of the pest population following a control operation, and to prevent environmental damage. Most conventional insecticides are relatively non-selective. Highly selective tactics include the SIT and other genetic techniques, pheromones, pest-resistant crop varieties, certain insect pathogens, parasitoids and predators, and certain artificial and naturally occurring attractants. Light traps, some attractant baits, "general" predators, parasitoids and pathogens, and certain insecticides, are only moderately selective.

3.1. Principles of Designing Pest Management Systems

The design of a pest management system requires information on the selectivity and efficiency of available tactics. When the suppression of populations to a very low level is required, methods that are effective against high populations, and methods that are effective against low populations, should be integrated so that the actions of the former potentiate those of the latter (Mangan, this volume). For example, the use of a selective insecticide potentiates the SIT by increasing the sterile:fertile ratio. Moreover, the release of both a pest-specific parasitoid and sterile insects is likely to be mutually potentiating (Carpenter et al., this volume). When the economic threshold of the pest is moderately high, several tactics that have additive effects against dense populations may be combined to give much more reliable suppression than from a single method. Knipling (1979) provided a thorough discussion of these principles.

3.2. SIT and Suppression of Local Populations

Since 1981, the SIT has been used commercially to control the onion maggot *Delia antiqua* (Meigen) on an area of 2600 hectares in The Netherlands (Loosjes 2000). The flies are reared year-round and stockpiled for release during the onion-growing season. To protect against immigrant flies, sterile flies are constantly maintained in the fields. Individual farmers contract for the SIT independently of their neighbours, many of whom use chemical control. Free-riders (growers in the release area who benefit but do not pay) weaken the programme, and the programme has not been able to expand beyond 16% of the onion-producing area. Some efficiency is lost because the fields with sterile flies do not form a contiguous block.

In about 20 counties in Florida, each year the SIT has been used to create grapefruit groves that are free of the Caribbean fruit fly *Anastrepha suspensa* (Loew) (Riherd 1993, 1994, Malavasi et al. 1994). The effectiveness of the suppression decreases greatly with the size of the areas. This programme is critically important to the industry, which depends on profits from shipments of fruit to other citrus-producing states and to Japan. A somewhat similar programme exists in south-eastern Texas to create citrus groves free of the Mexican fruit fly (Malavasi et al. 1994).

3.3. SIT and Suppression of "Total" Populations

The SIT can be applied for population suppression rather than eradication (Hendrichs 2001; Hendrichs et al., this volume). As a sequel to the Oslo Accords, an AW-IPM programme using the SIT against the Mediterranean fruit fly was established in fruit-producing areas of Israel, Jordan, and the Territories under the Jurisdiction of the Palestinian Authority. Enkerlin and Mumford (1997) concluded that, over a 9-year time frame, the net economic benefits from this SIT suppression programme would be greater than from an SIT-based eradication programme, or from an area-wide bait-spray programme. Assuming that sufficient sterile flies can be purchased from existing rearing facilities, SIT suppression is profitable beginning in the first year. In contrast, eradication would have a payback period of 4 years, because of the need for an upfront investment in a rearing facility, high quarantine costs, and the need for more intensive monitoring. A similar SIT management programme is being contemplated for southern Europe.

The SIT suppression programme against the codling moth in British Columbia, Canada, initiated in 1994, has reduced populations of the pest to non-detectable levels in most commercial orchards in the southern Okanagan and Similkameen Valleys (Bloem et al. 1981; Bloem et al., this volume).

3.4. SIT and Eradication of Well-Established Populations

Eradication of a pest population is the destruction of every individual of the pest species in an area surrounded by natural or man-made barriers sufficiently effective to prevent reinvasion except through the intervention of man (Newsom 1978). Although the eradication of a population may not endure forever, the total

elimination of a pest from a defined area, followed soon after by reinvasion, is not considered to be eradication.

By means of the SIT, through an unrelenting effort from 1957 to 2000, the New World screwworm was eradicated in its native range in the southern USA, Mexico and Central America. An area-wide programme was essential since the screwworm infested wild as well as domestic animals. Similarly, the SIT was the key to the eradication of Mediterranean fruit fly populations in areas of Argentina, Chile, USA, and Mexico, and the eradication of the melon fly *Bactrocera cucurbitae* (Coquillett) in Japan's southern islands. Tsetse populations were eradicated temporarily, with the use of the SIT, in several small areas of the African continent, and permanently in Unguja Island of Zanzibar. However, the goal of creating a number of tsetse-free zones in Africa (Feldmann et al., this volume) will require significant improvements in mass-rearing technology, an in-depth knowledge of the behaviour of released and wild flies, and a careful consideration of the design of pest suppression systems. In addition, besides technical concerns, considerable development of programme management, and political and financial support, is essential (Dyck, Reyes Flores et al., this volume).

3.5. SIT and Containment and Prevention of Pest Populations

As already noted, the SIT and quarantines are major tactics in systems used to implement the containment (exclusion) and the prevention strategies. Examples of the containment strategy include Panama, where a barrier of sterile New World screwworm flies is maintained to contain the screwworm at the Panama-Columbia border, and a barrier of sterile Mediterranean fruit flies is maintained to exclude this pest from Mexico (Villaseñor et al. 2000).

For the prevention strategy, examples include releases of sterile pink bollworms to prevent the pest from establishing on cotton in California's San Joaquin Valley, releases of the male-only strain of the Mediterranean fruit fly over southern California and major metropolitan areas in Florida, and releases of sterile Mexican fruit flies to prevent immigrant flies from crossing over from Mexico into the lower Rio Grande Valley of Texas. The SIT and quarantine stations were used, initially, to prevent the spread of the New World screwworm throughout Africa, and then to eradicate it in the coastal region of Libya (FAO 1992, Lindquist et al. 1993).

3.6. Lessons Learned from Using SIT to Achieve Area-Wide Integrated Pest Management

In most AW-IPM programmes that apply the SIT, the goal of containment or eradication has been threatened by the existence of untreated or inadequately treated refugia or microhabitats unusually favourable for the pest, i.e. "hot spots", from which recruits could come to reinfest cleared areas. In the screwworm campaigns, some hot spots were the ranches where livestock wounds were not being treated. Breaches of quarantine lines were very troublesome. The overarching lesson was that absolutely all segments of the population, i.e. the total population, must be suppressed.

More specifically, with respect to the SIT, the following conclusions were drawn:

- An extended lag period may occur between the initiation of the release of sterile insects and a noticeable effect on the density of the pest. This is inevitable since the wild population will include many mated females, sexually immature females, pupae, larvae, and eggs. The released sterile males can only prevent the reproduction of unmated females. As the immature forms mature, they become subject to the impact of the sterile flies. However, the time of one generation will pass before the progeny of previously mated females can be affected. Consequently, the release ratio must be sustained over a period of time equivalent to several generations.
- If a high proportion of the wild population is present as eggs and larvae when the release of sterile insects begins, then the wild population will increase for a time in spite of the releases of sterile insects. However, this period can be shortened if an insecticide application is made to kill females previously mated to wild males.
- Severe weather events, such as periods of cold weather, may reduce the density of the pest population, and also synchronize the development of the population by halting reproduction and killing exposed life stages. In this way the generation overlap may be eliminated.
- An influx of pests into the target area, even a few mated females, will greatly prolong an eradication programme. Great care must be taken not to underestimate the flight range of the pest. For most pest insects one can assume that an immigrant female will produce 10–20 adult progeny in a small area. However the progeny may disperse and be thinly distributed, and thus vulnerable to the SIT.

Krafsur (1998) asserted that the SIT is an underutilized and widely misunderstood technology (Whitten and Mahon, this volume). He refuted several misconceptions concerning the evolutionary responses to the SIT, the role of weather in AW-IPM programmes using the SIT, and the occurrence of undetected populations where eradication had been claimed.

3.7. Requirements to Achieve Area-Wide Integrated Pest Management Using SIT

The requirements for implementing the SIT to accomplish AW-IPM are complex and sophisticated. Of paramount importance is the recognition that AW-IPM must be preventive, offensive, and planned on a multi-year basis (Lindquist 2001; Dyck, Reyes Flores et al., this volume). Requirements include the following:

- The target pest must be a good candidate for suppression by the area-wide integration of the SIT with other methods, i.e. substantial overflooding ratios can be achieved, and sensitive methods of detection and sampling of sparse populations are available, and rearing, handling, irradiation, and release technologies have been developed.
- The ecology of the target pest must be thoroughly understood.
- There must be strong stakeholder cohesiveness, good leadership, and commitment to the campaign.

- An effective and knowledgeable programme leader, supported by an effective organization, is needed. This team must formulate and continuously update both technical and business plans.
- There must be a system of programme review, including external and independent experts.
- Research and methods-development to backstop the programme are needed.
- Legal authority is required to execute all aspects of the programme, e.g. conduct operations on private properties, and operate quarantines.

Up to the present time, the relative simplicity of the SIT has given it a great advantage over other genetic techniques such as the use of strains with multiple chromosomal translocations, compound chromosomes, cytoplasmic incompatibility, etc. (Whitten and Foster 1975). Since losses caused by pests rapidly mount with the passage of time, the time required to organize a programme is critically important, especially when it is financed in part by an industry under economic stress. Generally an AW-IPM programme that releases sterile insects can be mounted much more quickly than a programme for which a considerable amount of genetic research is still needed. For example, more than a decade of sophisticated genetics research was required to develop the exquisite genetically impaired female technique (GIFT) for use against the Australian sheep blow fly *Lucilia cuprina* (Wiedemann) in Australia (Foster et al. 1993). However this programme was cancelled in 1992 before the mass-rearing technology required for this promising strain had been developed (Krafsur 1998).

Nevertheless, it is very important to continue to develop additional genetic techniques to meet the problems of pest insects. For example, Curtis and Graves (1998) and Curtis and Andreasen (2000) proposed genetic approaches to replace malaria vectors with harmless strains, and Crampton et al. (2000) outlined some of the steps still needed to accomplish this goal.

4. IMPROVEMENTS IN SIT TECHNOLOGIES

- Separation of males from females. The removal of females prior to large-scale sterile insect releases is of great importance, both in terms of economics of production and biological efficiency in the field (Franz, this volume; Parker, this volume). In the Mediterranean fruit fly, where genetic sexing strains are in widespread use, there is a several-fold increase in field efficiency (Rendón et al. 2000, 2004; Caceres et al. 2004).

  Mechanical methods for separating males from females (Parker, this volume) exist in many species, e.g. *Culex pipiens fatigans* Wiedemann, *Aedes aegypti* (L.), *Anopheles albimanus* Wiedemann, and *L. cuprina* (LaChance 1979), and genetic methods have been devised in a variety of species, e.g. *Bombyx mori* (L.), *Tetranychus urticae* Koch, *Musca domestica* L., *An. albimanus, Culex tritaeniorhynchus* Giles, *Anopheles gambiae* Giles species A, *Anopheles arabiensis* Patton, *L. cuprina* (LaChance 1979), and *C. capitata* (Franz 2000; Franz, this volume). A full list of species where sexing methods have been developed is found in Robinson (2002). In tsetse flies *Glossina morsitans* ssp. (Zdarek and Delinger 1995) and *Glossina austeni* Newstead (Opiyo et al. 2000),

adult emergence is temperature dependent, with females emerging before males from pupae deposited at the same time. This phenomenon is now the basis for self-stocking of rearing cages with the desired ratio of males to females, and collecting males for irradiation and release (Msangi et al. 2000).

- Improvements in rearing. The replacement of crude liquefied larval diets with modified starch gels was of great importance to rearing the New World screwworm (Brewer 1992, Chaudhury et al. 1998); more efficient use of diet ingredients is made, major savings in labour costs are realized, and an offensive odour is no longer generated. A "filter rearing system" has been devised to maintain the genetic integrity of genetic sexing strains of the Mediterranean fruit fly (Fisher and Caceres 2000; Parker, this volume). During the past decade the cost per pupa of rearing tsetse flies has been reduced more than ten-fold (Opiyo et al. 2000).

- Improvements in sterile insect release and distribution technologies. Packing sterile flies in bags and boxes for release has, in some instances, been replaced by the use of machines in which the sterile insects are kept in bulk in a chilled hopper, and then metered into the atmosphere to achieve the desired number per unit area (Dowell et al., this volume).

- Global Positioning System (GPS). The introduction of the GPS, for precision navigation and georeferencing the location of insect traps, hosts and other information, has greatly reduced costs (Cox and Vreysen, this volume). The GPS technology provides a printout of the track of an aircraft as it distributes sterile insects. Inexpensive hand-held GPS units allow personnel to locate and service traps efficiently.

- Geographic information systems (GIS) and database management. Specialized software is now commonly used to manage data and chart developments in programmes (Cox and Vreysen, this volume).

- Molecular techniques for determining places of origin of immigrant insects. Using restriction endonucleases and the polymerase chain reaction, it is possible to characterize the mitochondrial DNA of individual insects. This approach was utilized to establish that the New World screwworm population in Libya had not originated in North and Central America (Narang and Degrugillier 1995). Such "genetic fingerprinting" has been applied to tropical fruit flies, tsetse flies, etc. in view of their importance for quarantine purposes (Krafsur, this volume).

- Sterilizing natural insect pest population by transferring chemosterilant during mating. Langley and Coates (1982) developed pheromone-baited traps fitted with a contaminating device that dispensed a mutagenic chemosterilant, bisazir, to tsetse flies that passed through it. Subsequently, Hargrove and Langley (1990) used pyriproxyfen, an insect growth regulator, as the sterilant. Field trials in Spain (Navarro-Llopis et al. 2004) showed that two formulations of a bait-chemosterilant (lufenuron) combination reduced Mediterranean fruit fly populations in citrus orchards. Autosterilization is entirely compatible with the release of sterile insects, although it has not been tested in conjunction with large-scale AW-IPM programmes.

5. RESEARCH NEEDS

- In operational programmes, correlate the frequency of matings of sterile males with wild females and the dynamics of the pest population. There is a paucity of published data that relate sterile male releases to population suppression (Vreysen, this volume).
- Determine if the competitiveness of sterile males is correlated with density. Rogers and Randolph (1985) suggested that the competitiveness of sterile males is strongly impaired by high density, and Hargrove (2003) made a similar claim without providing any mechanism.
- Study the detection and sampling of very sparse populations. Disagreement, on the interpretation of sample data, enveloped area-wide programmes against the boll weevil in an especially bitter and costly controversy (NRC 1975). Similar difficulties have been encountered in programmes against tsetse flies *Glossina* spp., screwworms, the gypsy moth, etc.
- Automate the collection of field data, resulting in substantial cost savings in area-wide programmes, for example, insect traps (baited with a species-specific attractant) that send a radio signal when a catch is made.
- Determine the full host range of many pest species.
- Identify sociological barriers, and opportunities to surmount them, in implementing AW-IPM programmes.

6. KNIPLING'S IMPERATIVE

When the World Food Prize was awarded to E. F. Knipling and R. C. Bushland, Knipling (1992b) stated:

> If major advances are to be made in coping with most of the major arthropod pest problems, then the tactics and strategies for managing such insects, ticks and mites must change. They must change from the current, limited scale, reactive, broad-spectrum measures to preventive measures that are target-pest specific and rigidly applied on an area-wide basis.

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CHAPTER 2.2.

BIOLOGICAL BASIS OF THE STERILE INSECT TECHNIQUE

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SUMMARY

In principle, the sterile insect technique (SIT) is applicable to controlling a wide variety of insect pests, but biological factors, interacting with socio-economic and political forces, restrict its practical use to a narrower set of pest species and situations. This chapter reviews how the biology and ecology of a given pest affect the feasibility and logistics of developing and using the SIT against that pest insect. The subjects of pest abundance, distribution, and population dynamics are discussed in relation to producing and delivering sufficient sterile insects to control target populations. Pest movement and distribution are considered as factors that influence the feasibility and design of SIT projects, including the need for population- or area-wide management approaches. Biological characteristics, that affect the ability of sterile insects to interact with wild populations, are presented, including the nature of mating systems of pests, behavioural and physiological consequences of mass production and sterilization, and mechanisms that males use to block a female's acquisition and/or use of sperm from other males. An adequate knowledge of the biology of the pest species and potential target populations is needed, both for making sound decisions on whether integration of the SIT into an area-wide integrated pest management (AW-IPM) programme is appropriate, and for the efficient and effective application of the technique.

1. INTRODUCTION

In principle, the sterile insect technique (SIT) is simple — the release of a large number of reproductively sterile male insects into a wild population of the same species so that they mate with, and block the reproduction of, wild females (Knipling 1955). Released insects are most often completely sterile (or nearly so), but inherited (F_1) sterility (IS) is an option with species (primarily Lepidoptera) in which appropriate substerilizing doses of radiation produce males that are partially sterile but sire completely sterile offspring (Carpenter et al., this volume).

The successful application of the SIT requires: (1) the ability to rear, sterilize, and distribute sufficient insects to achieve a sufficiently high "overflooding" (sterile:wild insect) ratio in the field, and (2) that the sterile males can successfully compete and mate with their wild counterparts. Although the concept of the SIT is simple, the implementation is complex (Seawright 1988). Insects are mass-produced in an artificial environment and, after being exposed to ionizing radiation (Bakri et al., this volume), are often densely packed and shipped to a distant facility. Their subsequent release into the field can involve procedures such as immobilization, chilling, and ejection from flying aircraft (Dowell et al., this volume). Through all of this the insects must remain "competitive", i.e. able to survive and perform behaviours that allow them to mate successfully with wild insects (Calkins and Parker, this volume).

Several key biological questions must be considered when deciding whether the use of the SIT would be warranted in a given pest situation (Table 1). Although economic and political considerations may drive decisions on when and where the technique is developed and deployed, these biological issues ultimately determine both the logistical feasibility and economics of suppressing a given pest population with the SIT. Understanding a pest's biology also allows programmes to optimize procedures and avoid pitfalls that could make the SIT impractical or ineffective.

Table 1. Key biological considerations affecting the decision to use the SIT

Question	Biological consideration
Is the pest an appropriate target for the SIT?	Role of pest in agroecosystems Existence of negating or complicating trait(s) Potential of integration into (typically area-wide) pest management system
Can adequate sterile:wild ratios be achieved?	Pest ecology and population dynamics Biological factors affecting production, distribution, and release Integration with other suppression methods
Will released sterile males be able to compete effectively against wild males in target populations?	Effects of mass rearing and sterilization on insect behaviour and physiology Evolution and the SIT Mating systems Post-copulatory factors Potential for enhancing sterile insect competitiveness

2. ECOSYSTEM, AGRONOMIC, LIFE HISTORY, AND BIONOMIC CONSIDERATIONS

2.1. Role of Pest in Agroecosystems

2.1.1. Pest Status

The SIT is mostly used when the selective removal of, or a great reduction in, a population of an individual species would have significant benefit. Examples (not inclusive) of applicable pest situations are shown in Box 1. Alternatively, the SIT would generally not be warranted if the suppression or elimination of a single pest population would not substantially reduce overall management costs or efforts. For example, the use of the SIT against the summer fruit tortrix *Adoxophyes orana* (Fischer von Röslerstamm) in The Netherlands would do little to reduce the number of required insecticide sprays in apple orchards since other tortricid pests are present (Ankersmit et al. 1977).

2.1.2. Pest Stage Producing Damage

In his original treatise on the SIT, Knipling (1955) suggested that:

> It probably would be impractical to release insects which are highly destructive in the adult stage.

Box 1. Examples of Pest Situations Where SIT Could be Considered for Control

- Incipient population of an exotic pest that, if established, would severely impact agricultural or environmental ecosystems, e.g. eradication of the Mexican fruit fly *Anastrepha ludens* (Loew) in California, USA
- Vector of a serious disease (plant or animal), e.g. tsetse fly *Glossina* spp. eradication programmes (Feldmann et al., this volume)
- Presence of a "key pest" that greatly increases management costs and/or is quarantined in potential export markets, e.g. New World screwworm *Cochliomyia hominivorax* (Coquerel) in North America; *Bactrocera* spp. in Okinawa (Kuba et al. 1996)
- Alternate methods of controlling a pest disrupt ecological processes that regulate populations of other pests, e.g. chemical control of the boll weevil *Anthonomus grandis grandis* Boheman disrupts the biological control of noctuids such as *Helicoverpa* sp.
- Preventing establishment of an important pest by maintaining a continuous population of sterile insects in an area of high risk of introduction, e.g. releases of sterile Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) in Los Angeles, California, USA (Dowell et al. 2000)

The theoretical basis of the SIT is largely unaffected by which stage(s) produces damage, but sterile insects themselves can be nuisances, disease vectors, or agricultural pests (Nagel and Peveling, this volume). For example, in the case of blood-feeding horn flies *Haematobia irritans* (L.) (Patterson and Miller 1982, Perotti and Lysyk 2003), large releases of sterile insects may preclude the use of the SIT. If the damage from sterile insects is primarily done by females, e.g. mosquitoes, the development of genetic sexing strains can allow the SIT to be used with few or no negative consequences (Franz, this volume).

2.1.3. Plant Part Attacked

In the case of plant pests, the SIT has most often been deployed against insects that attack marketable, especially fruiting, tissues. Small numbers of pests can cause substantial economic damage by attacking these high-value substrates, and, as a technology, the SIT is best used to drive small populations to very low levels or even to local extinction. In contrast, many agricultural crops can sustain modest levels of damage to vegetative tissues such as leaves or roots with little or no economic loss, and populations of pests attacking such tissues can, in some cases, be very large (Sutter et al. 1998), and hence the SIT is less appropriate for such pest situations.

2.2. Reproductive/Life History Strategy

Since the SIT relies on sterile males mating with wild females, most sexually reproducing insects are at least potential targets of the technique. Beyond that, various aspects of a species' basic biology tend to make that species relatively amenable to (Box 2), or a poor candidate for (Box 3), the SIT. Natural parthenogenesis, even at low levels, is a potential pitfall of the SIT, which could theoretically select for parthenogenesis in wild populations (Templeton 1978). Nevertheless, use of the SIT has been considered against facultatively parthenogenetic aphids (Steffan and Kloft 1973). Controlling eusocial insects, such

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Box 2. Examples of Biological Characteristics that Allow, or Increase the Feasibility of, Using SIT

- Sexual reproduction (exclusively)
- Methods of mass-rearing are available, or can be developed
- Species is holometabolous (quiescent pupal stage facilitates sterilization and handling)
- Males exposed to sterilizing doses of ionizing radiation can compete with wild males for mates
- Methods are available to monitor released sterile and wild populations
- Low intrinsic rate of increase

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as termites, with the SIT, while not theoretically impossible, would also present immense challenges in mass-rearing, sterilization, and release. Haplodiploidy, common in Hymenoptera, could make classical deployment of the SIT (rear, sterilize, release) problematic, but could open possibilities for simplified male-only release strategies. Broad taxonomically based generalizations about the applicability of the SIT are risky except, perhaps, that the technique may often be simpler with holometabolous than hemimetabolous insects (Box 2). The presence of the quiescent pupal stage tends to facilitate the harvest, sterilization, and transport of mass-reared insects. Also, larvae of many holometabolous insects have limited mobility and, in some cases, feed gregariously within a restricted unit of habitat such as a fruit. These habits tend to facilitate mass-rearing, whereas in other cases containerization is required to avoid cannibalism. However, the historical bias of area-wide integrated pest management (AW-IPM) programmes integrating the SIT toward Diptera, Lepidoptera, and to a lesser extent Coleoptera, is arguably, as much as anything, a reflection of the large numbers of pest species in these orders. In particular, they contain high percentages of pests of regulatory and, in the case of Diptera, public and veterinary health concern. Such pests can be prime targets for government-sponsored development of the SIT.

2.3. Potential of Integrating SIT with Other Control Strategies

In most applications of the SIT, it is a key component of AW-IPM programmes (Klassen, this volume). As such, the SIT is commonly integrated with other control methods, most often: (1) following pre-release suppression of the target population

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Box 3. Examples of Biological Characteristics that Could Negate or Severely Complicate Using SIT

- Parthenogenesis
- Highly synchronous, aggregated, ephemeral mating system (found in many eusocial insects and other groups such as some Ephemeroptera)
- Extended life cycle, e.g. typical of many cicadas
- Sterile insects themselves are a serious pest, disease vector, or nuisance pest, such as horn flies, locusts, house flies or cockroaches
- Migratory behaviour involving long-distance flight and/or movement along weather fronts, as in various moths, locusts, planthoppers and stable flies

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(often involving insecticides) to the point where the SIT becomes more effective, less costly, or even feasible at all (Knipling 1979), (2) during simultaneous suppression using SIT-compatible controls (for example, the use of larvicides against mosquitoes or screwworms), or (3) exploiting the specificity of the SIT to provide control without disrupting biological control of the target population and/or other species in the area (Knipling 1998). Thus the potential for area-wide integration of the SIT depends on a species' basic biology, the specific population situation, and the availability of effective and/or compatible suppression tools. Mangan (this volume) discusses supplementary control tactics used in AW-IPM programmes that release sterile insects.

3. PEST ECOLOGY AND SIT

The successful application of the SIT requires knowledge of the target population's ecology, including estimates of the absolute density of the adult population, and how that density changes over time (Lindquist 1969; Lindquist et al. 1974; Knipling 1979; Itô and Yamamura, this volume). For example, the number of insects needed for a given programme depends on the size of the target population, the area covered by the programme, the goal of the programme (Hendrichs et al., this volume), and the required ratio of sterile:wild insects in the field. When Knipling (1955) initially developed the theoretical basis of the SIT, he used a simple mathematical model to demonstrate that a wild population of 1 000 000 insects could be driven to extinction in 4 or 5 generations by maintaining a level of 2 000 000 sterile insects within the area (an initial 2:1 overflooding ratio). This model assumed that the wild population was stable, i.e. the average female produced one female offspring that survived to reproduce, and that sterile males were equivalent to wild males in their ability to mate with wild females. In practice, these assumptions are rarely true.

Subsequent models, which contain parameters that incorporate behavioural and ecological information, provide more realistic estimates of overflooding ratios needed for desired levels of suppression (Knipling 1968; Barclay, this volume; Klassen, this volume). In practice, when high rates of increase are involved, the required overflooding ratios can be quite high. Using empirical data, Brower and Tilton (1975) calculated an optimal sterile:wild ratio of about 100:1 for the almond moth *Cadra cautella* (Walker). In some programmes, ratios greater than 100:1 have not controlled rapidly increasing populations (Vargas et al. 1994; Rendón et al. 2000, 2004).

3.1. Abundance of Pest

3.1.1. Numerical Size of Population
The need to produce enough sterile insects to overflood a wild population places practical limits on the size of the target population that can be suppressed or eradicated, and has led to the assertion that the SIT is best applied against relatively small numbers of insects (Knipling 1955, 1979). This can include pests that are widely distributed but tend to occur at low densities, such as the New World

screwworm (Knipling 1968), and others that occur in higher densities but exist (at least in the programme area) in relatively small and somewhat isolated patches of habitat. An example of the latter would be populations of Mediterranean fruit flies as they exist in some Middle Eastern areas (Rossler et al. 2000). The SIT has also been used to eradicate highly isolated populations such as the melon fly *Bactrocera cucurbitae* (Coquillett) in Okinawa (Kuba et al. 1996), and incipient populations of the Mediterranean fruit fly and the Mexican fruit fly in the United States (Penrose 1996). For the latter types of programmes, pest surveys must be sensitive enough to detect and delimit a population before it grows beyond the capacity of the system and resources available for eradication (Lance and Gates 1994).

3.1.2. Pest Population Dynamics

Since the SIT interacts with a pest population at the point of reproduction, overflooding ratios must account for any tendency of the population to increase. As a simple demonstration, Knipling (1968) extended his 1955 model to show that, if a 2:1 overflooding ratio could reduce or eliminate a "stable" pest population, a ratio of 9:1 or 10:1, i.e. 2:1 X 5, would be needed for a population that was increasing 5X per generation. Such rates of increase are not uncommon among insects. For example, Bartlett and Butler (1979) documented a 10-fold increase per generation in pink bollworm *Pectinophora gossypiella* (Saunders) populations, and Cirio et al. (1972) reported generation-to-generation increases of greater than 40-fold in the Mediterranean fruit fly. Since rates of increase in the field are difficult to predict, operational programmes should be monitored carefully for effectiveness and to ensure that proper overflooding ratios are being maintained (Knipling 1979). Relationships of pest population dynamics to the SIT are discussed by Barclay (this volume) and Itô and Yamamura (this volume).

3.1.3. Seasonality and Voltinism

It is clear that pest populations do not continuously increase at high rates. In warmer regions, many insects breed year-round, or at least go through multiple generations annually, but the populations cycle in response to factors such as the abundance of food, e.g. host plants, weather (temperature, wet/dry cycles), and cropping cycles (Adkisson 1971, Wong et al. 1983). Conceptually, seasonal periods of low and declining pest numbers provide opportunities to effectively apply the SIT against pest populations that are too large during other seasons (Knipling 1968; Lindquist 1969; Adkisson 1971; Hendrichs et al., this volume); empirical data support this concept (Iwahashi 1976, Baumhover 2002). However, following reductions in target population numbers, maintaining adequate "pressure" from sterile insects on the pest population can be difficult when resources subsequently become abundant and pest populations rapidly increase in size. For example, Carpenter and Gross (1993) were not able to stop season-to-season increases in populations of the corn earworm *Helicoverpa zea* (Boddie) with releases of substerile males, although they consistently delayed or reduced the extent of those increases. If releasing sufficient sterile insects becomes impractical during some portions of the year, then alternating

sequences of the SIT and other methods of pest management (e.g. mass-trapping or cultural control) may prove more cost-effective than continual releases of sterile insects (Cirio 1974).

Many insect species, especially in temperate areas, have a dormancy period. Dormancy may involve diapause, induced by environmental factors such as photoperiod or temperature, and be either facultative or obligate (Leopold 2000). Temperate species are frequently univoltine (one generation per year); in some, a single generation requires two or more years. Mating, then, is restricted to specific periods within the year, and the production and release of sterile insects must be properly timed to ensure the maximum benefit (Mastro and Schwalbe 1988). If partial sterility carries across generations, conditions that initiate and break diapause in sterile insects must be appropriate, or sterile insects may not be present when needed. As examples, relatively normal diapause characteristics were observed in *H. zea* with inherited (F_1) sterility and in sterile backcross hybrids of *Heliothis subflexa* (Guenée) x *Heliothis virescens* (F.) (tobacco budworm), allowing appropriately timed activity and/or survival over winter (Stadelbacher and Martin 1981, Carpenter and Gross 1989).

3.2. Dispersion and Dispersal of Wild and Sterile Populations

Programmes that release sterile insects can be strongly affected by both the dispersion (distribution of organisms over an area) and the dispersal (movement, or displacement, of individuals) of wild and sterile populations. These two parameters are influenced by a variety of ecological and behavioural factors intrinsic to a species' basic biology, and that tend to make that pest species more or less amenable to the implementation of the SIT.

3.2.1. Dispersion

Most insect populations have clumped distributions with areas of relatively high density amid regions of substantially lower density. This clumping is often related to the distribution of resources, such as host plants, and may vary seasonally (Shiga 1986). For example, Nakamori and Shiga (1993) outlined zones of *B. cucurbitae* density in Okinawa based on the seasonal availability of host fruits, and identified "hot spots" where host fruits were abundant year-round. In such local areas with high densities of wild insects, the overflooding ratio is substantially lower than the overall ratio of sterile to wild insects throughout the programme area. As a result, other things being equal, the effectiveness of sterile insect releases would decrease as the degree of clumping in the target population increases (Sawyer et al. 1987, Barclay 1992). This effect will be overcome to the degree that the sterile insects are distributed, or redistribute themselves, to mirror the distribution of the wild insects. Knipling (1979) cited cases in which sterile insects concentrated themselves in areas of high wild-insect density, but poor correlations between the distributions of wild and sterile insects have been observed in other cases, such as trapping studies on *B. cucurbitae* (Shiga 1986) and *H. virescens* (Hendricks et al. 1973). The distribution of wild and sterile populations must be understood to be able to allocate and

distribute sterile insects optimally (Lindquist et al. 1974; Itô and Yamamura, this volume). Recent advances in geographic information (GIS) and database systems can facilitate the identification, monitoring, and differential treatment of population foci on relatively broad spatial scales (Cox and Vreysen, this volume). However, if the aggregations of target populations occur on finer spatial scales, or are not predictable, the overall release rates may have to be adjusted upwards to ensure that the local sterile:wild ratios are high enough to achieve the desired level of sterility.

3.2.2. Host Specificity

The distribution of an insect population will, of course, be influenced by the distribution of its hosts and, as a result, by the pest's degree of monophagy or polyphagy. Plant pests targeted in AW-IPM programmes that use the SIT have ranged from relatively monophagous or oligophagous pests such as the pink bollworm to highly polyphagous pests such as *H. virescens* and *C. capitata* (Proshold et al. 1983, Liquido et al. 1991, Staten et al. 1999). Monophagy should tend to simplify the application of the SIT, especially if host plants are restricted to discrete patches. For polyphagous pests, the widespread presence of alternate hosts can increase the area, logistical complexity, and costs required for effective control (Vargas et al. 1995). Movement of insects to and from sites of adult food, or other ecological requisites, can also influence pest distribution (Hendrichs and Hendrichs 1990). For example, adult male New World screwworms, when waiting for potential mates, will often perch near sources of adult food (nectar) rather than near the animals that are the larval hosts (Guillot et al. 1978).

3.2.3. Dispersal

The ability of wild insects to move within and between habitat patches influences the required size of release areas, the need for and size of barrier or buffer areas, and the pattern of insect release (Knipling 1979). The immigration of small numbers of mated females or large numbers of males into an SIT release area can potentially disrupt a programme (Prout 1978; Barclay, this volume). The magnitude of the impact from immigration depends on pest biology and on programme goals, with less isolation being required where moderate suppression rather than eradication is desired. For example, Ankersmit et al. (1977) reported that the SIT appeared to be capable of suppressing populations of the summer fruit tortrix in small orchards with a modest degree of isolation, even though the efficacy obtained was not sufficient for eradication. The dispersal capacity of a pest species determines the need to isolate treatment areas from immigration, and is the primary factor dictating a population-wide approach to AW-IPM programmes integrating the SIT (Hendrichs et al., this volume; Klassen, this volume).

In eradication programmes, the potential for reinvasion also needs to be considered. The melon fly was eradicated from subtropical Japan (Kuba et al. 1996), but continuous surveys are now needed in the region, with preventive sterile fly releases in the southernmost islands, because this species is capable of flying in from Taiwan (Koyama and Tanaka 1984, Kohama and Kuba 1996). The New World

screwworm was eliminated from North America, but because the flies are capable of dispersing more than 280 km, the eradication campaign had to be extended to cover most of Central America (Lindquist 1969, Jones et al. 1999, McGraw 2001), and a wide band of sterile-fly releases across eastern Panama is now required to prevent reinvasion from South America. Conversely, when the SIT is used in too small an area against an incipient, isolated infestation, undetected dispersal away from the site of the initial introduction can foil eradication efforts by producing satellite infestations beyond the SIT release zone (Penrose 1996).

Pilot-scale testing of the SIT also requires relatively isolated venues such as islands or oases (Proshold et al. 1983, Cayol and Zarai 1999, Baumhover 2002) or, alternately, plots that are buffered with wide barrier or treatment zones (Rendón et al. 2000). Such tests tend to be relatively large-scale, and can produce the added benefit of detecting logistical or biological problems that would not arise in smaller-scale studies (Seawright 1988, McInnis et al. 1996). However, large-scale tests are expensive, and logistical issues often force limits on size and/or replication. As a result, field data on relationships between sterile:wild ratios, sterile insect competitiveness and level of sterility, and effects on wild populations, are minimal in many cases (Krafsur 1998; Vreysen, this volume). In some instances, the initial stages of an operational programme have to function as a feasibility study (Lindquist et al. 1974).

Although long-distance movement can create problems for programmes that apply the SIT, dispersal on a more local scale is essential to the technique's effectiveness. Modelling studies indicate that a moderately high dispersal capability may tend to facilitate the SIT by reducing spatial heterogeneity in the pest population (Wehrhahn 1973; Barclay, this volume). Hence arthropods that are largely sedentary, such as ticks and mites, as well as various homopterans, are much less amenable to the SIT. Moreover, released sterile males must move sufficiently to locate resources such as food, mating arenas, and/or mates (Calkins and Parker, this volume; Vreysen, this volume). The dispersal capability of sterile males is a primary consideration when designing release methods and protocols for a specific pest species, since it is critical that sterile males are distributed throughout the release area, at least in habitats where wild insects may occur (Dowell et al., this volume).

3.3. Chemical Ecology

Chemical communication is often involved in mating, feeding, or other key ecological interactions of insects (Matthews and Matthews 1978). Accordingly, an insect's chemical ecology can have important implications for the SIT (Table 2). In particular, the common involvement of semiochemicals in intraspecific finding and/or recognizing mates means that sterile males must respond to, and in some cases produce, semiochemicals appropriately for the SIT to be effective (Table 2). In addition, an insect's chemical ecology can often be exploited to benefit AW-IPM programmes that integrate the SIT. Long-range attractants are very useful for assessing the distribution of wild and sterile insects (section 3.2.1.), monitoring overflooding ratios (Vreysen, this volume), and evaluating specific aspects of sterile male quality (Calkins and Parker, this volume). In addition, males of species that use

Table 2. Examples of types of semiochemicals utilized by insects, and their potential implications for programmes releasing sterile insects

Type of semiochemical	Implication for programmes	Reference
Sex attractant pheromone (female-produced)	Used for monitoring or evaluating programmes that apply the SIT Used for assessing sterile male quality	Staten et al. 1999 Bloem et al. 1998
Sex attractant pheromone (male-produced)	May be critical component of sterile male competitiveness (section 5)	Heath et al. 1994
Parapheromone (such as the "male attractants" of tephritids) or aggregation pheromone	Used for monitoring or evaluating programmes that apply the SIT Used for assessing sterile male quality (section 5)	McInnis and Cunningham 1986 FAO/IAEA/USDA 2003
Aphrodisiac and/or contact recognition pheromone	May play important role in mating process and affect mating competitiveness (section 5)	Hammack 1992
Host-plant or other food-related kairomones	Used for monitoring programmes that apply the SIT May play critical role in mating system May need to be provided in diet as precursor of pheromone component	Katsoyannos et al. 1999 Wood et al. 1982 Nishida et al. 1997

a long-range female-produced sex attractant are normally mobile enough to disperse well throughout the release area (sections 3.2.1. and 3.2.3.).

In some species, feeding, or otherwise exposing sterile males to an appropriate compound, can optimize mating competitiveness. Sometimes these chemicals are components (or precursors of components) of male-produced pheromones, which may be long-range sex attractants or close-range "aphrodisiacs" (Boppre 1990, Nishida et al. 1997). In other cases, reasons for the enhancement are not clear; for example, mating competitiveness is improved when sterile Mediterranean fruit fly males are exposed to the parapheromone α-copaene (Shelly and McInnis 2001).

3.4. Sterile Insect Longevity

The frequency of sterile insect releases will depend on each species, and vary according to the average longevity. The ability of sterile insects, to survive as long as wild insects in the field, is critical to the success of the SIT. If the longevity of sterile insects declines, the frequency of releases, and the number of insects released, must be increased to maintain the desired overflooding ratio (Dowell et al., this volume; Vreysen, this volume). A reduction in longevity can be a side effect of mass-rearing, strain genetics, sterilization, or handling and release methods (Fay and Meats 1987, Meats 1998). Assessment of the longevity of sterile insects has often

been conducted in the laboratory (Meats 1998, Thomas and Loera Gallardo 1998, FAO/IAEA/USDA 2003), in spite of the fact that the ability to survive in the field is critical, and is influenced by factors beyond the scope of laboratory tests. For example, released insects can suffer proportionately higher predation than wild insects if release methods concentrate or temporarily immobilize insects, or if mass production alters normal predator-avoidance behaviour (Schroeder et al. 1973, Iwahashi 1976, Hendrichs and Hendrichs 1998). The survival of sterile insects to reproductive age is especially critical, and, in many programmes, adults are not released into the field until they are sexually mature, or have at least acquired nutritional reserves (Dowell et al., this volume). Although obviously critical, the ability of sterile insects to survive in the field has generally not received as much attention as some other aspects of competitiveness (Calkins and Parker, this volume).

4. BIOLOGY AND STERILE INSECT PRODUCTION

4.1. Feasibility of Rearing and Cost of Production

Technical issues surrounding the production of sterile insects are reviewed elsewhere (Parker, this volume), but some biological factors that influence the feasibility of mass-rearing bear mention here. As noted above, sterile insect production should generally be easier with holometabolous than with hemimetabolous species. Also, dormancy periods must be taken into account when designing rearing systems (Parker, this volume), and, in some cases, they can be exploited to "stockpile" sterile insects (Mastro and Schwalbe 1988, Bloem et al. 2000). In some insects, developing specific components of rearing systems can prove intractable, e.g. the continued lack of useful artificial diets for rearing larvae of some bark beetles (Mattanovich et al. 1999), root-feeding beetles (Branson et al. 1988, Klein and Allsopp 1994), or parasites of mammals such as *Dermatobia hominis* (Linnaeus, Jr.) (Banegas et al. 1967, Arce 1968, Borja 2002). Obligatory diapause in a one-generation-per-year species, e.g. the western cherry fruit fly *Rhagoletis indifferens* Curran (Vankirk and AliNiazee 1982), may make continuous rearing difficult unless a solution to the obligatory nature of the diapause is found.

The cost of rearing insects, in particular, affects the economic feasibility of the SIT (Mumford, this volume), and is influenced by basic biological characteristics. Small insects with rapid life cycles can often be reared relatively cheaply. For example, Mediterranean fruit flies develop from the egg to prepupal stage in 6–9 days within large trays of relatively inexpensive diet, and several thousand sterile flies can be produced for USD 1 (Hendrichs et al. 2002). At that rate, hundreds of thousands of Mediterranean fruit flies can be produced for the current cost of rearing a single individual of the Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky), a large 3-cm-long cerambycid with a 10-month larval period (Dubois et al. 2002). The developmental and operational costs of mass-rearing are also affected by factors such as cannibalism, requiring individual containerization (Sparks and Harrell 1976), and the need for specialized environments such as artificial streams for simuliid larvae (Edman and Simmons 1985). In many cases,

innovation and automation help to reduce costs and other problems of mass production (Parker, this volume). Finally, while lower reproductive rates generally reduce overall SIT costs by requiring lower overflooding ratios in the field, they tend to increase the unit rearing cost since facilities needed to maintain such colonies are large relative to production output (Hendrichs et al., this volume).

4.2. Mass-Rearing and Competitiveness

A sterile male's ability to compete for mates against wild males is a function of its phenotype, which, as in all living organisms, is determined by the expression of its genotype within its environment. Insects in mass-rearing facilities typically experience biotic and physical environments that clearly are very different from those in which wild insects develop. These environmental differences can influence the phenotype of sterile insects both directly and, along with other factors, by inadvertently selecting for genetic differences between laboratory and wild populations. Careful monitoring of phenotype (manifest as sterile insect quality) is critical to the success of programmes that use the SIT (Calkins and Parker, this volume; Vreysen, this volume).

Many aspects of mass-rearing environments directly affect sterile insect quality. Characteristics of artificial larval or adult diets, such as nutritive elements, contaminants, moisture, texture, and pH, can influence body size, survival, longevity, flight ability, mating ability, and responsiveness to light (Economopoulos et al. 1990, Villavaso et al. 1998, Chang et al. 2000, Shelly and Kennelly 2002). Keena et al. (1998) found that, in the gypsy moth *Lymantria dispar* (L.), a deficiency of available iron in the diet of a female larva reduced survival and skewed the developmental rate of her offspring. Handling methods and environmental conditions that optimize, or are simply convenient for, mass-rearing do not always produce the most competitive insect. A classic example is the "droopy-wing syndrome" and poor flight ability that were found to occur in tephritid flies when pupae were sifted from the pupation medium during the time of flight muscle development (Ozaki and Kobayashi 1981). Lance et al. (1988) reported another example where holding *L. dispar* pupae at typically warm laboratory temperatures "programmed" sterile males with inappropriately timed mating activity. Conversely, temperature preconditioning (cold-conditioning) enhanced the survival and mating success of sterile male Queensland fruit flies *Bactrocera tryoni* (Froggatt) (Fay and Meats 1987). More details on rearing methodology and insect quality are presented by Parker (this volume) and Calkins and Parker (this volume).

Reproductive sterility is typically induced by exposure to X-rays, electron beams, or most commonly gamma rays from a Co-60 or Cs-137 source (LaChance 1975; Bakri et al., this volume; Robinson, this volume), which all cause chromosomal damage. Sterility is usually permanent, although irradiated males of some species may, over time, regain at least partial fertility, especially following multiple mating (Brower 1976). Nevertheless the irradiation process reduces insect quality in some measurable way. Various strategies minimize somatic damage and thus preserve quality: irradiating insects near, or after the completion of, adult

development, i.e. late-stage pupae or adults, irradiation in a reduced-oxygen atmosphere, "fractionating" the dose into several smaller doses, and using irradiators with small maximum to minimum dose ratios (which allow the minimum sterilizing dose to be achieved without substantially overdosing a large proportion of the insects) (Economopoulos 1977; ASTM 2005; Bakri et al., this volume; Calkins and Parker, this volume).

Radiation doses that sterilize males typically kill oogonial cells, but the radiation sensitivity of oocytes varies with such factors as maturity and meiotic stage. As a result, females of some species may retain a degree of fertility after irradiation, especially when treated late in development. For example, in *C. capitata*, careful monitoring is needed to ensure that pupae are not irradiated too early, resulting in poor quality, or too late, leaving females with residual fertility (Williamson et al. 1985). Some insect species that are irradiated as adults, such as the boll weevil, require alternative or augmentative sterilization strategies (McKibben et al. 2001).

Mass-rearing can also produce genetic differences between wild and laboratory populations (Cayol 2000). Genetic changes in rearing colonies have been cited as the likely causes of shifts in such traits as flight ability, mating age, age at first reproduction, cuticular hydrocarbons, and adult longevity (Spates and Hightower 1970, Itô and Koyama 1982, Pomonis and Mackley 1985, Hammack 1987, Mangan 1991, Miyatake and Shimizu 1999, Suenaga et al. 2000, Meats et al. 2004). Mating arenas, in particular, may differ greatly between field and laboratory environments, and inadvertent selection of inappropriate mating behaviours in mass-reared colonies could be especially detrimental to the SIT (Edman and Simmons 1985, Briceño and Eberhard 1998). Strategies for maintaining the competitiveness of mass-reared strains include holding colonies under "relaxed" conditions to minimize selection of undesirable traits, and regular replacement of mass-reared strains (Leppla et al. 1983, McInnis et al. 1985, McInnis et al. 2002). Of course genetic changes in mass-reared colonies can also result from factors other than inadvertent selection, such as founder effects, genetic drift, and "bottlenecking".

5. MATING SYSTEMS

Given that population suppression by the SIT is overwhelmingly a function of matings between sterile males and wild females (McInnis et al. 1994), the ability of released sterile males to compete for mates is critical. The mating competitiveness of sterile males is a function of their mating propensity and mating compatibility. Mating propensity, the tendency to locate a mate, copulate and inseminate, is primarily of concern as a component of sterile insect quality (Calkins and Parker, this volume). Mating compatibility is a relative measure of how readily two populations of insects are reproductively compatible, and, in relation to the SIT, most often refers specifically to matings of sterile males with wild females (FAO/IAEA/USDA 2003). Calkins and Parker (this volume) describe methods of assessing and quantifying compatibility. In programmes that release sterile insects, it is necessary to ensure that those insects are compatible with the target insect population (FAO 1992, Cayol et al. 2002).

Insect mating systems are almost as diverse as the insects themselves, and have been categorized in a variety of ways, such as their relation to ecological resources (Hendrichs et al. 2002), type or degree of aggregation, the type or extent of male-male competition (Robacker et al. 1991, Hendrichs et al. 2002), means by which females select mates (Eberhard 1996), or the involvement and type of semiochemicals. Insects use a variety of sensory modalities to locate, identify, and evaluate potential mates and related resources, including vision, sound, odours, contact chemoreception, and "touch" (Matthews and Matthews 1978). For the SIT, the modalities used in an insect's mating system have to be understood. Sterile males must be competent in their ability to communicate with females, as receiver and/or sender of signals, to be fully competitive (Table 3). Most mating systems, in themselves, do not preclude the use of the SIT, but they influence the efficiency and logistical difficulty of the technique. In general, greater levels of complexity in the role of the male in mating will require more effort in tracking male behaviour as a part of product quality control (Hendrichs et al. 2002; Parker, this volume), and will lower expectations of high mating competitiveness of mass-produced sterile males.

5.1. Simple Mating Systems

Relatively simple mating systems often involve scramble competition for females. For example, adult female gypsy moths emerge essentially mature, do not feed, and begin "calling" (releasing a single-component sex attractant) near their pupation sites, which are spread throughout their habitat. To mate successfully, a male moth must be active at the time of day when females begin calling (Lance et al. 1988), be

Table 3. *Characteristics of insect mating systems that are favourable or unfavourable for the development and operation of programmes releasing sterile insects*

Characteristic of mating system	Favourable	Unfavourable
Behavioural role of male, including any courtship ritual	Simple	Complex
Female choice of mates	Passive (accepts first male)	Active (chooses among males)
Sex pheromone	Female-produced, simple (1- or 2-component), long-range	Male-produced, complex
Characteristics of adult male	Long-lived, active disperser	Short-lived, sedentary
Male-male competition	Indirect (scramble for mates)	Contest for mates or resources
Mating in time and space	Distributed throughout habitat, asynchronous	Highly aggregated, e.g. termite swarms

capable of locating the source of the pheromone (before another male finds it), and then recognize and attempt copulation with a female when he, literally, steps on her (Charlton and Carde 1990). Mating is slightly more complex in the New World screwworm where male flies must locate and perch in sites where they can encounter flying females (Guillot et al. 1978). The males dart out and grab at small objects flying by, recognizing and attempting to mate with females if a contact pheromone is present (Hammack 1992). Such relatively simple mating systems are amenable to the SIT, and can lead to the production of highly competitive sterile males. Sterile male gypsy moths are typically nearly 100% competitive, based on their ability to locate pheromone sources (Mastro 1978), and on the relationship of egg sterility to the ratios of sterile:wild males trapped during pilot tests (Mastro and Schwalbe 1988; Bloem et al., this volume). One downside of simple mating systems, especially those with pure scramble competition, is that they are often associated with short adult lifespans and compressed mating periods (sections 3.4. and 3.1.3.).

5.2. Complex Mating Systems

The difficulty of producing highly competitive sterile males will, as a general rule, increase with the complexity of the males' mating-related behaviours. For example, Mediterranean fruit fly males attract females by releasing a very complex pheromone (Jang et al. 1989) from an appropriate microhabitat, which typically, but not always, is the underside of a sun-lit leaf. Males often call near other calling males at locations that have been referred to as leks (Prokopy and Hendrichs 1979). When a female approaches a male, he initiates a complex courtship ritual and, if the female remains to the end of the display, he attempts to mount her. Following mounting the female can mate, or reject the male by dropping from the leaf (Lance et al. 2000). Given the complexity of male behaviour, differences in pheromone composition and sexual behaviour between wild and sterile male Mediterranean fruit flies are not unexpected (section 4.2.), and in fact have been quantified (Heath et al. 1994, Briceño and Eberhard 1998). Accordingly, in small-scale mating assays through pilot-scale tests, sterile male Mediterranean fruit flies have usually been less than fully competitive, and in very extreme cases less than 1% competitive (Wong et al. 1986; McInnis et al. 1994; McInnis et al. 1996; Rendón et al. 2000, 2004). Such lapses in mating competitiveness can increase costs, and compromise the effectiveness of programmes that release sterile insects (Calkins and Parker, this volume; Vreysen, this volume; Whitten and Mahon, this volume). In spite of this, the SIT is increasingly being used against the Mediterranean fruit fly and other tephritids in eradication, exclusion and suppression contexts (Dowell et al. 2000; Rossler et al. 2000; Enkerlin, this volume; Hendrichs et al., this volume). Several potential methods of enhancing the competitiveness of sterile Mediterranean fruit flies are being investigated, such as the development of improved pre-release feeding regimes (Yuval et al. 2002, Niyazi et al. 2004), and hormonal (Teal et al. 2000) and semiochemical treatments (Papadopoulos et al. 2001, Shelly and McInnis 2001).

The mating behaviour of sterile males becomes especially critical when females, as in the example above, actively choose among mates based on male phenotype. In

these insects, seemingly minor differences in behaviour between wild and sterile insects can translate into poor competitiveness (Lance et al. 2000). In addition, selection can potentially favour wild females that are adept at identifying and rejecting sterile males, resulting in wild populations that are "behaviourally resistant" to the SIT (Itô and Yamamura, this volume; Whitten and Mahon, this volume). Apparently this occurred after several years of sterile insect releases against *C. capitata* on the island of Kauai, Hawaii, USA — the percentage of successful courtships dropped from about 10% to 1 or 2% for interactions between sterile males and wild females; however the compatibility of the sterile males with wild females from other islands was unaffected (McInnis et al. 1996, Lance et al. 2000). A similar erosion of mating compatibility had previously been noted and overcome during the SIT-based successful eradication of *B. cucurbitae* in Japan (Hibino and Iwahashi 1991).

6. POST-COPULATORY FACTORS

The effectiveness of mating between sterile males and wild females can be lost partially or entirely if the females also mate with wild males, and preferentially use sperm from the latter for fertilization. To determine if the SIT is appropriate for use against an insect species, Knipling (1955) proposed that one principle to consider was:

> Females must normally mate only once.

This assertion is still occasionally voiced dogmatically, at least as a question, although polyandry does not negate the basic principles of the technique (Barclay, this volume; Whitten and Mahon, this volume). However Knipling (1955) continued:

> If females of a species mate more frequently, the sperms from irradiated (sterile) males must be produced in essentially the same number and compete with sperms from fertile males.

Indeed, all else being equal, the overall sterility induced into a population of ten females by a sterile:wild overflooding ratio of 9:1 should be the same whether each female mates once — nine with a sterile male and one with a wild male — or each female mates ten times — nine times with sterile males and once with a wild male. This issue is broader than Knipling's assertions, but regardless of the number of times that a female "normally" mates, the competitiveness of sterile males will be influenced by post-mating factors, including the ability to induce mating refractoriness in females, sperm competition, and/or sperm precedence, depending on the species.

Patterns of female receptivity include variations on three basic themes: (1) monogamy, (2) females cycle through periods where they alternately are or are not receptive, and (3) continuous receptivity (Ringo 1996). In most insects, female receptiveness drops sharply after mating, typically due to a physiological response to materials passed from male to female during copulation (LaChance 1975, Eberhard

1996, Ringo 1996, Kraaijeveld et al. 2005). In many species the sperm or spermatophores themselves produce the effect, but in others (best documented among dipterans) an "anti-aphrodisiac" in the seminal fluid — often an accessory-gland protein — produces the change (Ringo 1996). An accessory-gland factor has been documented in *C. capitata*, and males from sterile and wild strains were found equally competent at inducing females to shift from mate-seeking to oviposition behaviours (Jang et al. 1998). Similarly female *B. cucurbitae* became unreceptive after mating either with virgin or with "spermless" (sperm-depleted) males (Kuba and Itô 1993).

In other species, the transfer of a full complement of sperm appears to be the critical factor that turns off female receptiveness. For programmes for these other species, this requires that sterility be based on dominant lethal mutations rather than, for example, on the elimination of sperm production (LaChance 1975). Male lepidopterans produce sperm that are apyrene (anucleate) as well as eupyrene (functional), and in particular the presence of eupyrene sperm appears to be important in shutting off receptivity (LaChance 1975). Radiation doses that cause reproductive sterility can also reduce the quantity and/or quality of a male's sperm (North et al. 1975, LaChance et al. 1979, Proshold et al. 1993). Also sperm are often depleted faster (after fewer matings) in radiation-sterilized males than in unirradiated males (Haynes and Mitchell 1977). The F_1 sterile progeny of substerilized males may also transfer less than a full complement of sperm (Carpenter et al. 1987; Proshold et al. 1993; Carpenter et al., this volume). Sterilization-related reductions in the amount of sperm transferred to females can reduce sterile male competitiveness by increasing the incidence of remating among females that mate with both sterile and wild males (Haynes and Mitchell 1977, Carpenter et al. 1987).

When females mate with both sterile and wild males, the proportion of eggs fertilized by the sperm of sterile males can be influenced by the species' patterns of sperm precedence and/or the competitiveness of the males' ejaculates. In many species sperm from recent matings takes precedence over sperm from earlier matings (Brower 1975, Etman and Hooper 1979, Saul and McCombs 1993), although other species show first-mating (El Agoze et al. 1995) or variable sperm precedence (Conner 1995, LaMunyon and Huffman 2001). In some species sperm precedence is complete, or nearly so (Brower 1975, Etman and Hooper 1979), and specialized mechanisms exist to expel or otherwise inactivate sperm from previous matings (Waage 1979). However, often sperm from different matings mix to various degrees, and the proportion of offspring a male sires will depend at least in part on the competitiveness of his ejaculate.

Ejaculate competitiveness can potentially be related to a variety of factors such as male age (LaMunyon 2001), but often the determinant is simply the quantity or quality of sperm transferred (Saul and McCombs 1993, Alyokhin and Ferro 1999, LaMunyon and Huffman 2001). The proportion of a multiple-mated female's eggs that any given male fertilizes can be reduced by sterilization procedures (LaMunyon 2001). This effect has been shown to be dose-dependent in the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Carpenter et al. 1997), and influenced by the age at irradiation in the boll weevil (Villavaso et al. 1998).

7. CONCLUSIONS

No insect is a "perfect" target for the SIT. Sterile Mediterranean fruit flies can be produced in large numbers at a reasonable cost, but the high release rates required, and the complex role of the male in mating, can create problems for operational programmes. New World screwworm flies have a relatively simple mating system but are not easy to rear, and in a mass-production situation the quality of a colony tends to degrade rapidly (Mangan 1991). Although numbers of tsetse required for release are much lower than for other species, it is also problematic to rear them in sufficient numbers (Opiyo et al. 2000). In spite of these problems, the SIT is being used successfully against all of these insects in AW-IPM programmes that, in some cases, are among the most extensive insect management programmes ever undertaken.

For other insects, such as the boll weevil and gypsy moth, functional SIT technology has been developed but is not being used (at least not on a significant scale) because simpler or more cost-effective alternative control methods are available. Agronomic, socio-economic, and biological factors must be weighed when deciding whether the SIT is an appropriate tool for managing a given pest. Probably the one generalization that can be made regarding pest biology and the SIT is this: when considering, developing, or conducting an AW-IPM programme integrating the SIT, an understanding of the pest's biology is critical to making appropriate decisions and to the overall success of the programme.

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CHAPTER 2.3.

GENETIC BASIS OF THE STERILE INSECT TECHNIQUE

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SUMMARY

The use of the sterile insect technique (SIT) for insect control relies on the introduction of sterility in the females of the wild population. This sterility is produced following the mating of these females with released males carrying, in their sperm, dominant lethal mutations that have been induced by ionizing radiation. The reasons why the SIT can only be effective when the induced sterility in the released males is in the form of dominant lethal mutations, and not some form of sperm inactivation, are discussed, together with the relationship of dominant lethal mutations to dose, sex, developmental stage and the

particular species. The combination of genetic sterility with that induced by radiation is also discussed in relation to the use of genetic sexing strains of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in area-wide integrated pest management (AW-IPM) programmes that integrate the SIT. A case is made to lower the radiation dose used in such programmes so as to produce a more competitive sterile insect. Increased competitiveness can also be achieved by using different radiation environments. As well as radiation-induced sterility, natural mechanisms can be recruited, especially the use of hybrid sterility exemplified by a successful field trial with tsetse flies *Glossina* spp. in the 1940s. Genetic transformation will make some impact on the SIT, especially regarding the introduction of markers for released flies, and the construction of genetic sexing strains. It is concluded that using a physical process, such as radiation, will always have significant advantages over genetic and other methods of sterilization for the large-scale application of the SIT.

1. INTRODUCTION

E. F. Knipling realized in the 1930s (Lindquist 1955) that, if male insects could be sterilized genetically without affecting their ability to mate, then they could be used to introduce a genetic load into a wild population in the field that would lead to its suppression or even eradication. For some time geneticists were aware that X-rays could induce mutations in insects (Runner 1916, Muller 1927), but it was not until A. W. Lindquist showed a publication by Muller (1950) to Knipling that applied entomologists realized the great potential it offered (Baumhover 2001, 2002). The results from the first experiments to sterilize the New World screwworm *Cochliomyia hominivorax* (Coquerel) were published in 1951 (Bushland and Hopkins 1951). This demonstration, that X-rays could indeed induce sterility, was the first small step on the way to the eradication of this important livestock pest in the southern states of the USA, and then in Mexico and all the countries of Central America as well as Panama (Wyss 2000). A permanent barrier of sterile insects has been established in eastern Panama to prevent the reinvasion of the pest from South America. Baumhover (2001, 2002) provided a historical account of the early days of the screwworm eradication programme, and Klassen and Curtis (this volume) describe the sterile insect technique (SIT), in general, from an historical perspective.

During the first field trials of sterile New World screwworms in Curaçao, the genetic basis of sterility was poorly understood, but it was realized that sterility resulted from the induction of dominant lethal mutations in the irradiated sperm (Bushland and Hopkins 1951, LaChance et al. 1967). At that time the level of understanding of the genetics of the screwworm led Bushland to comment that (quoted by LaChance 1979):

> . . . we eradicated screwworms from Curaçao and the south-eastern United States without knowing how many chromosomes it had.

Prior to the adoption of radiation to sterilize insects, chemical mutagens were evaluated (Borkovec 1966), but difficulties relating to toxicity, handling, and residues were considerable, and so radiation has usually been the method of choice. Even though field trials with chemosterilized *Anopheles albimanus* Wiedemann mosquitoes in El Salvador were successful (Breland et al. 1974), it is unlikely that today such releases could be carried out.

2. STERILITY REQUIREMENTS FOR SIT

It is very important that the word "sterility" be precisely understood in terms of its use in the SIT. The word "sterility" describes one of many possible end points of the reproductive process, but it can cover a multitude of causal factors. The following definitions of sterility were taken at random from three biological dictionaries:

- Structural or functional inability to reproduce
- Involuntary total inability to reproduce
- Any complete or partial failure to produce functional gametes or viable zygotes

These definitions cover genetic, physiological, morphological or even "psychological" factors, which can lead to a final end point of sterility, and clearly many of these manifestations would not be useful for sterility in area-wide integrated pest management (AW-IPM) programmes. For the SIT to be effective, females of the wild population in the field have to be permanently prevented from reproducing, and any factor(s) transferred by the released male that accomplishes this would, in fact, be sufficient. True genetic sterility in released male insects requires: (1) production of viable sperm, (2) their transfer to the wild female during mating, (3) their use in fertilization of eggs, and (4) the inability of the fertilized zygote to complete development to a fertile adult. In other words, an irradiated male insect must be able to carry out all the functions of a normal fertile insect — it must produce fully functional sperm that succeed in fertilizing eggs and initiating the development of fertilized eggs. In the SIT, the radiation-induced sterility is actually produced in the generation following the release of the males, i.e. with the death of the embryo, larva, pupa or adult, or the production of F_1 adults that themselves produce gametes that result in zygotes that do not develop. A male insect that cannot mate, is aspermic, or that transfers non-functional sperm, could be classed as sterile, but males with any of these defects would probably not be effective for the SIT.

Recently the International Plant Protection Convention (IPPC) (FAO 2005) provisionally adopted the following definition of a sterile insect:

> an insect that, as a result of an appropriate treatment, is unable to produce viable offspring.

and the following definition of the SIT:

> a method of pest control using area-wide inundative releases of sterile insects to reduce fertility of a field population of the same species.

Irradiated males must also be able to transfer the appropriate accessory gland fluid during mating, ensuring that female behaviour corresponds to that following mating with a fertile male. In some insects, this female post-mating response involves temporary or permanent refractoriness to further mating, and a change in female behaviour. In *Drosophila* sp. the peptides transferred in the accessory fluid, that are involved in the female post-mating behavioural changes, have been well studied (Chen 1996), and it has even been possible to sterilize females by the ectopic expression (i.e. gene expressed in all tissues) of a transgene which codes for the sex peptide (Aigaki et al. 1991). In fact, a male that only transferred accessory gland fluid, and which could elicit the correct post-mating female response, could theoretically "sterilize" the female. In the Mediterranean fruit fly *Ceratitis capitata*

(Wiedemann), it has been shown that irradiated sterile males produce the same kind of change in wild female behaviour, from mating to oviposition, as do fertile males (Jang et al. 1998, Jang 2002).

In species where females remate, sterility for use in the SIT must be efficiently induced in sperm without affecting sperm function and its capacity to compete with other sperm, and exert its effect only after fertilization of the female egg; dominant lethal mutations are such sterility factors. They are readily induced in all chromosomes by irradiation, and they have little effect on the phenotype of the sperm, at least at the doses usually used for the SIT (Bakri et al., this volume). Lethality occurs when the haploid nucleus, carrying such a mutation or mutations, is combined with a normal haploid nucleus, resulting in the death of the early embryo at the moment when the genetic information required for normal development is absent or incorrect (Muller 1927). In addition, cell division can become asynchronous and lead to the death of the zygote.

3.   DOMINANT LETHAL MUTATIONS

The mechanisms by which these mutations cause lethality in Diptera in the developing zygote are now well documented (Smith and von Borstel 1972, LaChance 1967); they are illustrated in Figure 1.
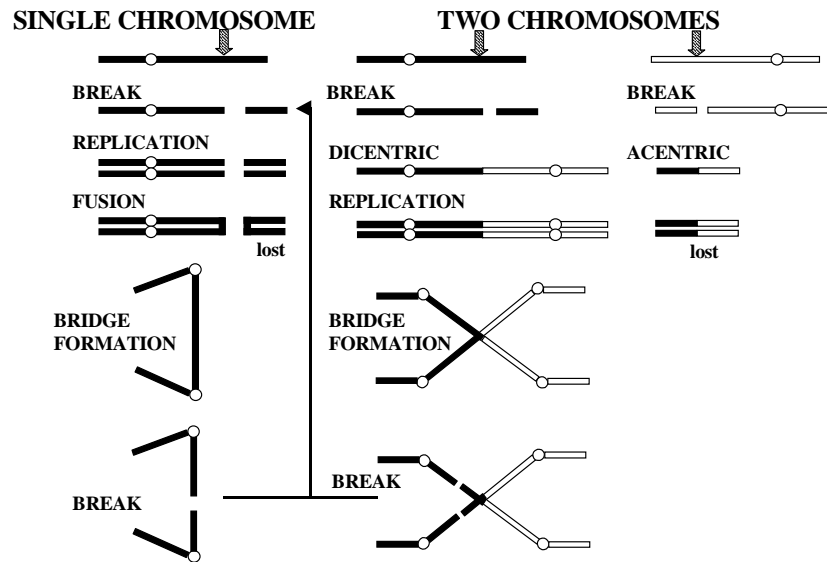
Figure 1. Schematic representation of the fate, during cell division in the embryo, of chromosomes with radiation-induced dominant lethal mutations, leading to the accumulation of serious imbalances in the genetic information of subsequent daughter cells.

The primary lesion leading to a dominant lethal mutation is a break in the chromosome, in this case, induced by radiation. When a break is induced in a chromosome in mature sperm, it remains in this condition until after the sperm has entered an egg. (This was shown very elegantly by mating queen bees to irradiated males, and then measuring fertility after storage of the sperm in the spermatheca for one year; there was no difference in the level of sterility in the same queen tested one year later (Lee 1958)). Following fusion, nuclear divisions begin, and a break in a chromosome can have drastic effects on the viability of the embryo as development proceeds. During early prophase the broken chromosome undergoes normal replication, but during metaphase the broken ends can fuse leading to the formation of a dicentric chromosome and an acentric fragment. The acentric fragment is frequently lost, while the dicentric fragment forms a bridge at anaphase leading to another chromosomal break. This whole process then repeats itself, leading to the accumulation of serious imbalances in the genetic information of the daughter cells. The accumulation of this genetic damage finally leads to the death of the zygote. If two different chromosomes are broken they can also rejoin in the way depicted in Fig. 1. These chromosomes produce the same problems for the dividing cells as those formed by a break in a single chromosome, by undergoing incorrect fusion and leading to the breakage-fusion-bridge cycle. In this way dominant lethal mutations can cause cell death, and the accumulation of genetic imbalance in the developing zygote leads to lethality.

3.1. Dose Response for Dominant Lethals

Dose-response curves for the induction of sterility by radiation are generally developed using measurements of hatchability of eggs, deposited either by irradiated females mated with non-irradiated males, or by non-irradiated females mated with irradiated males. The implicit assumption is that most dominant lethal mutations exert their effect during early embryonic development. Data from *Drosophila* sp. and other dipteran species containing chromosomes with monokinetic centromeres, have shown that, indeed, this is the case (Demerec and Fano 1944, Catcheside and Lea 1945, Lee 1958, Tantaway et al. 1966, Franz 2000), making egg hatch an appropriate stage to evaluate. In insect species with holokinetic chromosomes, many dominant lethal mutations exert their lethal effects only at later developmental stages (Carpenter et al., this volume).

Dose-response curves for dominant lethal mutations can be developed by irradiating insects with increasing doses of radiation and calculating the percentage egg hatch. The curves tend to show a characteristic shape, depending on the cell type and stage irradiated. The shape of the curve can be used to infer information about the types of initial chromosomal lesion producing the lethal effect. In irradiated sperm, there is an approximately linear relationship between the dose and the induction of dominant lethals at low doses, but at higher doses there is a noticeable departure from linearity, i.e. it tends to saturate at higher doses, and approaches 100% sterility asymptotically. This is due to the induction of multiple lethal events in the same cell, but only one is needed to cause the egg to die. The shape of the curve, as well as indicating the underlying causal factors of dominant lethality,

should help in selecting a dose that will be used to sterilize insects for release. Fig. 2 shows the dose-response curves for the house fly *Musca domestica* L. and the large milkweed bug *Oncopeltus fasciatus* (Dallas). The difference in the shape of the curves is due to the fact that the latter species has holokinetic chromosomes (section 3.4.), and this results in the need for much higher doses of radiation for sterilization.
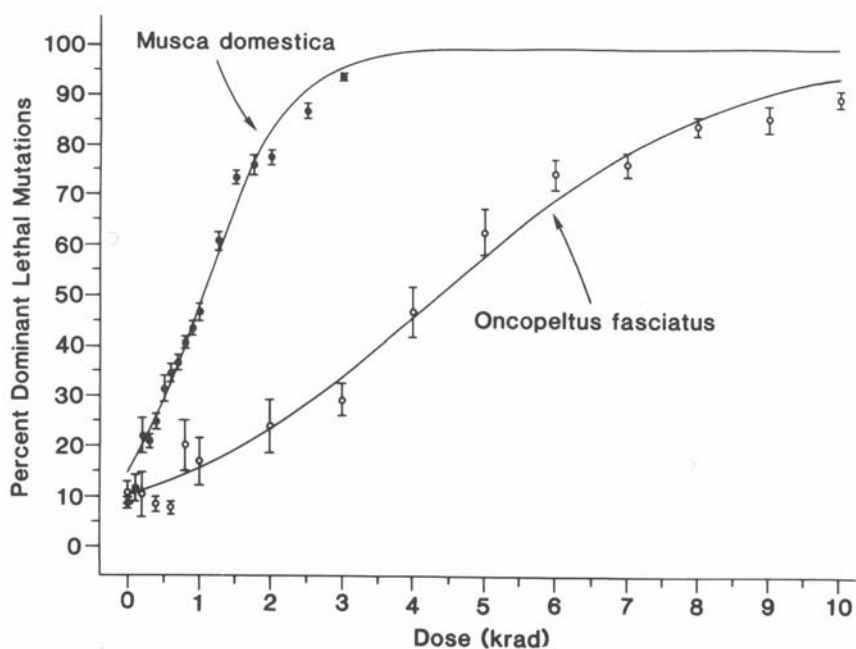
Figure 2. Dominant lethal dose-response curves for Musca domestica *and* Oncopeltus fasciatus. *(Figure from LaChance and Graham 1984, reproduced with permission from Elsevier.)*

Assuming that dose-response curves have been developed for both sexes, that both sexes have to be released, and that close to full sterility is required, then the minimum radiation dose chosen must lead to full sterility in females. Any residual fertility in released females can be extremely damaging, as this will actually contribute to the reproductive potential of the wild population. For example, a residual fertility of 2% in irradiated females, following a sterile:wild release ratio of 100:1, will actually double the number of insects in the next generation. Residual fertility in males is of less importance; it simply reduces the rate at which the population is suppressed. These simplistic assumptions ignore the impact of any density-dependent regulation in the population. The full impact that the decision — on the dose to be used, based on the shape of the curve (Fig. 2) — has on the effectiveness of the SIT is described below (section 4.).

3.2. Dominant Lethals and Sex

Male and female insects normally differ considerably in their sterility response to radiation, primarily because sperm are haploid post-meiotic cells, whereas mature eggs are pre-meiotic. Eggs usually develop in the female to metaphase 1, and then are arrested until oviposition when meiosis is completed with the production of a single pro-nucleus and three polar bodies. Mature eggs in the female are much more resistant to radiation than are the earlier oocytes, with the consequence that a radiation dose which induces full sterility in mature eggs often leads to the cessation of oogenesis at a later date. This can lead to an increase in the lifespan of females as energy is redirected away from reproduction to survival (White and Hutt 1970, Hafez and Shoukry 1972). In most cases this is of no consequence, but it can be important if the sterile females that are released are themselves damaging in some way. In tsetse, for example, it is essential to remove sterile females from the released insects as their increased lifespan (Dean and Clements 1969) might enable them to be efficient vectors of disease.

In addition to this basic difference between male and female germ cells, there are also species-related differences in the overall sensitivity of male and female insects to radiation sterilization. In most species, females are more sensitive than males to radiation sterilization, and therefore it is possible to identify the sterilizing dose for the SIT based on the acceptable dose for males (section 4). Use of this dose will ensure that all released females are fully sterile. In the minority of species where the female is more resistant (Haynes et al. 1977, Crystal 1979), the sterilizing dose for females must be used, and this can compromise the competitiveness of the sterilized males. These patterns of differential sensitivity of germ cells in males and females are important to investigate when the radiation dose for an AW-IPM programme using the SIT is identified.

3.3. Dominant Lethals and Developmental Stage

To maximize genetic damage to mature germ cells and minimize somatic damage, insects should be irradiated as late as possible in the development pathway, ideally as fully differentiated adults. In the adult stage, insects are most resistant to somatic damage as somatic cell division is at a minimum.. However, even in a fully differentiated insect, certain somatic cells, especially in the gut, continue to divide, and sterilizing radiation doses can compromise viability and hence the fitness of the treated insect (Flint et al. 1966).

In spite of these biological relationships, operational considerations in implementing the SIT often play the major role in determining the stage at which insects are irradiated. The operational decision is based on identifying the most convenient stage for radiation, from the point of view of mass-rearing, handling and release protocols. In most insects, this is the pupal stage, and consequently radiation is applied to pupae as late in development as possible. In New World screwworm and Mediterranean fruit fly AW-IPM programmes applying the SIT, pupae are irradiated 2 days before emergence, enabling the shipment of irradiated pupae to emergence facilities far from the production plant, where adults emerge and are fed

and processed for release. In the successful eradication of the screwworm outbreak in Libya, irradiated pupae were shipped twice weekly from Mexico (FAO 1992).

In AW-IPM programmes integrating the SIT for some other species, adult insects have to be irradiated. In Lepidoptera, larvae generally pupate within a cocoon that attaches itself to a substrate, and in these cases it is impractical to irradiate this stage, and thus teneral adults are irradiated (Proverbs et al. 1975). In tsetse, before release, adult males are fed blood containing a trypanocide and irradiated at 3-4 days of age (Dyck et al. 2000, Vreysen et al. 2000). In general, operational factors tend to be decisive in determining the developmental stage that is irradiated (Bakri et al., this volume).

3.4. Dominant Lethals and Species

The class Insecta comprises 29 orders (Richards and Davies 1977), and pest species are found in most of the major orders. This very diverse class exhibits widely different sensitivities, both within and between orders, to the induction of dominant lethal mutations (IDIDAS 2004, Bakri et al. 2005), and to the somatic effects of radiation (Willard and Cherry 1975). Probably there are many factors that contribute to these differences, including the centromere structure (Smith and von Borstel 1972), the degree of chromosome condensation (Israelewski 1978), radiation repair (Beatty and Beatty 1967), and the chromosome environment (Thoday and Read 1947). This is certainly not an exhaustive list, and other factors, as yet unidentified, could still be important.

Insects can, however, be divided into two main groups which show major differences in sensitivity to the induction of dominant lethals, and for which experimental evidence is available that identifies the underlying biological and genetic factors responsible. Orders such as Diptera, Hymenoptera, and Coleoptera can be classed as radiation-sensitive, while orders such as Lepidoptera and Homoptera are radiation-resistant. A major difference between these two groups of insects is that the former group has a localized centromere (monokinetic), while the latter has a diffuse centromere (holokinetic) (Bauer 1967), and this difference plays a major, although not exclusive, role in radiation sensitivity (Tothova and Marec 2001). Lepidoptera also do not show the classical breakage-fusion-bridge cycle that is a characteristic of dominant lethals induced in Diptera. It appears that lepidopteran chromosomes can tolerate telomere loss without the drastic effects that this has on chromosomes in other orders. Discussions on this phenomenon are found in Bakri et al. (this volume) and Carpenter et al. (this volume).

4. APPROPRIATE RADIATION DOSE FOR SIT: IS 100% STERILITY REQUIRED?

The word "sterile" in the acronym "SIT" is generally perceived to imply a requirement for full sterility, and hence a radiation dose is sought that achieves 100% sterility. However, since there are varying levels of sterility that can be induced, the word "sterile" is not an absolute term, and it might not always be required that released insects are fully sterile. As described above, the relationship of

radiation dose to dominant lethal induction in the mature sperm of insects approximates to linearity at low radiation doses, but not at high doses, producing an asymptotic approach towards full sterility (Fig. 2). This curve is used as a decision-making tool to identify the radiation dose appropriate for a specific insect in a specific AW-IPM programme using the SIT. The fact that the curve approaches 100% sterility asymptotically makes it difficult to select the dose that gives the required full sterility. The equation used to describe the curve predicts that, in theory, given a large enough sample of eggs, it will always be possible to find one that hatches, and hence the goal of full sterility will never actually be met. In practical terms, however, a dose is chosen which prevents the hatch of over 99% of eggs in a large sample. This decision is often taken solely on the basis of the dominant lethal-induction curve; the effects of radiation on somatic tissues, and hence on the final ability of the insect to introduce sterility into wild females in the field, are generally ignored.

In fact, the goal of attempting to achieve full sterility in treated insects can seriously compromise their competitiveness in the field. This follows from the shape of the dose-response curve. At high doses, increasing amounts of radiation are required for proportionally smaller increases in sterility. LaChance and Graham (1984) described the dominant lethal-induction curve for several insect species, and, using equations derived from their curves, it can be shown that, for example, to decrease egg hatchability from 2 to 1%, an 11% increase in dose is required. This marginal increase in sterility, obtained at the expense of a disproportionate increase in the radiation dose, can have major negative effects on the competitiveness of the released insects. Since the shape of the curve relating somatic damage to dose is not known, some assumptions have to be made in arriving at this conclusion.

The proportion of wild females rendered sterile by a given number of males released depends on both their sterility and their success in competing with wild males to mate with wild females. Therefore, to optimize the balance between somatic fitness and genetic sterility, it is in the interest of programmes applying the SIT to choose radiation doses that maximize the genetic load introduced into the wild populations. This means that chosen optimal radiation doses to achieve this objective may give sterility levels well below 100% (Toledo et al. 2004).

A word of caution is required here. It is known that, in *Drosophila* spp., males receiving substerilizing radiation doses can recover fertility over time (Luning 1952), and it is important to evaluate this aspect if lower doses of radiation are used. Currently all operational programmes consistently err on what may be considered the side of caution, and use radiation doses that induce close to 100% sterility in the treated insects. Unfortunately, high levels of somatic damage, and hence lower sterile male performance, normally result from such high doses.

5. RADIATION IN DIFFERENT ENVIRONMENTS

Usually radiation is the final treatment that insects receive at the mass-rearing facility before being transported to, and released in, the field. Any technique that reduces the somatic damage induced by the treatment would be advantageous. Mass produced insects are expensive, and need to function as well as possible in the field.

The amount of genetic damage produced by radiation, both in reproductive and somatic tissues, is related to the environment in which the tissue finds itself, in particular, the oxygen tension. It is well known that treatment in low-oxygen tension, e.g. irradiating in nitrogen or anoxia, reduces radiation damage (O'Brien and Wolfe 1964; Bakri et al., this volume). However, if low-oxygen tension gives the same protective effect for somatic tissue (competitiveness) and sperm (sterility), then there would be no net gain. To achieve the same level of sterility as irradiation in air, irradiation in nitrogen would have to be at a higher dose.

The differential protection afforded to somatic cells by irradiation in nitrogen is related to the fact that somatic tissue is diploid and still undergoing cell division, whereas sperm are fully differentiated and haploid. This means that damage induced in somatic cells can manifest itself during the further life of the insect, whereas damage induced in sperm cells is only realized following fertilization of eggs in the wild females. The positive effects on competitiveness of irradiation in nitrogen have been demonstrated for several insect species (Hooper 1971, Curtis and Langley 1972, Hallinan and Rai 1973, Wakid 1973, LaChance and Richard 1974, Economopoulos 1977). Two successful fruit fly AW-IPM programmes integrating the SIT were carried out in Australia using pupae irradiated in nitrogen (Hooper 1971, Fisher et al. 1985, Fisher 1996), and this approach is still being used for Mediterranean fruit fly programmes in that country (Fisher 1997). At present, no other operational programmes are irradiating insects in nitrogen.

6. COMBINATION OF RADIATION AND GENETIC STERILITY

Genetic sexing strains are now being used in almost all AW-IPM programmes that use the SIT for the Mediterranean fruit fly (Robinson et al. 1999; Franz, this volume). Since these strains are constructed using male-linked translocations, they are semi-sterile (Laven 1969). To obtain a more competitive insect, it has been suggested (Steffens 1982, 1983) that this genetically contrived sterility be combined with a lower dose of radiation-induced sterility. At lower doses of radiation, the overall sterility of males from a genetic sexing strain is, of course, higher than that of males from a normal strain. However, as the radiation dose increases, the contribution from genetic sterility gets progressively less, and eventually disappears as the sterility increases. For a male with a normal karyotype, and a male carrying a translocation, the radiation dose close to full sterility is the same. Nevertheless, the use of males produced from a genetic sexing strain offers the opportunity to seriously re-examine the radiation strategy of Mediterranean fruit fly programmes to maximize the genetic load introduced into wild populations.

7. HYBRID STERILITY

When hybrids are formed between closely related species, or even between some populations of the same species, sterility is observed. The sterility phenotype can include the total absence of viable progeny, the production of hybrids of both sexes with varying levels of sterility, or the production of a unisexual sterile F_1 generation that is usually male. Examples of all these are given below.

This array of different hybrid phenotypes has a corresponding wide range of underlying genetic and cytoplasmic causes, which in many cases overlap to produce a very complex phenotypic picture. The causes of hybrid sterility can be grouped roughly into those factors that are carried on the nuclear genome of the insects themselves, and those that are maternally (cytoplasmic) inherited. Many nuances and interactions are possible within these two major groups. Currently the picture is far from complete. An early paper by Haldane (1922) reviewed cases of classical hybrid sterility in many different animal groups, including a large number of lepidopteran species. His analysis showed that there was preferential sterility or inviability in the hybrids of the heterogametic sex. This observation has become known as Haldane's Rule, and has largely stood the test of time (Orr 1997). For pest insects, this means that, for example in Lepidoptera where the female is heterogametic, the major hybrid effects would be seen in the resulting female hybrids, whereas in Diptera where the male is heterogametic, the F_1 males will be more affected.

7.1. Cytoplasmic Incompatibility in Culex pipiens L.

Mosquitoes of the genus *Culex* are important vectors of filarial worms and some arthropod-borne viral diseases such as arboviruses. As early as 1938, Marshall (1938) showed that certain crosses of populations of *Culex pipiens* from England and France failed to produce progeny. In the 1950s Laven (1967a) carried out a worldwide survey of compatibility among many different populations of this complex. He showed that this phenomenon was cytoplasmic in origin, and that incompatibility could be uni- or bi-directional (Laven 1967a). The causative agent was a rickettsia-like bacterial symbiont; its removal by antibiotic treatment abolished the sterility phenotype (Yen and Barr 1973). The symbiont has been classified as *Wolbachia pipientis* Hertig, and is widely distributed in arthropods, with up to 76% of insect species so far examined showing evidence of infection (Jeyaprakash and Hoy 2000). It has a wide variety of effects on arthropod reproduction (Bourtzis and O'Neill 1998), and has been implicated in maintaining the viability of filarial worms that cause river blindness. In insects, females infected with *Wolbachia* can successfully reproduce with males that are uninfected, but the reciprocal cross is sterile. This, coupled with the maternal inheritance of the infected state, enables the bacterium to spread through a population and carry with it any other factor that is exclusively maternally inherited (Pettigrew and O'Neill 1997, Curtis and Sinkins 1998). The release of males infected with *Wolbachia* into a naive population would be equivalent to the release of radiation-sterilized males.

In 1967 an experiment, to use cytoplasmic incompatibility to suppress a small isolated population of *Culex fatigans* Wiedemann, was carried out in Okpo, a small village near Rangoon (Laven 1967b). Over a period of 4 months, each day about 5000 infected or incompatible males were released into a population estimated to fluctuate between 2000 and 10 000 mosquitoes. After 4 months of releases, all the remaining egg rafts collected were sterile. Unfortunately the arrival of the monsoon season prevented any further observations. Curtis et al. (1982) carried out a much larger field trial. Although releases of incompatible males did reduce the population

build-up, it was not possible to increase the percentage of sterile egg rafts above 70%. It was concluded that immigration of fertilized females from outside the village caused the stagnation in the numbers of sterile egg rafts observed. The use of this approach for suppression requires that exclusively males of the incompatible strain be released; any females that are co-released would be compatible with the males and would lead to population increase. To solve the problem of error-free sexing as well as escaping females, a low dose of radiation can be given; female mosquitoes are generally more sensitive than males (Arunachalam and Curtis 1985).

7.2. Hybrid Sterility in Tsetse

The first evidence that hybrids are formed by crossing different tsetse species was provided by Corson (1932), who obtained progeny from mass matings involving female *Glossina morsitans centralis* Machado and male *G. swynnertoni* Austen, but as he did not make observations on mating, he concluded that it was due to parthenogenesis. Potts (1944) repeated these experiments, and concluded that true hybrids were obtained as evidenced by the morphology of the hybrid male genitalia. He also was able to backcross F_1 females and produce progeny. Vanderplank (1944) confirmed these observations, but noted that, when no choice of mates was offered, inter-specific crosses took place as readily as the intra-specific crosses. By catching and identifying each member of copulating pairs, Jackson (1945) confirmed that this was true also in the field. Extensive work by Curtis (1972), Rawlings (1985), Gooding (1993; 1997a, b; 2000), and Gooding and Krafsur (2005) expanded the knowledge of tsetse hybridization phenomena, and indicated how this might, or might not, be used to develop methods of pest population suppression. Interspecific crosses in tsetse produce sterile male hybrids and partially sterile female hybrids, and there is a strong asymmetry in the sterility phenotype of the reciprocal crosses. In some crosses no F_1 progeny are produced. The male hybrids can copulate with and inseminate females, but the hybrid sperm cannot always succeed in fertilising eggs. For other crosses, Curtis (1972) showed that, following multiple mating of females, the two types of sperm were equally competitive. However Gooding (1992) showed that, in multiple-mated females of the *G. morsitans* subspecies, the sperm from the conspecific male was used preferentially. The inability of hybrid males to fertilize females, and the preferential use of conspecific sperm, would be serious handicaps to the use of hybrid sterility for pest suppression.

Hybrid sterility in tsetse appears to be mediated by both genetic and maternally inherited factors. A very complex picture has emerged, with many factors interacting, and it has proved difficult to tease apart the differing contributions to the total picture. Apart from the expected chromosomal and genic interactions, two interesting observations have been made. Firstly, the fertility of reciprocal crosses between the different taxa show high levels of asymmetry. This is reminiscent of phenotypes induced by *Wolbachia* symbionts, and it is known that many tsetse species carry *Wolbachia* (Chen et al. 1999, Cheng and Aksoy 2000). Secondly, it appears that in some hybrid crosses there is a form of interaction between the mother and hybrid offspring that determines whether a pregnancy will be successfully completed. (Tsetse flies reproduce by adenotrophic viviparity, where the fertilized

egg hatches into a larva that is fed within the female by milk glands, and a mature third-instar larva is produced every 9–10 days.) Recent results (Olet 2000) have shown that females mated to males of a different taxon, though initially sterile, can as they get older begin to produce viable progeny.

Using hybrid sterility for tsetse control would involve either the release of fertile males into a non-compatible population, or the release of F_1 sterile males into a population of either of the parental species. The use of F_1 males has the disadvantage that two taxa have to be maintained in the laboratory, and that males and females from the respective taxa have to be obtained to set up the appropriate cross. The release population must be sexed to prevent the release of semi-sterile F_1 females, and the F_1 males need to be good inseminators. For the release of fertile males of one taxon into a wild population of a second taxon, only the release population must be sexed (however 100% accuracy is required).

In a large field experiment, Vanderplank (1947) eliminated a population of *G. swynnertoni* from the Shinyanga area, Tanzania, by releasing into it fertile *G. m. centralis* from Kondoa-Irangi, Tanzania (Klassen and Curtis, this volume). About 140 000 *G. m. centralis* pupae were released over a 7-month period, and the population of *G. swynnertoni* fell, presumably due to the reduced fertility of the hybridized *G. swynnertoni* females and the subsequent matings with sterile hybrid F_1 males. *G. swynnertoni* was eradicated, but *G. m. centralis* did not become established because of the harsh climatic conditions, and therefore the area remained tsetse-free for some time. These field trials were carried out before it was known to applied entomologists that sterility could be readily induced by radiation, and clearly demonstrated the potential of hybrid sterility to suppress tsetse flies. They were carried out before mass-rearing of these species was possible, and all the flies released, both males and females, were from field-collected pupae (there was no way to separate the sexes). Recent improvements in tsetse mass-rearing procedures and sex-separation methods (Opiyo et al. 2000, Dowell et al. 2005) should encourage a re-examination of this form of pest suppression for tsetse.

7.3. Heliothis *Hybrids — an Exception to Haldane's Rule*

Laster (1972) demonstrated that, when *Heliothis subflexa* (Guenée) females and *Heliothis virescens* (F.) males are hybridized, sterile male and fertile female progeny are produced, and the hybrid females continue to produce sterile male and fertile females through many generations of backcrossing to *H. virescens* males (Lance and McInnis, this volume). In Lepidoptera, females are heterogametic, and would be expected from Haldane's Rule to suffer more from hybridization, but in this case this is not true, suggesting that novel factors may be involved. Hybrid males can mate and transfer a spermatophore, but no eupyrene sperm reach the female spermathecae, even though they are produced in the testis. However, as the number of backcross generations increases, the ability of the males to produce eupyrene sperm decreases. This pattern of hybrid sterility suggests a very strong maternal component, but treatment of the moths with agents known to be lethal for *Wolbachia* failed to remove the sterility syndrome (LaChance and Karpenko 1981, 1983). An analysis of mitochondrial biogenesis in hybrid males failed to identify elements of

protein synthesis or transport as being the cause of the sterility, but sperm mitochondria were implicated in the phenomenon (Miller and Huettel 1986). Hybrid males of the type generated in these crosses would unlikely be effective "sterile males" in the field. Nevertheless, field trials on the island of St. Croix were conducted using the release of backcross males (Proshold 1983, Proshold et al. 1983), and temporary sterility could be demonstrated in the target population. Subsequently, there has been no field evaluation of this technology.

7.4. Anopheles gambiae *Complex*

The *Anopheles gambiae* Giles complex in Africa consists of seven sibling species, and crosses between every combination led to hybrid males, with some crosses producing no females (Davidson et al. 1967). The sterility is due to chromosomal interactions, and cytoplasmic factors have not been implicated. Davidson (1969) carried out a successful series of laboratory cage experiments to evaluate the use of these sterile males, and was able to demonstrate the induction of the expected levels of sterility in target mixed-sex populations. However, a field trial (Davidson et al. 1970) failed to demonstrate any substantial degree of mating between released sterile hybrid males and wild females. Pre-mating isolation mechanisms played a decisive role in the failure, illustrating the behavioural constraints that underlie the use of hybrid sterility in the field.

8. TRANSFORMATION AND MOLECULAR STERILITY

The successful demonstration of transformation in *Drosophila melanogaster* Meigen (Rubin and Spradling 1982) encouraged the development of similar systems in other insects, including important pest species. A review by Handler (2001) illustrates the current state of this successful technology for introducing foreign genes into pest insects. The use of transgenic techniques in traditional SIT will probably involve the introduction of molecular markers into the released insects (Robinson and Franz 2000), and the development of genetic sexing strains (Franz, this volume). Recently, constructs have been tested in *Drosophila* sp. that might be used to produce both an elimination of females from the insects to be released, and sterility induced by matings with the released males (Heinrich and Scott 2000, Thomas et al. 2000). These molecular sterility systems rely on conditional lethality in F_1 females that are produced following the mating of released transgenic males with wild females. The systems have now been demonstrated in *Drosophila* (Heinrich and Scott 2000, Thomas et al. 2000) and the Mediterranean fruit fly (Gong et al. 2005), but only in small laboratory experiments. To date, there is only a limited amount of data available on transgenic pest insects, and what is known is giving some cause for concern in two areas related to the technology itself, namely stability of the insertion and expression of the transgene. Currently there are insufficient data to predict if transgenic strains will retain the appropriate expression patterns of the constructs under large-scale mass-rearing over many generations, and following their release in the field. This will be particularly relevant for systems based on molecular sterility where permanent and absolute sterility of the system is essential.

Transformation in insect pests is carried out using transposable elements. Initially the "*P*" system from *Drosophila* was extensively tested, but without any success. Subsequently other elements were identified in different genomes, including those of insects, and some of these have proved to be successful. There is, however, growing concern that, as most of these elements are members of gene families, it will be difficult to predict the long-term stability of the transgene, and hence its expression in the transformed strain. This problem could be magnified once fertile laboratory transgenic strains are released into a wild population, the genomic diversity of which is unknown. There is also the slightly disconcerting idea that a transgene, once released in a fertile insect, cannot be "recalled" or destroyed. The integration of transgenic technology with the SIT, in the form of a sexing methodology, would be an option to reduce risk, since radiation-induced sterility would at least prevent vertical transmission of the transgene.

Public concern related to genetically modified organisms needs to be addressed, and a regulatory framework is required, so that when transgenic insect strains eventually become available they can be properly field-tested. Nevertheless risk assessment of the use of these strains in the field will not be straightforward.

9. CONCLUSIONS

Due to the extensive studies on the radiation biology of *Drosophila* sp. carried out in the 1950s and 1960s, there is a very good understanding of the genetic basis of radiation-induced sterility in that species. However, even though the underlying mechanisms are known and are probably of wide relevance, there still are many unexplained observations, especially in regards to the different dose-response curves found for different species, even within the same order (Bakri et al. 2005). For one type of mutation, i.e. specific locus mutations, there is a very good correlation between the dose-response curve and the DNA content of the haploid genome (Abrahamson et al. 1973); these studies ranged from bacteria to man. There is as yet insufficient information on genome size, in a large enough number of insect species, to assess whether such a simple relationship holds for dominant lethal induction. In addition, in Lepidoptera and other species with holokinetic chromosomes, the mechanism(s) of dominant lethal induction has/have still to be fully explained (Carpenter et al., this volume).

Radiation is usually one of the last procedures that insects undergo before leaving mass-rearing facilities for release in the field, and it is important that it be applied in a way that minimizes its detrimental effects on insect competitiveness. Firstly, it is essential that the dosimetry of the radiation source be checked to ensure that all the insects receive the required minimum dose, and that none is unnecessarily overdosed (Bakri et al., this volume). Secondly, a dose should be chosen that maximizes the level of introduced sterility in the wild females in the field, i.e. both the sterility level in the released males and their competitiveness has to be taken into account. Thirdly, numerous studies have shown that irradiation in nitrogen can provide protection against the detrimental somatic effects of radiation, but unfortunately no large-scale programmes make use of this procedure.

Currently, the development of molecular methods to sterilize pest insects in the field, by the release of fertile insects carrying transgenes, is very much in vogue, in spite of the many unknowns inherent in such a system and the current negative public opinion on transgenic technology. There is also no regulatory framework in place for any eventual release of transgenic insects. Using biological/molecular methods to sterilize insects is quite different from using a physical process, such as radiation. In the former, the essential biological interaction required to generate the sterility phenotype is subject to biological variation in both components, and is therefore unpredictable in the long term. A physical process such as radiation is not subject to this variation, and hence, in some ways, is an ideal methodology. Insects cannot become resistant to ionizing radiation as it is used in the SIT (Whitten and Mahon, this volume).

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CHAPTER 2.4.

INHERITED STERILITY IN INSECTS

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SUMMARY

The unique genetic phenomena responsible for inherited sterility (IS) in Lepidoptera and some other arthropods, as compared with full sterility, provide advantages for pest control. Lepidopteran females are usually more sensitive to radiation than males of the same species. This allows the radiation dose to be adjusted to suit programme requirements. When partially sterile males mate with wild females, the radiation-induced deleterious effects are inherited by the F_1 generation. As a result, egg hatch is reduced

and the resulting offspring are both highly sterile and predominately male. Compared with the high radiation required to achieve full sterility in Lepidoptera, the lower dose of radiation used to induce F_1 sterility increases the quality and competitiveness of the released insects as measured by improved dispersal after release, increased mating ability, and superior sperm competition. F_1 sterile progeny produced in the field enhance the efficacy of released partially sterile males, and improve compatibility with other pest control strategies. In addition, F_1 sterile progeny can be used to increase the production of natural enemies, and to study the potential host and geographical ranges of exotic lepidopteran pests.

1. INTRODUCTION

Lepidopteran species are among the most destructive pests of annual and perennial crops, forests, and stored products throughout the world. Following the successful area-wide integrated pest management (AW-IPM) programme, integrating the sterile insect technique (SIT), against the screwworm fly *Cochliomyia hominivorax* (Coquerel) (Bushland 1971), studies were conducted on the possibility of suppressing lepidopteran pest populations through the release of radiation-sterilized moths. However, because Lepidoptera are radioresistant compared with most other insects (LaChance et al. 1967; Bakri et al., this volume), a fully sterilizing dose of radiation reduced the ability of sterile moths to compete with wild moths. To increase the competitiveness of irradiated Lepidoptera, Proverbs (1962) investigated the effects of substerilizing doses of radiation on the codling moth *Cydia pomonella* (L). He noted that male moths treated with substerilizing doses and then mated to fertile females produced reduced numbers of F_1 progeny, the majority of which were males with very low fertility. This discovery prompted numerous investigations in many lepidopteran pests. North (1975) and LaChance (1985) provide thorough reviews of the early investigations on inherited sterility (IS), and discuss potential advantages of using IS in suppressing pest populations and its possible genetic basis.

IS is also referred to as inherited partial sterility, partial sterility, delayed sterility, semi-sterility and F_1 sterility. Although it is difficult to find a satisfactory definition for IS, LaChance (1985) described several attributes that are common to IS in Lepidoptera: F_1 male and female offspring are more sterile than the irradiated parental (P_1) generation, and more F_1 male progeny than female progeny are produced. Other attributes may include longer developmental time and reduced sperm quality in the F_1 generation. Radiation-induced deleterious effects can be inherited for several generations; however, the majority of the inherited deleterious effects are expressed in the F_1 generation.

2. HISTORICAL OVERVIEW

IS was first reported in the Soviet Union in the mid-1930s by Astaurov and Frolova (1935) while studying radiation-induced genetic anomalies in the silkworm *Bombyx mori* (L.). A few years later Ostriakova-Varshaver (1937) reported IS in the greater wax moth *Galleria melonella* (L.). In North America, Proverbs (1962) was the first to describe IS in the codling moth. Within the order Hemiptera, LaChance and Degrugillier (1969) reported IS while conducting genetic studies on the large milkweed bug *Oncopeltus fasciatus* (Dallas), and Delrio and Cavalloro (1975) and Maudlin (1976) documented IS in *Gonocerus acuteangulatus* (Goeze), a coreid pest

of hazelnuts, and in *Rhodnius prolixus* (Stål), a reduviid vector of Chagas disease, respectively. IS has also been reported in mites of the family Tetranychidae (Henneberry 1964). Although Curtis (1969) and Curtis et al. (1973) found low levels of sterility (5–15%) in the F_1 generation of irradiated tsetse flies (Diptera: Glossinidae), this level of sterility was less than that found in the irradiated parents, and other attributes common to IS in Lepidoptera were not demonstrated in these insects.

The genetic basis for IS has been reviewed and discussed by many authors (Bauer 1967, LaChance 1967, 1974, 1985, LaChance et al. 1970, North and Holt 1970, North 1975, LaChance and Graham 1984, Anisimov et al. 1989, Marec et al. 1999, Tothová and Marec 2001). In this chapter, emphasis will be given to the more recent research findings in the order Lepidoptera. Also the use of genetic sexing, together with IS for the suppression of lepidopteran populations, will be discussed briefly. The advantages of IS as compared with full sterility, the potential for IS to suppress pest populations, and the compatibility of IS with other pest control tactics, particularly with biological control, are also discussed. Table 1 provides a comprehensive list of arthropod species where IS has been documented, and includes key references that deal with radiation biology and field studies.

3. GENETICS AND INHERITED STERILITY

No comprehensive review of lepidopteran genetics has been published in the last 30 years. Robinson (1971) provided useful information on formal genetics (including karyology) for many species in this large order. Additional information can be extracted from published research on three economically important species: the silkworm (Tazima 1964, Goldsmith 1995, Fujii et al. 1998, Nagaraju 2000), the Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Caspari and Gottlieb 1975, Leibenguth 1986), and the pink bollworm *Pectinophora gossypiella* (Saunders) (LaChance and Ruud 1980, Bartlett 1989, Bartlett and Del Fosse 1991).

The lepidopteran genome exhibits a number of peculiarities that distinguishes it from the genomes of other insect orders, except perhaps from that of the closely related order Trichoptera. Chromosomes in Lepidoptera are usually small, numerous, and possess few differentiating features. Most species are reported to have haploid numbers close to 30 (n = 28–32). However, karyological studies have identified species with lower or higher chromosome numbers that are probably the result of chromosome fusion or fission (Suomalainen 1969a, Robinson 1971). Lepidopteran chromosomes are usually spherical and uniform in shape, and consequently not much is known about their morphology, kinetic organization and behaviour during mitotic and meiotic cell division. Lepidopteran chromosomes lack distinct primary constrictions (centromeres) and, as a result, sister chromatids separate by parallel disjunction during mitotic metaphase. Many researchers have concluded that these genomic peculiarities are an indication that lepidopteran chromosomes are holokinetic (Murakami and Imai 1974). However, recent work by Wolf (1996) suggests that lepidopteran chromosomes are intermediate between holokinetic and monocentric chromosomes.

Table 1. Arthropod species, including key references of radiation biology and field studies, in which inherited sterility (IS) has been documented

Family, species, and common name	Key references	
	Radiation biology	Field studies

Arachnida – Acari

Tetranychidae
Tetranychus urticae Koch   Henneberry 1964
  twospotted spider mite

Insecta – Hemiptera

Coreidae
Gonocerus acuteangulatus   Delrio and Cavalloro 1975
(Goeze)

Lygaeidae
Oncopeltus fasciatus (Dallas)   LaChance and Degrugillier 1969
  large milkweed bug   LaChance et al. 1970

Reduviidae
Rhodnius prolixus (Stål)   Maudlin 1976

Insecta – Lepidoptera

Bombycidae
Bombyx mori (L.)   Sugai and Mirumachi 1973
  silkworm   Murakami 1976

Gelechiidae

	Radiation biology	Field studies
Pectinophora gossypiella (Saunders) pink bollworm	Cheng and North 1972 Graham et al. 1972 LaChance et al. 1973, 1976 Henneberry and Clayton 1981 Miller et al. 1984 Qureshi et al. 1993a	Bariola et al. 1973 Flint et al. 1974 Qureshi et al. 1993b
Phthorimaea operculella (Zeller) potato tuberworm	Makee and Saour 1997	
Sitotroga cerealella (Olivier) Angoumois grain moth	Cogburn et al. 1966	

Lymantriidae

	Radiation biology	Field studies
Lymantria dispar (L.) gypsy moth	Mastro et al. 1989 Proshold et al. 1993 Proshold 1995	Maksimovic 1972 Mastro et al. 1989 Mastro 1993 Strom et al. 1996
Teia anartoides Walker painted apple moth	Suckling et al. 2002 Wee et al. 2005	Suckling et al. 2002

Table 1. Continued

Family, species, and common name	Key references	
	Radiation biology	Field studies
Noctuidae		
Agrotis ipsilon (Hufnagel) black cutworm	Elnagar et al. 1984	
Helicoverpa armigera (Hübner)	Saifutdinov 1989 Ocampo 2001	
Helicoverpa zea (Boddie) corn earworm bollworm tomato fruitworm	Carpenter et al. 1987c Carpenter and Gross 1989 Carpenter 1991, 1992 Carpenter and Wiseman 1992a Hamm and Carpenter 1997	North and Snow 1978 Carpenter et al. 1987a, 1987b, 1989 Carpenter and Gross 1993 Mannion et al. 1994, 1995
Heliothis virescens (F.) tobacco budworm	Proshold and Bartell 1970, 1972a, 1972b, 1973 Guerra and Garcia 1976	North and Snow 1978
Spodoptera exigua (Hübner) beet armyworm	Debolt 1973 Carpenter et al. 1996	
Spodoptera frugiperda (J. E. Smith) fall armyworm	Carpenter et al. 1983, 1986, 1997 Carpenter and Young 1991 Arthur et al. 1993 Hamm and Carpenter 1997	Carpenter et al. 1985 Carpenter and Wiseman 1992b
Spodoptera littoralis Boisduval	Wakid and Hayo 1974 Sallam and Ibrahim 1993	Sallam and Ibrahim 1993
Spodoptera litura (F.)	Seth and Sehgal 1993 Sutrisno Apu et al. 1993 Seth and Sharma 2001	
Trichoplusia ni (Hübner) cabbage looper	North and Holt 1968, 1969 Ercelik and Holt 1972 Karpenko and North 1973	Toba et al. 1972
Pieridae		
Pieris brassicae (L.)	Bauer 1967	
Plutellidae		
Plutella xylostella (L.) diamondback moth	Omar and Mansor 1993 Sutrisno Apu and Hoedaya 1993 Sutrisno Apu et al. 1993 Nguyen Thi and Nguyen Thanh 2001	Sutrisno Apu and Hoedaya 1993 Okine et al. 1998 Mitchell et al. 1999 Nguyen Thi and Nguyen Thanh 2001 Sutrisno Apu 2001

Table 1. Continued

Family, species, and common name	Key references	
	Radiation biology	Field studies
Pyralidae		
Amyelois transitella (Walker) navel orangeworm	Husseiny and Madsen 1964	
Cactoblastis cactorum (Berg) cactus moth	Carpenter et al. 2001b	Bloem et al. 2003a
Cadra cautella (Walker) almond moth	Ahmed et al. 1971 Gonnen and Calderón 1971 Brower 1980, 1982 Al-Taweel et al. 1990 Makee 1993	
Corcyra cephalonica (Stainton)	Chand and Sehgal 1982	
Crocidolomia binotalis Zeller	Sutrisno Apu and Hoedaya 1993	Sutrisno Apu and Hoedaya 1993 Sutrisno Apu 2001
Diatraea saccharalis (F.) sugarcane borer	Walker and Quintana 1968a, 1968b Walker et al. 1971 Sanford 1976, 1977 García and González 1993 González and García 1993	
Ephestia kuehniella Zeller Mediterranean flour moth	Riemann 1973 Marec et al. 1999 Tothová and Marec 2001	
Galleria mellonella (L.) greater wax moth	Nielsen 1971 Nielsen and Lambremont 1976 Nielsen and Brister 1980	
Ostrinia furnacalis (Guenée) Asian corn borer	Li et al. 1988 Zhang et al. 1993 Wang et al. 2001	Wang et al. 2001
Ostrinia nubilalis (Hübner) European corn borer	Shang and Lo 1980 Nabors and Pless 1981 Rosca and Barbulescu 1990 Barbulescu and Rosca 1993 Rosca and Barbulescu 1993	Barbulescu and Rosca 1993 Rosca and Barbulescu 1993
Plodia interpunctella (Hübner) Indian meal moth	Cogburn et al. 1966 Ashrafi et al. 1972 Ashrafi and Roppel 1973 Brower 1976, 1979, 1981	

Table 1. Continued

Family, species, and common name	Key references	
	Radiation biology	Field studies
Sphingidae		
Manduca sexta (L.) tobacco hornworm	Seth and Reynolds 1993	
Tortricidae		
Cryptophlebia leucotreta (Meyrick) false codling moth	Schwartz 1978 Bloem et al. 2003b	
Cydia pomonella (L.) codling moth	Proverbs 1962 Fossati et al. 1971 Charmillot et al. 1973 Pristavko et al. 1973 White 1975 Anisimov et al. 1989 Bloem et al. 1999a	Charmillot et al. 1973 Charmillot 1977 Proverbs et al. 1978 Bloem et al. 1999b, 2001
Grapholita molesta (Busck) oriental fruit moth	Genchev 2001	Genchev 2001

Two other groups of arthropods have shown IS, mites (Acari) and Hemiptera; both possess holokinetic chromosomes and are radioresistant (Brown and Nelson-Rees 1961, Hughes-Schrader and Schrader 1961, LaChance and Degrugillier 1969, Wrensch et al. 1994). Gassner and Klemetson (1974) showed that the kinetochore (centromere) in the large milkweed bug covers more than 70% of the chromosomal surface. Gonzales-Garcia et al. (1996) provided indirect evidence of the holokinetic nature of hemipteran chromosomes while working with *Graphosoma italicum* (Muller) (Pentatomidae). Nonetheless, Wolf (1996) suggests that further investigation is needed to verify the holokinetic nature of hemipteran chromosomes.

The sex chromosomes of Lepidoptera are of the WZ type, in which females are heterogametic (WZ) and males homogametic (ZZ). Lepidopteran species, where sex chromosomes have been identified, show a typical ♀WZ/♂ZZ system, or variants such as Z/ZZ, W_1W_2Z/ZZ or $WZ_1Z_2/Z_1Z_1Z_2Z_2$ (Suomalainen 1969b, Robinson 1971, Nilsson et al. 1988, Traut and Marec 1997, Rishi et al. 1999). Male chromosomes display a normal sequence of meiotic events. In contrast, female chromosomes undergo normal meiosis until they approach the pachytene stage (Fig. 1), when they pair by means of synaptonemal complexes to form bivalents, and synaptonemal complexes become visible (Marec 1996). From this point onwards, female meiosis proceeds without meiotic recombination, and is achiasmate (Traut 1977, Nokkala 1987). The synaptonemal complexes in the female transform into elimination chromatin that later detaches from the bivalents and persists during chromosome segregation in the metaphase plate (Rasmussen 1977). The female W and Z chromosomes, although often non-homologous and of different size, pair completely

during meiosis and form a regular bivalent.

Another peculiarity of the lepidopteran genome is the presence of one or more heterochromatic bodies in female somatic cells during interphase. This female specific heterochromatin (also known as W- or sex-chromatin) is derived from the W chromosome. Since sex chromatin is easily identified in interphase nuclei and is especially visible in highly polyploid somatic cells, it can be used as a marker to determine the sex of embryos and larvae and also to identify sex chromosome aberrations in mutagenesis screens (Traut and Marec 1996).
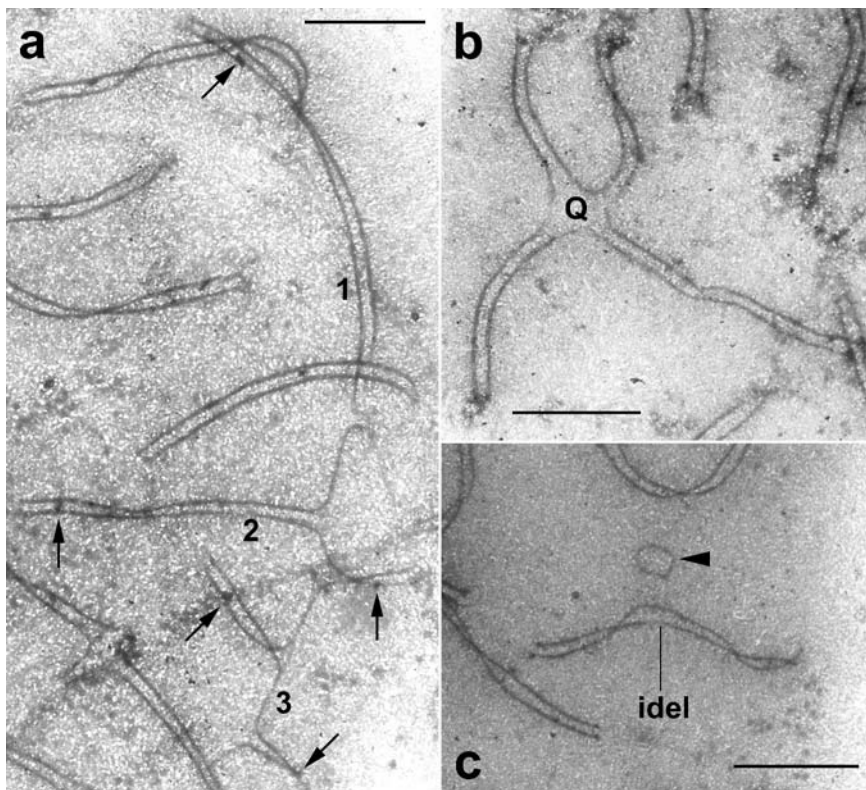
Figure 1. Examples of pachytene configurations in aberrant chromosomes of Ephestia kuehniella *in microspread spermatocytes of F$_1$ males after the parental male was irradiated with 150 Gy.* ***a****: Multiple chain translocation that involves 7 lateral elements, each representing the protein axis of one chromosome, where 1–3 represent structurally normal chromosomes inherited from the untreated female parent; arrows indicate recombination nodules;* ***b****: Quadrivalent (Q) typical in reciprocal translocations;* ***c****: Ring fragment (arrow) plus a bivalent with one shorter lateral element (idel) that indicates interstitial deletion. All are electron microscope (EM) micrographs stained with phosphotungstic acid. Scale = 2 µm.*

Lepidopteran males undergo two distinct modes of spermatogenesis/meiosis that result in the production of two different types of sperm (Wolf 1994, Friedländer 1997): the larger, nucleate and fertile eupyrene sperm, and the smaller, anucleate and non-fertile apyrene sperm. Apyrene sperm are more abundant and contain less mitochondrial material (Kawamura et al. 1998), while eupyrene sperm are less abundant and typically comprise 10–15% of the total sperm transferred to a female during mating (Gage and Cook 1994, Cook and Wedell 1996). The role of apyrene sperm is not fully understood, although it has been suggested that they aid the transfer of eupyrene sperm to the female (Cook and Wedell 1996), have a nutritive function (Friedländer 1997), or may be involved in sperm competition. The latter function was recently studied in *Pieris napi* (L.), where the presence of apyrene sperm was shown to delay female remating (Cook and Wedell 1999).

3.1. Radioresistance in Lepidoptera

A high resistance to the effects of ionizing radiation is a characteristic feature of moths and butterflies (LaChance 1985). Cultured lepidopteran cells are 50–100 times more resistant to radiation-induced death than similarly cultured mammalian cells. In contrast dipteran cells are only three to nine times more resistant than mammalian cells (Koval 1996, Chandna et al. 2004). This high radioresistance in Lepidoptera also applies to germ cells, and in particular to mature sperm. As a consequence very high doses of radiation are required to fully sterilize lepidopteran males (LaChance and Graham 1984).

LaChance and Graham (1984) and Koval (1996) suggested that possible molecular mechanisms responsible for the high radioresistance in Lepidoptera might include an inducible cell recovery system and a DNA repair process. Even though lepidopteran chromosomes are not truly holokinetic, a significant role in their radioresistance can be attributed to their holokinetic "nature" (as first suggested by LaChance et al. 1967) and to the fate of the radiation-induced chromosome fragments during mitotic cell cycles as explained below (Tothová and Marec 2001). Lepidopteran chromosomes possess a localized kinetochore plate to which the spindle microtubules attach during cell division (Gassner and Klemetson 1974, Traut 1986, Wolf and Traut 1991, Wolf et al. 1997). The kinetochore plates are large and cover a significant portion of the chromosome length (Wolf 1996), ensuring that most radiation-induced breaks will not lead to the loss of chromosome fragments as is typical in species with monocentric chromosomes. In species with large kinetochore plates, the fragments may persist for a number of mitotic cell divisions, and can even be transmitted through germ cells to the next generation (Marec and Traut 1993a, Marec et al. 2001). The plates also reduce the risk of lethality caused by the formation of dicentric chromosomes, acentric fragments, and other unstable aberrations (Tothová and Marec 2001) (Fig. 2).

3.2. Radioresistance in Hemiptera

A difference in radiosensitivity between males and females has also been documented in several hemipterans including species of economically important

leafhoppers (Shipp et al. 1966, Ameresekere and Georghiou 1971) and mealybugs (Brown and Nelson-Rees 1961). However, LaChance and Degrugillier (1969) were the first to document IS in the order Hemiptera when they induced chromosomal fragments and translocations in the large milkweed bug. These authors demonstrated that the induced fragments were both mitotically and meiotically stable and could be transmitted through three generations of outcrosses to normal females.
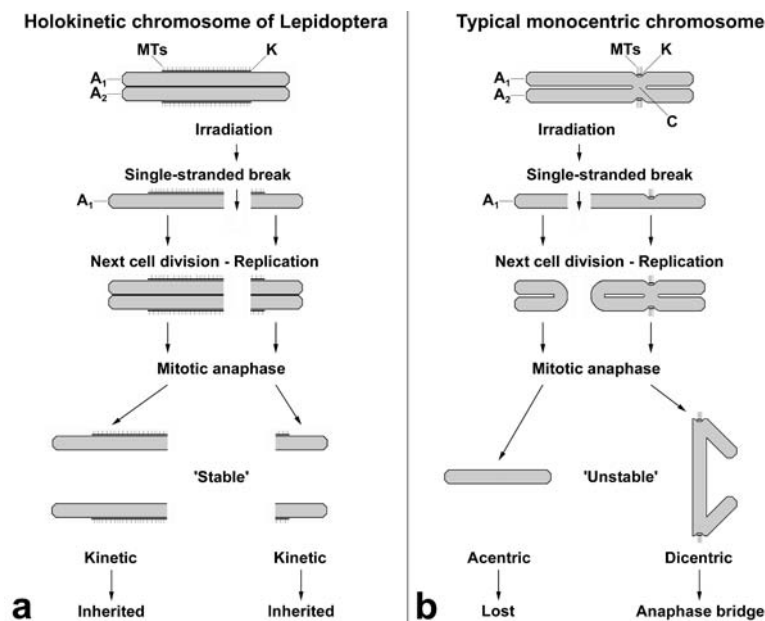
*Figure 2. Kinetic structure of lepidopteran chromosomes during mitotic metaphase, and consequences of chromosome breakage; **a**: Holocentric chromosome with two sister chromatids (A_1 and A_2), each with a kinetochore plate (K) covering about 50% of the chromosome surface; spindle microtubules (MTs) are attached to the kinetochore; **b**: Typical monocentric chromosome, where sister chromatids are joined by the centromere (C); the kinetochore is localized on the surface of the centromere.*

3.3. Radiation-Induced Sterility in Parental (P_1) Generation

Radiation-induced sterility is generally a consequence of dominant lethal mutations (DLMs) that result in the death of the zygote or the embryo. The chromosomal damage responsible for DLMs is characterized by the formation of anaphase chromosome bridges, chromosome fragments and other abnormalities in the dividing nuclei. In most insects DLMs are expressed during early embryogenesis (LaChance 1967). In Lepidoptera, however, the frequency of DLMs is much lower than in other insects and the majority are expressed very late in embryonic

development (LaChance 1974, Berg and LaChance 1976). Furthermore, no chromosomal bridges, indicating the presence of dicentric chromosomes, are evident during embryonic development in Lepidoptera (LaChance and Graham 1984) and Hemiptera studied thus far (LaChance et al. 1970, Maudlin 1976).

For males of four insect species, LaChance and Graham (1984) constructed dose-response curves for the induction of DLMs in mature sperm. The species exhibited a wide range of radiosensitivity, with Hemiptera showing intermediate and Lepidoptera high radioresistance. When analysed mathematically, the dose-response curves approximated an S-shape (LaChance and Graham 1984, Marec et al. 1999). For highly radioresistant Lepidoptera, the curves approximated those expected for 8–16-hit kinetics while in Hemiptera the curve exhibited 4-hit kinetics. These data suggest that multiple chromosome rearrangements must be induced in lepidopteran males to be manifested as DLMs, explaining why lepidopteran males require very high radiation doses (350–500 Gy) to be fully sterilized.

Radiation-induced sterility in lepidopteran males may also have other causes. Anisimov et al. (1989) observed a dose-dependent increase in the number of matings that produced no eggs, or the number of females that laid only unembryonated eggs, following mating with irradiated male codling moths. A significant proportion of unembryonated eggs (that might represent unfertilized eggs and/or eggs with early embryonic mortality) were also observed after treated males were mated to females of *Manduca sexta* (L.) (Seth and Reynolds 1993), *E. kuehniella* (Marec et al. 1999), and *Spodoptera litura* (F.) (Seth and Sharma 2001). Furthermore, Koudelová and Cook (2001) demonstrated with *E. kuehniella* that the volume of sperm transferred during copula decreased, and mating times increased, as the dose increased. Taken together, the above data suggest that an important component of male sterility can be due to physiological disruptions during copulation, including the inability to copulate and abnormal sperm transfer.

Lepidopteran females are considerably more radiosensitive than males. In a number of species, a dose of 100 Gy is sufficient to achieve almost full sterility in treated females (Anisimov et al. 1989, Marec and Mirchi 1990, Bloem et al. 1999a) (Fig. 3). It appears that this difference in radiosensitivity between males and females is related to the stage of development of the reproductive cells at the time of irradiation. Lepidoptera are usually irradiated as mature pupae or newly emerged adults. At this stage of development eupyrene spermiogenesis in the male has been completed, and the nuclei in eupyrene sperm are in interphase. In contrast, female meiosis is arrested at metaphase I in the nuclei of mature oocytes and the process does not resume until the eggs have been laid (Traut 1977). As a consequence irradiation of newly emerged females or of mature female pupae may disrupt the normal course of meiosis including chromosome segregation. Finally, various secondary detrimental effects can be expected in oocytes, which have a large amount of cytoplasm (that contains many components required for embryonic development) in comparison with the essentially cytoplasm-free sperm. Almost nothing is known about the radiosensitivity of female Hemiptera, although Delrio and Cavalloro (1975) showed that *G. acuteangulatus* females were fully sterilized at a dose of 50–60 Gy. At this dose males were about 70% sterile.
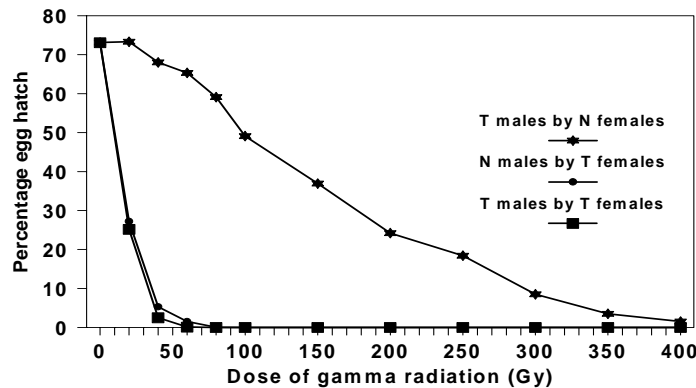
*Figure 3. Percentage egg hatch obtained when codling moth adults were treated with
increasing doses of gamma radiation, and either inbred or outcrossed with fertile moths.
T = Treated; N = Non-treated. (Figure from Bloem et al. 1999a.)*

3.4. Radiation-Induced Sterility in F_1 Generation

Recent work by Tothová and Marec (2001) showed that various types of
translocations (non-reciprocal, reciprocal and multiple) are responsible for the
production of genetically unbalanced gametes in F_1 progeny and, as such, represent
the main chromosomal mechanism of IS. In addition, they demonstrated that two
types of intra-chromosomal aberration, fragmentation, and interstitial deletion
increase the frequency of unbalanced gametes; however, their contribution to overall
sterility appears to be less significant. The study also revealed that the predicted
level of F_1 sterility, based on the observed frequency of aberrations, was much
higher than the sterility observed in a previous study (Marec et al. 1999). This
suggests that Lepidoptera possess a mechanism that corrects the predicted
unbalanced state towards a balanced segregation of chromosomes. The authors
suggested that the increased number of chiasmata might facilitate a balanced
disjunction of chromosomes from translocation multivalents in F_1 males. The
modified synaptonemal complex, which ensures regular disjunction of homologous
chromosomes during female meiosis in Lepidoptera (Rasmussen 1977, Marec
1996), might play a similar role in F_1 females (Tothová and Marec 2001).

Several authors have documented the effects of radiation on the incidence of
visible chromosomal aberrations in F_1 males using light microscopy (North and
Snow 1978, Saifutdinov 1989, Al-Taweel et al. 1990, Carpenter 1991, Carpenter et
al. 1997). More recently Tothová and Marec (2001) used a modified micro-
spreading technique, first employed by Weith and Traut (1980), to study radiation-
induced chromosome aberrations in F_1 individuals of *E. kuehniella*. The micro-
spread chromosomes were viewed with an electron microscope and several types of
aberrations were documented. In F_1 individuals from male parents treated with 100
and 150 Gy, the overall frequency of aberrations varied between 4.2 and 4.8 per F_1

larva in both sexes. A significant increase in aberrations was found in F_1 males from male parents treated with 200 Gy (6.2 per male). Fragmentation and several types of translocations (non-reciprocal, reciprocal, and multiple) were the most common aberrations, while interstitial deletions and inversions were rare. Multiple translocations forming complicated configurations were found with increasing radiation dose. In males the mean number of chromosomal breaks resulting in aberrations increased linearly with dose, from 8.4 to 16.2 per nucleus. In females this value reached a maximum of 11.2 breaks per nucleus when male parents were treated with a dose of 200 Gy (Fig. 1).

3.5. Sex-Specific Differences in Inherited Sterility

Two consequences of radiation-induced IS are sex-specific and positively correlated with treatment dose. First, the sex ratio of the F_1 generation is skewed toward males (Lepidoptera — Proverbs 1962; Hemiptera — LaChance et al. 1970) and, second, the level of IS in F_1 female progeny is lower than in F_1 males (Lepidoptera — Anisimov et al. 1989, Al-Taweel et al. 1990, Seth and Reynolds 1993, Bloem et al. 1999a).

Marec et al. (1999) suggested that the sex ratio distortion in the F_1 generation in Lepidoptera occurs as a result of recessive lethal mutations induced in the Z sex chromosomes of treated male parents. Since lepidopteran females are heterogametic, all female F_1 progeny will be hemizygous for Z and, as a consequence, any deleterious Z-linked mutations will result in F_1 female mortality. In contrast, the F_1 male progeny will inherit one Z chromosome from the treated father and the other from the mother and, as such, will be heterozygous for any Z-linked mutation.

Induction of F_1 sterility by the transmission of complex chromosome translocations to the progeny of treated males was first suggested by North (1967) and North and Holt (1968) for Lepidoptera and by LaChance and Degrugillier (1969) for Hemiptera. More recently Tothová and Marec (2001) suggested three factors that might explain the higher level of sterility found in F_1 male progeny of Lepidoptera:

- The ability of F_1 males to survive even though they inherit large numbers of chromosome breaks. The authors found that in F_1 males of *E. kuehniella*, the mean frequency of chromosome breaks was positively correlated with a dose-dependent increase in sterility, whereas a clear correlation was lacking for F_1 females. They suggested that this difference was due to higher mortality in F_1 females that inherit a high number of breaks. Furthermore, at higher treatment doses, there is increased probability that the sex chromosome (Z) will be damaged and, as a consequence, any resulting recessive lethal mutation would kill the F_1 females but not the males. Those F_1 females that survive carry a smaller number of chromosome breaks and, therefore, are more fertile than the F_1 males.
- The occurrence of crossing-over during spermatogenesis. In F_1 males crossing-over during spermatogenesis might increase the number of unbalanced gametes produced, but only if it occurs at the crossover point between an aberrant chromosome (that arose by two or more breaks) and its structurally normal

homologue. This situation might occur when inversions and multiple translocations are formed. However Tothová and Marec (2001) rarely detected inversions in their study on *E. kuehniella*. They concluded that crossing-over contributes to the sterility in F_1 males mostly through the formation of multiple translocations. Since female meiosis is achiasmate during oogenesis, this factor cannot play a role in the sterility level of F_1 females (Rasmussen 1977, Traut 1977, Nokkala 1987, Marec and Traut 1993b).

- A higher impact of radiation-induced deleterious effects on the fertility of F_1 males. Some studies have reported finding a higher number of F_1 male crosses that are fully sterile or that have resulted in the female laying a large number of unembryonated eggs, whereas most F_1 female crosses laid embryonated eggs (Anisimov et al. 1989, Marec et al. 1999). The data suggest that induced genetic changes impaired the fertilizing ability of some F_1 males while the F_1 females were not similarly affected. Recently Koudelová and Cook (2001) reported great variability in the number of sperm that were transferred by F_1 males of *E. kuehniella*. They found that, on average, the number of eupyrene sperm decreased, whereas the number of apyrene sperm increased, resulting in an abnormally high ratio of apyrene to eupyrene sperm. The ratio fluctuated between 9.5:1 for untreated males and as high as 100:1 for treated males. These results suggest that chromosomal rearrangements in F_1 males may have altered the mechanisms regulating dichotomous spermiogenesis or those underlying copulation and sperm transfer.

3.6. Genetic Sexing in Lepidoptera

Genetic sexing has been documented in only two lepidopteran species. Strunnikov (1975) reported that genetic sexing was possible in the silkworm, *B. mori*. Recently Marec (1990), Marec and Mirchi (1990), and Marec (1991) were successful in constructing in *E. kuehniella* a balanced lethal genetic sexing strain, according to the scheme of Strunnikov (1975). This strain, called BL-2, results in males that are trans-heterozygous for two sex-linked recessive lethal mutations, *sl-2* and *sl-15*. When BL-2 males are mated to wild-type females, the F_1 generation consists almost exclusively of male progeny. The F_1 females die during embryogenesis because they inherit one of the lethal mutations from their father, i.e., females are hemizygous for *sl-2* or *sl-15* (Fig. 4).

BL-2 males of *E. kuehniella* could be released directly into nature to introduce lethal mutations into the wild population (Marec et al. 1996), or they could be maintained in the laboratory and used to generate a male-only mutant strain that could be irradiated and released into the environment (Marec et al. 1999). Marec et al. (1999) suggested that the production of male-only colonies through the use of balanced lethal strains would reduce rearing costs and enhance population suppression when used alone or in combination with F_1 sterility. Furthermore, outcrosses of balanced lethal males with wild-type females one generation before irradiation and release would improve the competitiveness of males through positive heterosis. Finally, in addition to the genetic changes induced by treating the males with gamma radiation, the released mutant males would introduce sex-linked
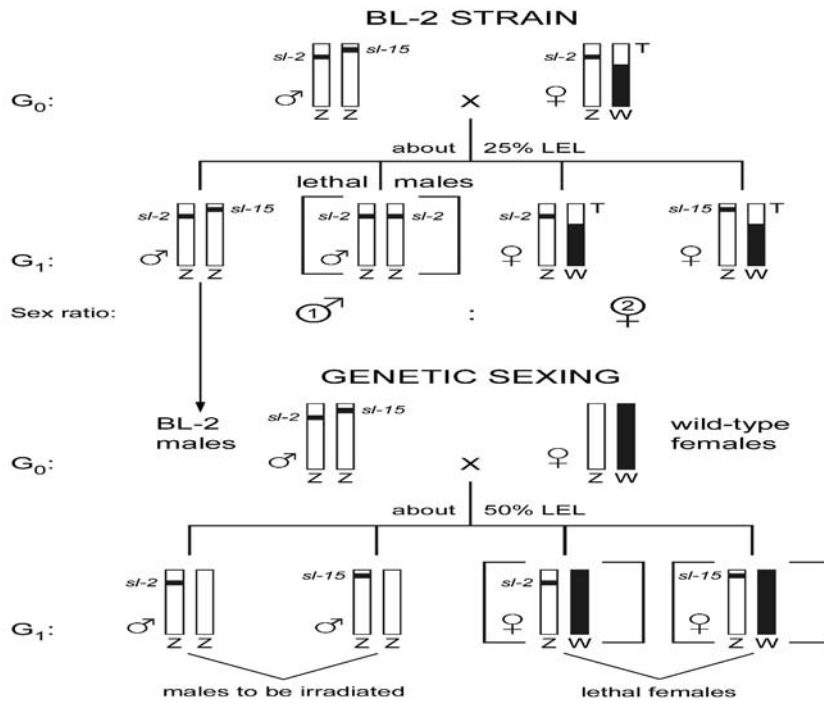
Figure 4. Genetic sexing system developed in the Mediterranean flour moth; the cross
between BL-2 males and wild-type females produces only male progeny. Z and W
represent the sex chromosomes; sl-2 and sl-15 are sex-linked recessive mutations. LEL =
late embryonic lethality. (Details in text.)

recessive lethal mutations into the wild population that would further reduce the
number of F_1 females produced in the field. However, this system relies on sexing
two different strains and making directed crosses, and would be impractical in an
operational programme.

Some significant obstacles still need to be overcome before these types of
genetic sexing strain can be used against pest Lepidoptera. For example suitable
markers are currently lacking for the construction of similar mutant strains in other
economically important species. Also the genetic sexing system requires the mass-
rearing of two different colonies, a wild-type strain and a balanced lethal strain, for
which sex separation somehow has to be carried out under mass-rearing conditions.
Finally, the mutant strain must be routinely checked to prevent the loss of its genetic
structure through genetic recombination or colony contamination (Marec 1991).

Discovery of the sex-determining mechanism in Lepidoptera might facilitate the
development of a more convenient and sophisticated sexing system. Although little
is known about sex determination in Lepidoptera, and the exact locations of the
primary sex-determining factors are unknown (Traut and Marec 1996), some
progress is being made that will undoubtedly facilitate future research on

lepidopteran genetics. Ohbayashi et al. (2001) recently found a homologue of the *Drosophila doublesex* (*dsx*) gene in *B. mori* called *Bmdsx*. A sex-specific alternative splicing of the primary *Bmdsx* transcript suggests that the gene might be involved in the control of sexual differentiation, as does the *Drosophila dsx* gene. Also, germ-line transformations, with a *piggyBac* transposable element, have been demonstrated — in the pink bollworm by Peloquin et al. (2000), and in the silkworm by Tamura et al. (2000). It has been proposed that genetic sexing strains based on a transgenic approach may be a possible solution to this problem (Marec et al. 2005), where females would be transgenic, but not the sterile males for release.

4. ADVANTAGES OF INHERITED STERILITY VERSUS FULL STERILITY

The unique characteristics of IS in Lepidoptera and other arthropods provide some inherent advantages over the use of full sterility in pest control programmes (North 1975). Since lepidopteran females generally are much more radiosensitive than males of the same species, the radiation dose may be adjusted to suit programme requirements, i.e. treated females are completely sterile and males partially sterile. When these partially sterile males mate with fertile females the radiation-induced deleterious effects are inherited by the F_1 generation. As a result egg hatch is reduced and the resulting (F_1) offspring are both highly sterile and predominately male (Table 2). The lower dose of radiation used to induce F_1 sterility increases the quality and competitiveness of the released insects (North 1975) as measured by improved dispersal after release (Bloem et al. 2001), increased mating ability (Carpenter et al. 1987a), and superior sperm competitiveness (Carpenter et al. 1987a, 1997). In addition, because F_1 sterile progeny are produced in the field, the release of partially sterile males and fully sterile females is more compatible with other pest control mechanisms or strategies (Carpenter 1993).

Table 2. Typical attributes of male lepidopteran insects (and their progeny) receiving substerilizing doses of radiation

Dose applied to P_1 (Gy)	1 Egg hatch (%)	2 Larval mortality (%)	3 Sex ratio ♂:♀	4 Egg hatch (%)	
				F_1♂	F_1♀
0	71.8	20.0	1.0:1	82.5	76.8
100	46.1	51.1	2.6:1	10.8	13.9
200	30.8	69.5	5.1:1	0.9	7.5
250	19.1	75.1	7.0:1	0.8	6.1

1. Reduced F_1 egg hatch resulting from P_1 (parental) generation
2. Increased mortality during F_1 development
3. Skewed sex ratio in favor of males in the F_1 generation
4. Reduced F_2 egg hatch resulting from F_1 generation; sterility in F_2 generation higher than in F_1 generation

Data for codling moth (Bloem et al. 1999a)

Knipling (1970) used a mathematical model to explore the application of IS for control of lepidopteran pests (Barclay, this volume). He found that the release of partially sterile insects offered greater suppressive potential than the release of fully sterile insects, and suggested that the partially sterile-to-wild overflooding ratio could be as low as a one-quarter of what is normally required for fully sterile insects. Population models using data collected from several pest species (Walker and Pederson 1969, Brower and Tilton 1975, Carpenter et al. 1987a, Carpenter and Layton 1993, Anisimov 1993) corroborate Knipling's findings.

5. POTENTIAL FOR INHERITED STERILITY TO SUPPRESS PEST POPULATIONS

Field releases of partially sterile insects have demonstrated the usefulness of IS to control many lepidopteran pests, including the cabbage looper *Trichoplusia ni* (Hűbner) (North and Holt 1969), corn earworm *Helicoverpa zea* (Boddie) (Carpenter and Gross 1993), gypsy moth *Lymantria dispar* (L.) (Mastro 1993), codling moth (Proverbs et al. 1978, Bloem et al. 1999b, Bloem et al. 2001), and many others (Bloem and Carpenter 2001; Bloem et al., this volume). The effect of F_1 sterility to influence pest populations has been most convincing when irradiated insects have been released in the field throughout the entire growing season. Season-long releases of irradiated (100 Gy) *H. zea* in mountain valleys of North Carolina, USA, delayed and/or reduced seasonal increases of wild *H. zea* males (Carpenter and Gross 1993). The incidence of *H. zea* larvae with chromosomal aberrations indicated that irradiated males were very competitive in mating with wild females, and were successful in producing F_1 progeny, which further reduced the wild population. Release ratios averaged less than 5:1 overall, but reduced the wild population of *H. zea* by more than 70%. In another case, season-long field studies of the codling moth were conducted in apple orchards in Washington State, USA, that compared: (1) twice-weekly releases of partially sterile codling moths treated with either 100 or 250 Gy, and (2) combinations of mating disruption plus the release of partially sterile (100 Gy) codling moths, to control wild populations (Bloem et al. 2001). The results showed that fruit damage was significantly lower in all treatment plots when compared with control plots located outside the treatment areas.

6. COMPATIBILITY OF INHERITED STERILITY WITH OTHER PEST CONTROL TACTICS

The success of releasing insects irradiated with substerilizing doses of radiation for the suppression of pest populations is influenced by the ability of released insects and their progeny to survive and interact with insects of a wild population. Field survival rates for F_1 larvae from irradiated parents should be comparable with field survival of wild larvae because many of the deleterious effects induced by radiation are manifested and therefore eliminated during the F_1 egg stage (Carpenter et al. 1985). Any mortality agents such as insecticides, entomopathogens, and natural enemies (parasitoids and predators) could potentially interfere with the effectiveness of F_1 sterility if the agent killed a higher proportion of treated than wild larvae.

Likewise host-plant resistance could potentially interfere with the effectiveness of F_1 sterility if the host-plant defenses somehow prevented a higher proportion of treated than wild larvae from establishing and developing on the host plant (Carpenter 1993).

The compatibility of different pest control tactics with F_1 sterility has been investigated in both laboratory and field studies. Examples include the use of nuclear polyhedrosis viruses with F_1 sterility for controlling *H. zea* and *Spodoptera frugiperda* (J. E. Smith) (Hamm and Carpenter 1997), host-plant resistance and F_1 sterility in *H. zea* and *S. frugiperda* (Carpenter and Wiseman 1992a, 1992b), F_1 sterility and insecticide resistance in *S. frugiperda* (Carpenter and Young 1991), F_1 sterility and synthetic pheromones to reduce wild populations of the codling moth (Bloem et al. 2001), and the use of parasitoids and F_1 sterility (Mannion et al. 1994, 1995; Carpenter et al. 1996; Bloem and Carpenter 2001; Mangan, this volume). All studies have shown that F_1 sterility is compatible with other pest control tactics.

7. POPULATION MODELS COMBINING THE EFFECTS OF F_1 STERILITY WITH OTHER CONTROL TACTICS

Knipling (1964), Barclay (1987), and Wong et al. (1992) recognized the potential benefit of combining sterile insects with conventional pest control methods. According to population models (Barclay 1987, Knipling 1992), combining inundative releases of natural enemies and sterile insects should yield additive or synergistic effects. Although natural enemies and the SIT have different modes of action, the effectiveness of the SIT increases the ratio of natural enemies to adult hosts, and the effectiveness of natural enemies increases the ratio of sterile to fertile insects. Therefore greater suppression could be expected if parasitoid releases were combined with the F_1 sterility technique (Carpenter 1993). Not only is F_1 sterility theoretically more effective than full sterility in reducing population increase (Carpenter et al. 1987a), but F_1 sterility results in the production of sterile F_1 larvae that provide an increased number of hosts for the parasitoids. As a result, the number of parasitoids produced should increase even if the rate of parasitism remained the same (host-density independent), and whether or not additional parasitoids are released. Although population models, that independently consider augmentative releases of parasitoids (Knipling 1992) and F_1 sterility (Carpenter et al. 1987a), suggest that both tactics are highly efficacious, integrating lepidopteran F_1 sterility and augmentative biological control results in synergistic effects (Carpenter 1993). Therefore the greatest impact of F_1 sterility and augmentative parasitoid releases as area-wide tactics against lepidopteran pests can be realized only when the two methods are integrated (Barclay, this volume).

Population models also provide insight into how different control strategies could be combined for greatest efficiency. Although the effectiveness of F_1 sterility continues to increase as the ratio of irradiated to non-irradiated insects increases, the efficiency per released moth declines. A similar loss of efficiency occurs in parasitoid releases (Carpenter 1993). According to these models the economic benefit of combining F_1 sterility and parasitoid augmentation would be greatest when the ratios of irradiated to non-irradiated, and parasitoid to host, are low (i.e.

equal to or less than 10:1). The model presented in Fig. 5 demonstrates that population suppression is increased when F_1 sterility and parasitoid releases are combined, and that the percentage reduction in population growth is greater when parasitized hosts produce adult parasitoids than when no parasitoids are produced (Carpenter 2000).
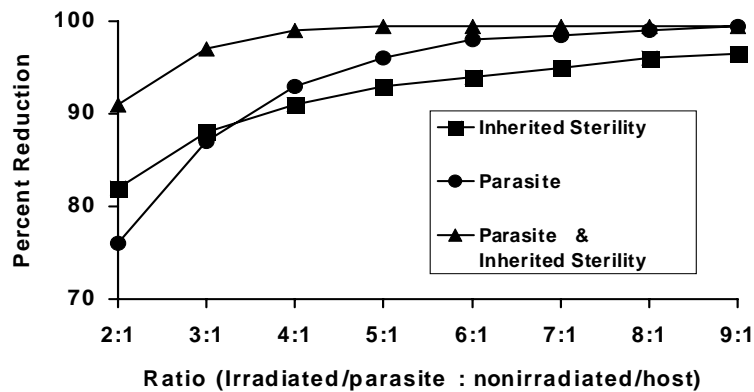
Figure 5. Comparison of the projected reduction in normal population growth when only parasitoids [parasites] are released (Knipling 1992), when only irradiated (100 Gy) male moths are released (Carpenter et al. 1987c), and when releases are equally divided between parasitoids and irradiated male moths.

8. INHERITED STERILITY IN COMBINATION WITH BIOLOGICAL CONTROL

Fully successful integration of F_1 sterility and parasitoid augmentation into a management approach can occur only if parasitoids do not negatively impact irradiated insects and their progeny more than that of the wild population, and if F_1 sterility does not negatively impact the efficacy and reproduction of parasitoids. Knowledge of any negative impact of F_1 sterility on parasitoids would be important before implementing an AW-IPM programme using F_1 sterility. For example, if parasitoids that attack the F_1 sterile progeny are unable to develop normally, and most of the hosts present are F_1 sterile progeny, then there could be a negative impact on subsequent parasitoid populations. Conversely, if parasitoids develop normally on F_1 eggs, larvae, and pupae, then the greater number of hosts available would allow for an increase in the parasitoid population. Since many hosts of the F_1 generation would experience genetically-induced mortality before they reached the adult stage, any parasitoids able to develop on these hosts would result in a positive and synergistic increase in the efficacy of an AW-IPM programme (Carpenter 2000).

Field, greenhouse and laboratory studies compared the acceptability and suitability of progeny from irradiated (100 Gy) and untreated *Spodoptera exigua*

(Hűbner) males as hosts for the braconid larval parasitoid *Cotesia marginiventris* (Cresson) (Carpenter et al. 1996), and progeny from irradiated (100 Gy) and untreated *H. zea* males as hosts for the tachinid *Archytas marmoratus* (Townsend) (Mannion et al. 1994, 1995). Results from these studies demonstrated that progeny of irradiated males and untreated females were acceptable and suitable hosts for parasitoid development. Female parasitoids showed no oviposition preference for progeny from females paired with either irradiated or untreated males. Other studies on different lepidopteran pests also have reported compatibility between the two control tactics (Bloem and Carpenter 2001).

There are many different scenarios in which F_1 sterility could be integrated with natural enemies to suppress pest populations (Carpenter 1993) (Box 1). The release of partially sterile males and females would produce large numbers of F_1 eggs and larvae that could be field-reared on early-season host plants or crop plants that tolerate some larval feeding damage, e.g. whorl-stage corn. Natural enemies (native and/or released) could use the F_1 eggs, larvae and pupae as hosts and thereby substantially increase the natural enemy population for the next generation of the pest insect (Proshold et al. 1998). Also surviving sterile F_1 progeny would produce sterile adults that would negatively impact the next generation of the pest insect. If the economic injury level of cultivated host plants indicated that the additional sterile F_1 larvae were undesirable, then the dose of radiation could be increased to a level that would reduce or eliminate the number of progeny from irradiated females, or releases could be limited to irradiated males.

Box 1. Opportunities for Combining Inherited Sterility with Biological Control

For pest suppression:
- Simultaneous or sequential inundative releases of irradiated insects and natural enemies
- Irradiated insects and their sterile progeny serve as host/prey for wild natural enemies

For research:
- Elucidation of the potential host range of exotic pests
- Prediction of the potential geographic range of exotic pests
- Delineation of the potential impact of native natural enemies on invading pests

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Although the compatibility of F_1 sterility with the application of synthetic organic insecticides has been demonstrated (Carpenter and Young 1991), parasitoids and/or predators generally are not compatible with these products. When insecticides are required to reduce pest infestations, insect growth regulators or other formulations that are compatible with natural enemies should be considered. Another management option would be to establish host plants for the pest in insecticide-free areas adjacent to insecticide-treated crops. Host plants could be artificially infested with pest larvae to provide natural enemies (native and/or released) with an adequate supply of hosts. If the pest larvae used in the artificial infestations (nursery crops) were sterile, i.e. the progeny of irradiated parents, then non-parasitized larvae would not contribute to the increase of the wild population but would produce sterile adults that would negatively impact the next generation of the pest insect (Okine et al. 1998, Carpenter 2000).

In addition to using F_1 sterility as a direct pest control tactic, there are opportunities to use F_1 sterility to facilitate the development of other pest suppression tactics. For example the F_1 sterile progeny (eggs, larvae and pupae) of a pest may be used as hosts/prey for natural enemies that are shipped commercially, especially in quarantine-sensitive shipments. The use of sterile insects in commercial shipments would ameliorate concerns regarding the reproductive viability of non-parasitized and non-consumed pests upon arrival at the shipment destination. Also the use of F_1 sterile progeny as hosts for parasitoids would eliminate the need to wait for non-parasitized pests (either eggs or pupae) to emerge before shipment of the parasitized pest (Greany and Carpenter 2000).

9. ADDITIONAL APPLICATIONS OF F_1 STERILITY FOR RESEARCH AND MANAGEMENT

Greany and Carpenter (2000) reported that F_1 sterility could provide a new risk management tool for assessing the safety of exotic lepidopterans being considered as biological control agents against invasive weeds (Box 1). Production of F_1 sterile progeny would allow for developmental and behavioral observations to be made under actual field conditions without concern that a breeding population would be established (Carpenter et al. 2001a). This would facilitate field observations on oviposition behaviors and host associations, larval feeding preferences, and larval development and survival on both target and non-target plant species. Also the impact that native natural enemies might have on exotic candidate species being considered as biological control agents for invasive weeds, and the ability of these candidate species to survive and overwinter under various climatic conditions could be studied in the field through this innovative application of F_1 sterility.

F_1 sterility could be used to conduct research on exotic pests that are expanding their geographical range. Strom et al. (1996) suggested that gypsy moth host preferences, or the quality of potential hosts outside the generally infested area, could be investigated using releases of F_1 sterile larvae of *L. dispar*. In addition to host range studies, Carpenter et al. (2001a) suggested that F_1 sterility could be used to predict the potential geographic range and to evaluate the potential impact of native natural enemies on the rate of spread of exotic lepidopteran pests.

10. CHALLENGES AND OPPORTUNITIES

Challenges are inherent in all pest management tactics, and Whitten and Mahon (this volume) discuss difficulties unique to the SIT. In addition to difficulties in common with the SIT, the major challenge to the use of IS is the perception that sterile F_1 larvae cause economic damage to crops, especially high-value crops such as fruit. Consequently, low doses of radiation, which would certainly result in more competitive insects, are often avoided. For example, Proverbs et al. (1978) found that codling moths treated with 250 Gy were more competitive in the field, and provided better control, than did fully sterile moths (400 Gy). Nevertheless, in spite of these findings, Proverbs et al. (1982) continued to be concerned that F_1 larvae would cause economic damage, and used 350 Gy to irradiate moths released in a

pilot study conducted in British Columbia, Canada, from 1976–1978. Also, in the current codling moth programme in British Columbia, Canada, which began field operations in 1994, moths treated with 350 Gy are released, even though doses as low as 100 Gy have been suggested (Anisimov 1993; Bloem et al. 1999a, 1999b). It was found in field studies that season-long releases of moths treated with 100 Gy did not cause fruit injury (Fig. 6), and moths receiving 100 Gy were more competitive than those receiving 250 Gy (Bloem et al. 2001). These studies indicate that fears of increased fruit (or plant) injury resulting from F_1 larvae are ill founded, especially when the radiation dose causes partial sterility in males and full sterility in females. Therefore the production of sterile F_1 larvae should be viewed not as a problem but rather an opportunity to enhance the production of natural enemies and to produce sterile moths in the field.
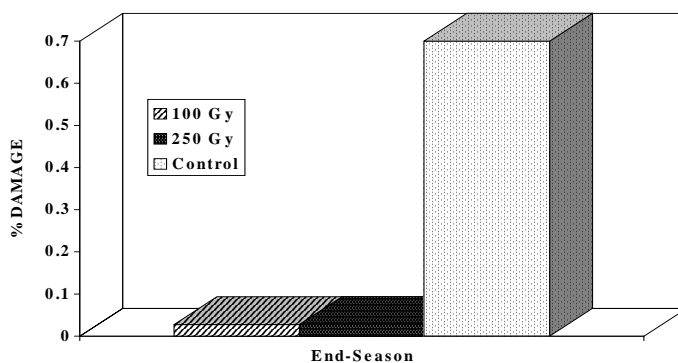
Figure 6. Percentage fruit damage at harvest caused by the codling moth in apple orchards in Washington State, USA. One series of three plots received season-long releases of partially sterile codling moths treated with 100 Gy (female moths were 100 % sterile, and males 50% sterile). A second group of plots received 250-Gy-treated moths (females 100% sterile, and males 75% sterile). The control areas were treated with six applications of azinphosmethyl insecticide. At the end of the fruiting season, 2500 fruit per treatment were sampled (Bloem et al. 2001).

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CHAPTER 2.5.

MATHEMATICAL MODELS FOR THE USE OF STERILE INSECTS

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SUMMARY

This chapter begins with a consideration of simple population models. The sterility formulation proposed by Knipling is then included into the population models, and these are elaborated in systematic fashion to include the major biological factors that will affect the success of the sterile insect technique (SIT) control programme. These factors include residual fertility, differential competitive ability of wild and sterilized males, mating patterns, immigration, and various combinations of these features. Also examined are density-dependence, age structure, population aggregation, biotic interactions with other species, and then integration of the SIT with other control methods. It was found that combinations of factors are synergistic: combinations of detrimental factors such as residual fertility and inferior competitive ability put severe limits on the probable success of the control programme, while combinations of control methods are much more likely to succeed than single control methods. This is because each control method needs only to account for a smaller proportion of the total mortality when combined with other methods than when acting alone.

1. BRIEF OVERVIEW OF MODELLING

1.1. Types of Models

Modelling is the abstraction of processes or states of being. Mathematical models involve equations, graphs or algorithms behind computer code. Virtually all models of the sterile insect technique (SIT) are population models, either analytic (just with equations) or computer models (often called numerical models and in which the equations are usually implicit, rather than being made explicit). Population models keep track of population numbers, and include various features that influence population size and trend, such as birth rate, mortality, age structure, immigration and emigration, competition, etc. Population growth can be either density-independent, in which birth rate and mortality are independent of population size, or density-dependent, in which either or both of birth rate and mortality depend on population size, usually in such a way as to eventually stabilize the population around some long-term mean value.

Mathematical models of populations are typically posed as difference equations or as differential equations. Difference equations are discrete and use some meaningful time step, such as days, years, generations, etc. These are popular with

entomologists, since many insects breed seasonally, such as most temperate forest insect pests (bark beetles, budworms, tent caterpillars, etc.). Differential equations are continuous, involving an infinitesimal time step, and rely on calculus to solve them. They are useful in species that breed continuously within some period of time, such as aphids, stored products pests and animal parasites. However, difference equations do not involve calculus, and generally are easier for the non-mathematician to understand. Another dichotomy is between deterministic models, which always yield the same model result, and stochastic models, which involve randomness. Most of the models of the SIT have so far been deterministic models, involving no random elements.

In this chapter, only models that predict some aspect of system behaviour (population dynamics) will be explored. Thus purely statistical analysis of data, however valuable and relevant that might be to the release programme, will not be considered here. Likewise the derivation of regressions for use in models will not be considered here as SIT modelling. However, a section on model parameter estimation, in which such techniques are mentioned, is included.

1.2. Simple Population Models

For density-independent population growth, the simplest models are geometric growth for a species with non-overlapping generations, and its continuous counterpart, exponential growth (Box 1). A simple modification to these models, to include resource limitation, puts an upper limit on growth. Many formulations exist for limiting geometric growth; a few were provided by Hassell (1978). This small complication makes some formulations insoluble analytically, and it is a common feature of population models that non-linearities render the models insoluble analytically; it is then necessary to resort to numerical solutions using a computer.

Box 1. Simple Growth Models

Geometric and Exponential Growth

The geometric model is $N_{t+1} = \lambda N_t$. Here N_t is the size of the population at time t, where t is scaled to generations and λ is the rate of increase each generation. In each generation the population size is λ times the size it was in the previous generation. In this model, generations are discrete and non-overlapping. This model is easy to solve. At any time t, $N_t = N_0\lambda^t$, where N_0 is the size of the population at time $t=0$. The exponential growth model is $dN/dt = rN$. The solution to this model is $N = N_0\exp(rt)$, where $\exp(rt) = e^{rt}$, e being the base of natural logarithms, r is the instantaneous rate of growth, and N_0 is the initial size of the population at time zero.

Density-Dependent Growth

With density-dependence, the geometric model becomes $N_{t+1} = \lambda N_t\exp(-gN_t)$, in which the exponential term has no real biological meaning, and is simply a convenient device to limit population numbers. The continuous version is the logistic equation: $dN/dt = rN(K-N)/K$, where K is the carrying capacity, imposed by resource limitation.

1.3. Host-Parasitoid and Predator-Prey Models

Modelling host-parasite and predator-prey systems in insect population dynamics has a long history, and the "workhorses" are the Nicholson-Bailey difference equation model and the Lotka-Volterra differential equation model (Box 2). Hassell (1978) described such models, and these models have been used in modelling sterile insect releases for species under the influence of biotic interactions with other species.

Box 2. Predator-Prey and Host-Parasitoid Model

Nicholson-Bailey Model

Without density-dependence $N_{t+1}=\lambda N_t\exp(-aP_t)$; $P_{t+1}=\lambda N_t[1-\exp(-aP_t)]$ where N_t and P_t are the host and parasite population sizes at time t.

    With density-dependence $N_{t+1}=\lambda N_t^{(1-b)}\exp(-aP_t)$; $P_{t+1}=\lambda N_t^{(1-b)}[1-\exp(-aP_t)]$ where b is a parameter for imposing density-dependence and has no obvious biological meaning, and $\exp(-aP_t)$ is the zero term of a Poisson series, representing those hosts not found each generation by a group of randomly searching parasitoids.

Lotka-Volterra Model

Without density-dependence $dN/dt=rN-bNP$; $dP/dt=P(cN-e)$ in which the first equation gives the rate of change of the prey population (N) in terms of the intrinsic rate of increase, r, and a predation rate per predator, b; the second equation gives the rate of change of the predator population (P) in terms of the rate of increase per prey, c, and a death rate, e.

    With density-dependence $dN/dt=rN(1-aN)-bNP$; $dP/dt=P(cN-e)$, where a is a density-dependent death rate.

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2. MODELS OF STERILE INSECT RELEASES

2.1. Three Kinds of Control Programmes Using Sterility

There are three methods of using sterile insects for population control. These are: (1) the standard method of releasing sterile males (or males and females) that have been reared and sterilized; earlier work in modelling of sterile releases was previously summarized by Hamada and Miyai (1985); (2) the treatment of insects with sub-sterilizing doses of radiation or chemosterilants so that the matings are partially sterile, but the offspring of matings involving treated insects are sterile, called inherited sterility (Carpenter et al., this volume); and (3) the deployment of chemosterilants in field traps to sterilize insects that are attracted to the traps. It is mainly the first of these three methods that will be dealt with here. Chemosterilants are seldom used in the field because of their carcinogenic potential, although modelling has been done on this technique by Knipling (1960), Lawson (1967), Staley et al. (1971), Hawkes and Coaker (1977), Barclay (1981a), and Wall and Howard (1994). In addition, although the SIT is usually used for insect control, in some cases the concept can apply to other animals (Klassen et al. 2004).

2.2. Initial Contribution of Knipling to Modelling SIT

Knipling produced a simple numerical model that foreshadowed most future modelling developments (Knipling 1955, 1959). The central feature of Knipling's model, and one found in almost all subsequent models, is the ratio of fertile males to all males in the population: $(M/(S+M))$ where M is the number of fertile males (or females, assuming a 1:1 sex ratio) and S is the number of sterile males. This gives the proportion of the population, under ideal conditions, that results in fertile egg production as a result of some fertile females mating with fertile males. Knipling's (1955) model for the release of sterile insects was a simple modification of the geometric model in Box 1 using the sterility factor above:

$$F_{t+1} = \lambda F_t(M_t/(S+M_t)) \tag{1}$$

where F_t and M_t are again the population size (fertile females and males) at time t, λ is the rate of increase per generation, and S is the release rate of sterile males each generation. This yields a stable steady state at $F=0$ and an unstable positive steady state for F when $S=S^*$, the critical release rate, where $S^*=F(\lambda-1)$, the value of sterile release rate that holds the population at the steady state (Berryman 1967). If $S>S^*$, then the pest population will collapse and be eliminated. If $S<S^*$, then the population in this model will increase indefinitely.

2.3. Sex Ratio

One question asked early in the use of sterile release programmes was, "Is there an optimal sex ratio for the insects being released?" It was initially thought that the release of females would be counterproductive. This question was addressed by Ailam and Galun (1967) and by Lawson (1967); using probabilistic models of mating, they found that the release of females is never detrimental (assuming they are all fully sterile), and in fact may assist the control programme if males are limited in their mating ability, in which case some fertile females might not get mated. However, there are limited field data to support this suggestion.

2.4. Residual Fertility of "Sterile Insects"

If some of the treated insects are not completely sterilized, then the situation becomes more complicated. Klassen and Creech (1971) constructed a simple numerical model in which a certain proportion of the released males remained fertile. They found an upper limit to this "residual fertility" that was compatible with the success of the release programme. Their model can be put into algebraic form and generalized. When there is incomplete sterilization of the released insects, a fraction, q, of males remains fertile. In that case, Knipling's model can be modified as in Box 3. The critical sterile release rate is then only finite for $q<1/\lambda$. If $q>1/\lambda$, then the population is not controllable by sterile releases. Thus, for example, if the rate of increase, λ, is 10, then q must be less than 0.1, i.e. the released males must be

greater than 90% sterile in order for control by the SIT to be possible. Also, if the residual fertility is more than about three-fourths of the limiting value, then the required rate of sterile releases is much higher than with complete sterility (Fig. 1).

If both males and females are released and neither sex is completely sterile, then the fertile male X fertile female matings can be modelled as in Box 3. If residual fertility exists in both sexes following release, it becomes impossible to eliminate the pest population by sterile releases alone; the best that can be done is to suppress it to a low level with continuing sterile releases. In addition, control is impossible unless $q_m < F/\lambda(F + q_f S_f)$, where q_m and q_f are the residual fertilities of males and females, respectively. This value of q_m is smaller than that without the release of females (Fig. 2). Thus less residual male fertility can be tolerated with the release of residually fertile females (Barclay 2001).

Box 3. Residual Fertility of "Sterile Insects"

Here Knipling's model becomes: $F_{t+1} = \lambda F_t (M_t + qS)/(M_t + S)$, where it is assumed that either only males (M_t) are released or that released females are completely sterile and only males display residual fertility. This model has a stable steady state at $F = M = 0$, and an unstable positive steady state for F and M when $S = S^*$, the critical release rate, where $S^* = M(\lambda-1)/(1-\lambda q)$. Here, S^* is only finite for $q < 1/\lambda$.

If both males and females are released and neither sex is completely sterile, then the fertile male X fertile female matings can be modelled by the equation: $F_{t+1} = \lambda (F_t + q_f S_f)(M_t + q_m S_m)/(M_t + S_m)$, in which F_t is the number of wild fertile females in generation t, q_m and q_f are the proportions of treated males and females, respectively, that remain fertile, and S_m and S_f are the number of treated males and females, respectively, that are released each generation (Barclay 2001). So far there is no restriction on the sex ratio. Thus $q_f S_f$ released females that remain fertile are added to the wild fertile females each generation, and $q_m S_m$ released males are added to the number of wild fertile males each generation, with the assumption that treated insects are equally competitive for mates with wild insects. This model has a lower stable steady state and an upper unstable steady state for F and $M > 0$ when $S = S^*$, and $S^*_m = (\lambda-1)(FM + \lambda q_f S_f M)/(F(1 - \lambda q_m) - \lambda q_m q_f S_f)$, and this equation is only soluble if the sex ratio is known. If we assume a one-to-one sex ratio (where $M_t = F_t$ and $S_m = S_f = S$), then we obtain a quadratic equation: $\lambda q_m q_f S^2 - [1 - \lambda(q_m + q_f)]FS + (\lambda-1)F^2 = 0$ which gives two roots when solved for either S or F. The upper root of F is unstable, and represents the size of the population before initiating sterile releases. The lower root of the equation for F is stable, and is the value at which the population would be maintained by residual fertility after collapse due to suppression by sterile releases. Thus it is impossible to eliminate the pest population by sterile releases alone; also control is only possible if $q_m < F/\lambda(F + q_f S_f)$. The relationship between the maximum values of q_m and q_f is hyperbolic (Fig. 2).

2.5. Competitive Ability of Males

The ability of sterile males to compete with wild males for mates can be affected by sterilization through the debilitating effects on either sperm competition or the behaviour of the adults (Calkins and Parker, this volume; Lance and McInnis, this volume). This problem was modelled by Berryman (1967), Bogyo et al. (1971), Berryman et al. (1973), Itô (1977), and Barclay (1982a). Their models are summarized by the model in Box 4. All of their models show that the critical release rate increases as the competitive ability of sterilized insects decreases.
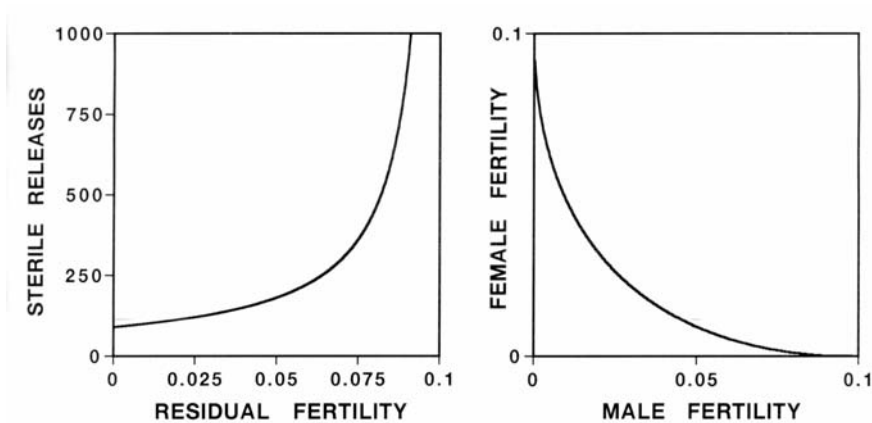
Figure 1. The critical sterile release rate, S*, as a function of residual fertility, q. λ = 10.

Figure 2. Allowable residual fertility when it is in both males and females. λ = 10.

Box 4. Competitive Ability of Males

We define c as a coefficient of competitive ability, with 0 being completely non-competitive and 1 being fully competitive. Then $F_{t+1} = \lambda F_t(F_t/(F_t+cS))$. This model has a stable steady state at $F=M=0$ when $S>0$. The positive (unstable) steady state for F occurs when $S=S*$, the critical value, where $S*=(\lambda-1)F/c$, which is greater than $(\lambda-1)F$, with full competitive ability (Itô and Yamamura, this volume).

2.6. Interactions of Residual Fertility and Competitiveness

In this model only males display residual fertility; females are either completely sterilized or not released.

2.6.1. Residually Fertile Insects Are Fully Competitive

Here the insects that remain fertile after treatment are fully competitive with wild insects (Box 5). The allowable residual fertility of males is an almost linear function of the competitive ability of released sterile males (Fig. 3A, solid line), unless λ is very small. Also, for a given degree of residual fertility, $S*$ becomes larger as c becomes smaller (Fig. 3B). Thus the extent of residual fertility, and lack of competitive ability, compatible with control are each more restricted in the presence of the other (Barclay 2001).

Box 5. Residual Fertility and Competitiveness

If residual fertile insects are fully competitive, $F_{t+1}=\lambda F_t(F_t+qS)/(F_t+qS+cS(1-q))$, then this model has a stable steady state at $F=M=0$ when $S>0$. The positive (unstable) steady state occurs when $S=S^*=(\lambda-1)F/(c(1-q)-q(\lambda-1))$, and S^* is finite only if $q<c/(\lambda-1+c)$.

If residual fertile insects are of reduced competitiveness, then the model becomes $F_{t+1}=\lambda F_t(F_t+cqS)/(F_t+cS)$. If there is no residual fertility, there is a stable steady state at $F=M=0$, if $S>0$, and a positive unstable steady state when $S=S^*=(\lambda-1)F/c$.

If there is both residual fertility and unequal male competitive ability, the steady state for F is at $S^*=(\lambda-1)F/(c(1-q\lambda))$, and this is finite only if $q<1/\lambda$.
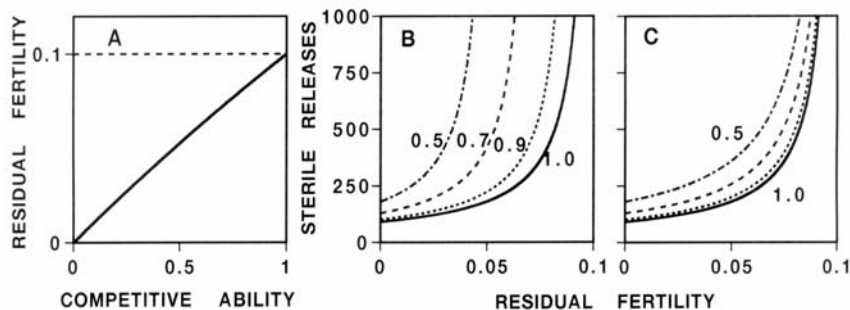
Figure 3. A: Changes in allowable residual fertility with changes in competitive ability of sterile males. Residually fertile insects are of reduced competitive ability (broken line) or are fully competitive (solid line). B and C: Critical sterile release rates for residual fertility (0 to 0.1) and competitive ability (0.5, 0.7, 0.9, 1.0). In B, residually fertile insects are fully competitive, and in C, residually fertile insects are of reduced competitive ability. $\lambda = 10$ in all cases. (Figure from Barclay 2001, reproduced with permission.)

2.6.2. Residually Fertile Insects Have Reduced Competitiveness

Here the insects that remain fertile after treatment are not fully competitive with wild insects (Box 5). The critical sterile release rate in this case (Fig. 3C and Box 5) is not as large as the corresponding value when residually fertile insects are of full competitive ability (Fig. 3B).

2.7. Mating Patterns

Another question asked early in the use of sterile releases was, "Should the females of the target species in a sterile release programme mate once or more than once?" The question has been addressed by Knipling (1964), and in the models of Berryman (1967), Lawson (1967), Zouros (1969), and Barclay (1984). The answer appears to be that female remating (polygamy) is quite compatible with the SIT, as long as mating is random, with sterilized males being fully competitive. In addition, in polygamous species, it doesn't matter whether sperm is diluted, replaced or excluded

after the first mating, again as long as mating is random, and sterile males are fully competitive (Lance and McInnis, this volume).

2.7.1. Interaction of Polygamy with Competitive Ability

If sterile males are equally competitive for mates with fertile males, sterile sperm is fully competitive with fertile sperm, and there is no residual fertility, then the effects of polygamy (multiple female mating) are simply to reshuffle the sperm at each mating, and polygamy has essentially no effect. On the other hand, if sterilization is incomplete, and/or sterilized males (or their sperm) are of inferior competitive ability, then the situation is more complex. The work of Berryman (1967) (also addressed by Zouros (1969)) is particularly insightful in this matter. If only the first mating of a female results in sperm retention, or if all of the sperm of previous matings is replaced at each successive mating, and if all matings occur before oviposition begins, then the effective number of matings is just one. If the sperm from all matings mixes and is retained, then the effect of multiple matings depends on sperm competition as well as on competition between sterile and fertile males for mates. Berryman (1967) addressed this important problem, and it is worthwhile revisiting his results, making appropriate changes in his notation to make it consistent with the development above. Berryman considered three cases, depending on sperm action (Box 6).

2.7.2. Non-Functional Sperm

If the sperm of sterilized adults is either nonexistent or immotile, then a female mating m times will only produce sterile eggs if all the matings were with sterile males. The resulting critical values (Box 6) of the sterile release rate, $S*$, are shown in Fig. 5 for several values of M, the maximum number of matings, values of the adult competition coefficient from 0.5 to 1.0, two values of the probability of mating, and assuming a binomial distribution of mating frequencies.

2.7.3. Dominant Lethal Mutations with Fully Competitive Sperm

If sterility is caused by dominant lethal mutations, and the sperm of sterilized adults is fully competitive with that of fertile adults, then it can be shown that the probability that an egg is fertilized by a sterile sperm is independent of the number of matings, and the results from section 2.5. on competitive ability still hold with polygamy, and correspond to the case of $M=1$ in Fig. 5.

2.7.4. Dominant Lethal Mutations with Reduced Sperm Function

If sterility is caused by dominant lethal mutations, and the sperm of sterilized adults is of reduced competitive ability compared with that of fertile adults, then it can be shown that the probability that an egg is fertilized by sterile sperm depends on the number of matings. The values of the critical release rates, $S*$, in Box 6, are shown in Fig. 4A and B against the adult sterile competitive ability, c_a, and for the sperm competitive ability, c_s. In addition, the values of c_a and c_s are shown for given values of the critical release rate (250, 500), $S*$, when it is held constant (Fig. 4C).

Box 6. Mating Patterns

Interaction of Multiple Female Mating and Competitiveness

The probability of a fertile female mating with a sterile male is defined as $P_s=c_aS/(F_t+c_aS)$, and the probability of a fertile female mating with a fertile male as $P_f=F_t/(F_t+c_aS)=1-P_s$, where c_a is the competitive ability of sterile adults (equivalent to c in Box 4). Berryman (1967) considered the joint distribution of the number of matings, and the number of sterile matings, as a sequence of marginal distributions of the number of sterile matings given the number of matings. Thus a female can mate from zero to M times, and for a given number, m, of matings the number of sterile matings is binomially distributed. If $_mC_n$ is defined as the number of combinations of m things taken n at a time ($=m!/n!(m-n)!$), then the conditional probability that a given female mates with n sterile males, given that she mates m times, is $P(n/m)=_mC_nP_s^n\ P_f^{m-n}=_mC_nP_s^n(1-P_s)^{m-n}$. This is one term of a binomial distribution that describes the number of sterile matings given the number of matings, and there will be $M+1$ such distributions, including one for no matings. Berryman considered three cases, dependent on sperm action.

Non-Functional Sperm

If the sperm of sterilized adults is either nonexistent or immotile, then a female mating m times will only produce sterile eggs if all the matings were with sterile males. The probability of this occurring is P_s^m, and so the probability of at least one fertile mating is $(1-P_s^m)$. Then the probability of at least one fertile mating, over the range of mating frequencies, is $\Sigma P_m(1-P_s^m)$ for $m=1,2,3, …, M,$ and so the population equation becomes $F_{t+1}=\lambda F_t\Sigma P_m(1-P_s^m)$ for $m=1,2,3, …, M$, where P_m is the probability of mating m times.

Dominant Lethal Mutations

In the binomial expansion of the probabilities of m matings, where m goes from 0 to M, each of the terms representing mixed fertile and sterile matings will be weighted by a factor, c_s, representing the competitive ability of sterile sperm. Thus the probability of an egg being fertilized by a sterile sperm, taken over all mating frequencies, will be $P(e)=P_s^m+c_s((m-1)/m)\ _mC_{m-1}\ P_s^{m-1}(1-P_s)+c_s((m-2)/m)_mC_{m-2}P_s^{m-2}(1-P_s)+….+(0/m)_mC_0(1-P_s)^m$, which can be reduced to $P_s^m+c_sP_s(1-P_s^{m-1})$, where $P_s=c_aS/(F_t+c_aS)$, as above. We can write the equation as $F_{t+1}=\lambda F_t\Sigma P_m[1-(P_s^m+c_sP_s(1-P_s^{m-1}))]$ for $m=1,2,3, …, M$. The values of the critical release rates, S^*, are shown in Fig. 4A and B against the adult sterile competitive ability, c_a, and for the sperm competitive ability, c_s. In addition, the values of c_a and c_s are shown for given values of the critical release rate (250, 500), S^*, when it is held constant (Fig. 4C).
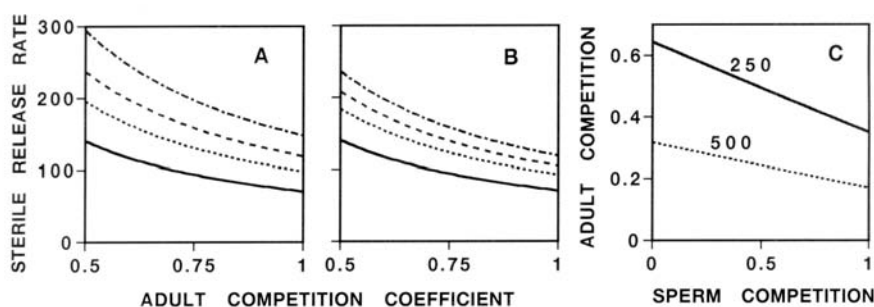
Figure 4. A and B: Values of the critical release rates, S*, for values of adult sterile competitiveness, c_a, four mating frequencies (1,2,4,8), and two probabilities of mating — A: 0.6, B: 0.8. C: Limits on adult sterile competitiveness compatible with control of the pest population; these are shown for a range of values of sperm competitiveness and two values of sterile releases (250,500). (Figure generated from equations, Berryman 1967.)
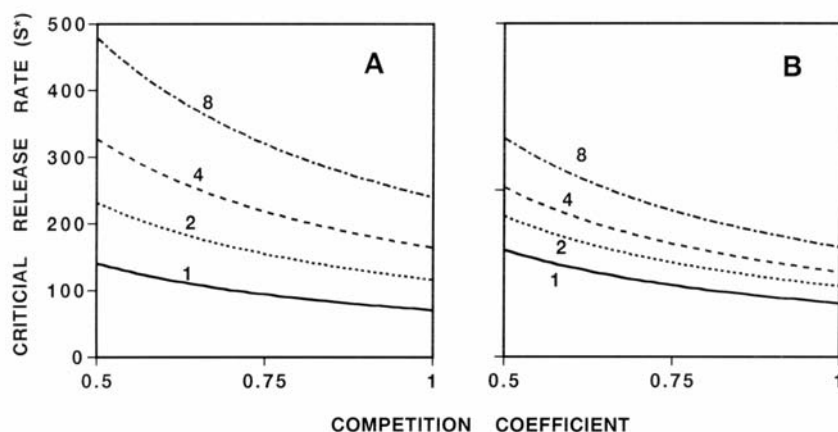
Figure. 5. Critical sterile release rates when sterile males produce non-functional sperm. The maximum possible number of matings, M, is 1,2,4,8, and the probability of mating in A is 0.8, and in B is 0.9. The adult competition coefficient ranges from 0.5 to 1.0. $\lambda=10$ in all cases. (Figure generated from equations, Berryman 1967.)

2.8. Interaction of Polygamy with Residual Fertility

A similar analysis on residual fertility (not shown) can be performed. Starting with the equation for residual fertility, and assuming that sterile sperm are fully functional, one proceeds as with the case of dominant lethal mutations and fully functional sperm. The result is that the probability that a female will mate with a sterile male is independent of the number of matings (Barclay 2001), and the probability that an egg will be fertilized by a sterile sperm is also independent of the number of matings. Thus multiple female mating (polygamy) and residual fertility have no interaction, and the results derived above for residual fertility alone apply both to monogamy and polygamy.

2.9. Population Movement

The release of sterile insects, together with immigration from outside the control area, can be modelled by a simple modification of the model in Box 4 (Dietz 1976, Prout 1978). Following Prout, two cases must be accommodated here: (1) immigration before mating, and (2) immigration after mating. In these two models it is assumed that all sterilized insects that are released are completely sterile.

2.9.1. Immigration Before Mating
Assuming that *V* males and *V* females immigrate each generation prior to mating, the female immigrants are thus available for mating with the released sterile males

as well as the wild males, and the male immigrants are available for competing with the sterile males. The model (Box 7) has two positive roots for F, with the upper one being unstable (the population as it existed just prior to sterile releases) and the lower one being stable. This lower steady state represents a population in a state of collapse due to sterile releases, but which is replenished each generation by immigrants. Note that zero is not a steady state solution here. The required sterile release rate grows rapidly with V, but there is no value of immigration that disallows control by sterile releases. The values of S^* depend only modestly on V, if the immigration rate each generation is only a small proportion of the total population.

Box 7. Immigration

Immigration Before Mating

If we include immigration into the model, we obtain $F_{t+1}=\lambda(F_t+V)(F_t+V)/(F_t+S+V)$. Solving for steady state, we obtain the quadratic: $(\lambda-1)F^2-[S-(2\lambda-1)V]F+V^2=0$ which has two positive roots for F, with the upper one being unstable and the lower one being stable. Note that zero is not a steady state solution here.

The critical release rate is $S^*=[(\lambda-1)F+\lambda V](F+V)/F$.

Immigration After Mating

The equation here is $F_{t+1}=[\lambda F_t^2/(F_t+S)]+\lambda V$. Note that, if the wild population is reduced to zero, it will be reconstituted the following generation, as then $F_{t+1}=\lambda V$. Again solving for steady state, we obtain $(\lambda-1)F^2-[S-\lambda V]F+\lambda VS=0$.

The critical sterile release rate is given by $S^*=F[(\lambda-1)F+\lambda V]/(F-\lambda V)$, and sterile releases can only control the population if $V<F/\lambda$.

For a given immigration rate, the required sterile release rate is much higher if immigration is after mating than before mating.

2.9.2. Immigration After Mating

In this case it is assumed that V males and V females immigrate each generation after mating. The female immigrants are thus not available for mating with the released sterile males or the wild males, however the male immigrants are available for competing with the sterile males. Thus, immigrating females remain fully fertile. Note that, if the wild population is reduced to zero, it will be reconstituted the following generation, as $F_{t+1}=\lambda V$. For a given value of immigration rate, the required sterile release rate, S^*, is much higher if immigration is after mating than before mating, due to the fully fertile nature of the immigrating females.

2.9.3. Large-Scale Population Movement

The problem of large-scale population movement was addressed by Manoranjan and van den Driessche (1986), Lewis and van den Driessche (1993), Plant and Cunningham (1991), and Marsula and Wissel (1994). They showed, using diffusion equations, that dispersal of insects, coupled with non-linear growth terms, can result in waves of invasion or extinction. Both the velocity and direction of these waves depend critically on the rate of release of sterile insects. With low rates of release, the travelling wave advances as an invasion; when the density of sterile insects exceeds a critical density, the wave recedes, giving rise to local extinction. This is

likely to have considerable relevance to programmes releasing sterile insects such as the New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programme in the southern USA, Mexico and Central America, in which the pest population has been pushed back to Panama and a sterile insect buffer zone created. Matlock and associates are pursuing this approach with the screwworm, assessing the size of the buffer zone needed to ensure that insect invasion into the eradicated area does not occur (R. B. Matlock, Tulane University, personal communication).

2.10. Combinations of Residual Fertility, Reduced Competitiveness, and Immigration

Four models can be considered, being the four combinations of: (1) those sterilized insects that show residual fertility can be either of reduced competitive ability (reduced) or fully competitive (equal) with the wild insects, and (2) insects can immigrate either before mating or after mating.

Barclay (2001) provided equations for the four cases, and the values of the limiting residual fertilities are shown in Table 1 and Fig. 6. In Table 1, the allowable residual fertility for the case of residually fertile insects being of reduced competitive ability is the same as for the case involving sterile insects being fully competitive and immigration occurring. The other two cases yield more stringent limits on allowable residual fertility than with no immigration. It is apparent that there is a strong interaction among residual fertility, competitive ability, and immigration, with the feasible limits on each factor becoming much more restrictive in the presence of the other factors.

Table 1. Limits on residual fertility (q) when competitive ability of "sterilized but residually fertile insects" is either reduced or equal to that of wild fertile insects, and immigration is either before, or after, mating

	Reduced	Equal
Before	$q < F/\lambda(F+V)$	$q < cF/[\lambda(F+V)-F(1-c)]$
After	$q < (F-\lambda V)/\lambda F$	$q < c(F-\lambda V)/[\lambda F-(1-c)(F-\lambda V)]$

2.11. Density-Dependence in Population Regulation

Density-dependence in a population has been shown by modelling to predispose it to control by sterile releases (Miller and Weidhaas 1974, Itô 1977, Prout 1978, Barclay and Mackauer 1980a). There are several formulations that include density-dependence in a model of a population, and all yield the same qualitative results. The two distinct ways that density-dependence assists the SIT are: (1) reduces the

effective biotic potential of a species by increasing the natural mortality at higher densities, and (2) introduces a bifurcation (splitting of one root of the equation into two) into the model whereby the population suddenly collapses as the sterile insect release rate is increased above the level required at the bifurcation (Fig. 7). This avoids the necessity of the high levels of release needed to reduce the population below the unstable steady state in the model with no density-dependence. This bifurcation occurs in all the models involving density-dependence, and appears to result from the interaction of the depressing effects of density-dependence and the unstable equilibrium created by the SIT formulation in section 2.2., which results in the release of sterile insects being more effective at low density than at high population density, and thus the efficiency of the SIT increases as the population declines. The sudden collapse of a population under attack by sterile insect releases has indeed been observed in the programme against the melon fly *Bactrocera cucurbitae* (Coquillett) in Okinawa, Japan (Iwahashi 1977). Thus this predicted bifurcation appears to be a robust result, and one that apparently mimics nature.
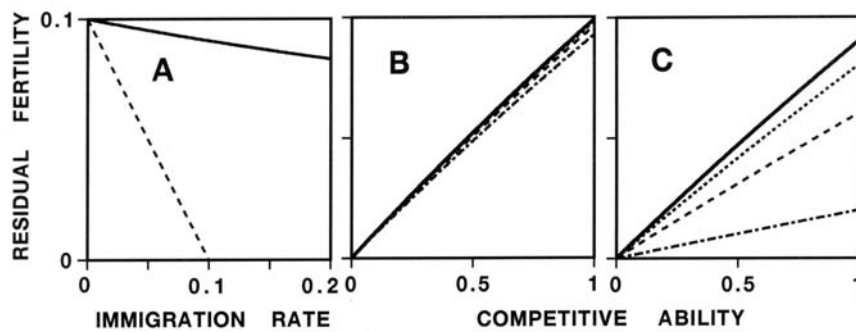
Figure 6. A: The allowable residual fertility (q) when the released insects that are fertile are also fully competitive with wild insects. Immigration occurs before mating (_____); immigration occurs after mating (- - - - - - -). B and C: Allowable residual fertility as a function of competitiveness of released insects. Immigration occurs at 1% (_____), 2% (…………), 4% (- - - - -), and 8% (- . - . - .-) of the wild population size F. B: Immigration occurs before mating. C: Immigration occurs after mating. (Figure from Barclay 2001, reproduced with permission.)

The exact behaviour of the SIT under density-dependence appears to depend sensitively on the biology of the system. Lawson (1967) and Berryman et al. (1973) pointed out that overcrowded populations may deplete their resources sufficiently such that survival to the adult stage is low. In this case, killing some of them (or lowering egg production) might actually result in a higher survival to the adult stage, making the use of the SIT counterproductive in such a situation. Another situation might be encountered in the case of an insect species wherein egg production is much higher than the resource allows, e.g. the olive fruit fly *Bactrocera oleae*

(Gmelin) in which one egg per fruit is laid. If sterile eggs did not deter insects from laying further eggs in a fruit already containing a sterile egg, then reduction in fertile egg production would have to be substantial before any effect would be noticed.
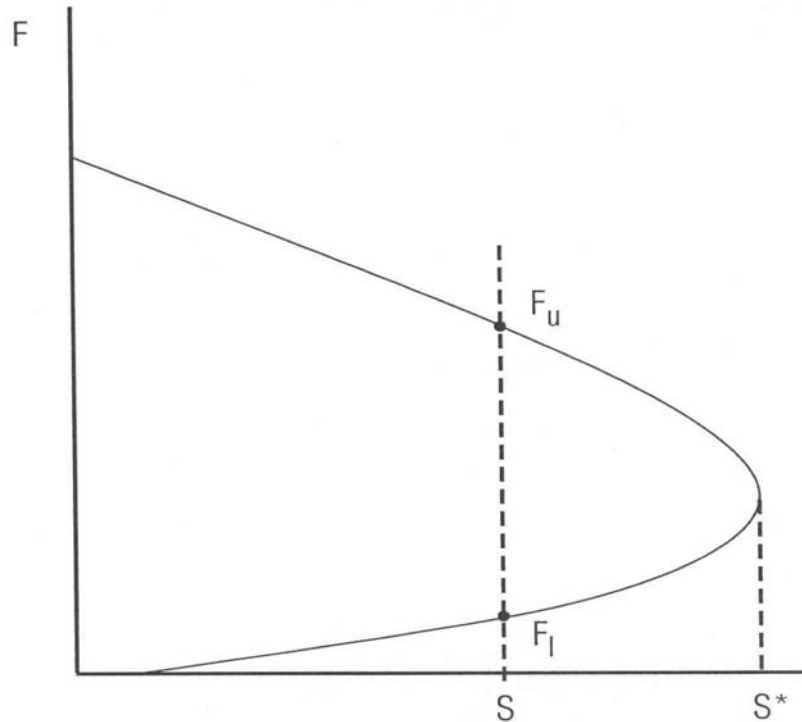
Figure 7. Isoclines formed by setting the equations for sterile insects (S, vertical line) and fertile insects (F) to zero; where they cross are the two steady states. The upper one is stable, and the lower one unstable. As sterile releases increase, the sterile isocline moves to the right. The bifurcation is at the point where the isoclines are tangent to each other. Larger values of sterile insect releases result in a sudden collapse of the population. (Figure generated from equation 12, Barclay 2001.)

2.12. Age Structure

The existence of two or more life stages of a species complicates the dynamic responses of a population to mortality factors, especially if the two stages are ecologically different, as they are in mosquitoes and other pest species in which the two active stages occupy different habitats. If density-dependence is strong in one stage and weak or absent in the other stage, then the density-dependent stage is strongly buffered against mortality factors and tends not to vary greatly, while the

other stage may vary more but the mean size is a linear function of the buffered stage. As such, somewhat different responses to mortality affecting mainly one stage would be expected, and indeed this appears to be the case. In the case of the SIT, the sterile insects released always affect the adult stage, reducing fertile egg production.

Prout (1978) modelled the SIT for species with identifiable age structure and subject to immigration. His results indicated that, if the larval stage caused the pest problem, a higher level of immigration of mated adults was tolerable.

Barclay (1980b) showed that the critical release rate ($S*$) is a larger proportion of the larval equilibrium size when density-dependence is in the larval survivorship than when density-dependence is in the adult survivorship. Thus, relative to a given larval equilibrium, the population requires fewer sterile insect releases when density-dependence is in the adult stage.

2.13.   Population Aggregation

In nature most populations are not dispersed evenly over the available habitat. Some processes, such as territoriality, result in dispersion patterns that are more regular than one would expect of a random spatial distribution. However most populations will tend to have a somewhat aggregated dispersion pattern. Aggregation is the most difficult pattern to deal with in making sterile insect releases, as one has to know where the clumps are located.

Modelling of spatial aggregation has been done by Wehrhahn (1973) and Barclay (1992a). Wehrhahn used a mosaic of patches, inhabited by differing numbers of insects, and compared the required release rates for various patterns of aggregation. He used Monte-Carlo simulation, which introduces random numbers to allow stochastic variation, in this case, of migration rates among patches. Wehrhahn pointed out that the control programme itself will probably change the nature of the spatial distribution.

Another approach has used probability distributions to describe the extent of aggregation (Barclay 1992a). There is a long history of using these distributions in ecology, summarized by Pielou (1969) and Patil and Stiteler (1974). The most common distribution to quantify aggregation is the Negative Binomial Distribution in which the parameter k measures clumping. If aggregation is extreme, then k is close to zero; as k goes off to infinity, the dispersion approaches a random pattern. Another approach uses $1/k$, which increases with the degree of clumping. Barclay (1992a) used the negative binomial distribution to derive required sterile insect release rates of an aggregated population as a function of the clumping parameter, k. For moderately aggregated populations ($k=0.25$), it was found that the required release rate was about four times that for a randomly dispersed population. Shiga (1986) analysed spatial distributions in the context of fruit fly eradication using male annihilation and the SIT.

Many aspects of aggregation involve behavioural components. Horng and Plant (1992) modelled the impact of lek mating on the SIT, using a Poisson binomial distribution. They found that the sterility effect, presence or absence of female mate-choice, and sterile male mating competitiveness were the most important factors in their model in determining the success of a programme releasing sterile insects.

2.14. Predation, Parasitism, and Competition

The effects of predation on the efficiency of the SIT were first modelled by Knipling (1979) using a simple numerical model. His model predicted a synergistic interaction between predation and sterile insect releases, such that the net effect would be considerably greater than either alone. Barclay and Mackauer (1980b) included sterile insect releases in the Lotka-Volterra predator-prey model, and demonstrated that, not only was the critical release rate lower with than without predators, the system was also greatly destablized, and population collapse often occurred with release rates well below the critical value. This model was subsequently shown by Harrison et al. (1982) to have a very complicated dynamic behaviour, and this is presumably related to the inherent instability. This model was then extended, and an even greater array of dynamical behaviour was found (Barclay and van den Driessche 1990). In addition, the general features of the predator-prey system were found similar to the situation involving hosts and parasitoids (Barclay 1987a). Knipling (1998) analysed extensively the effects of augmentation of predators and parasites on the efficiency of the SIT.

If the pest species is in competition for resources with another species, then this is of some value to the release programme, as it reduces the initial pest population size, but apart from that there seems to be little effect of the competing species on the release programme (Barclay 1981b).

2.15. Stochastic Models

Stochastic models involve the specification of certain variables as being random. If the processes involved are well known and the extent of variation is known, then stochastic models can give additional information on the expected variability of the resulting control, as well as deviations of mean values from those predicted by deterministic models. This is especially true in areas like genetics in which the mechanisms of variation, e.g. meiosis, are clear. However this information is often not well-known in animal ecology, and therefore stochastic models may be of limited use. In fact, if the wrong features are allowed to vary (e.g. birth rate is variable in the model, whereas in reality it is mortality that varies), then stochastic models can give misleading results. In addition, unless they are solved numerically, stochastic models are usually much more difficult to analyse than deterministic models, and for these reasons the history of stochastic population modelling has been rather disappointing.

Stochastic models of sterile insect releases were developed by Kojima (1971), Bogyo (1975), Costello and Taylor (1975), Taylor (1976) and Kimanani and Odhiambo (1993), and they confirmed the results of Knipling (1955) and others that used deterministic models. They also derived a threshold release rate that leads to local extinction, and showed that much greater release rates above this threshold will not result in a greatly reduced time to extinction, although Lawson (1967) and Itô and Kawamoto (1979) offered evidence to the contrary using both a deterministic model and a probabilistic model.

2.16. Stability under Various Conditions

Stability is a very important aspect of populations, and is of special interest to pest managers. If, for any reason, the population is likely to collapse, this is important. Barclay (1982b) examined four systems or cases for their relative stability under sterile insect releases. These were all differential-equation models and represented: (1) a simple one-species model with only one identifiable life stage (adult), (2) a one-species model with two life stages (larval and adult), (3) a model of two competing species, one of which is the pest, and (4) a predator-prey model in which the prey is the pest. There are many possible definitions of stability in ecology, and they involve various dynamic characteristics of the system. None has yet emerged as definitive, although an extensive analysis of the topic is now available (Mueller and Joshi 2000). Barclay (1982b) examined five criteria of stability for each system, and ranked the systems. These stability criteria involved characteristics such as extinctions, time to extinction, amplitude of fluctuations, and time until return to equilibrium. The most stable was the single species – single stage model, followed by the competing species model, then the two-stage model, and by far the least stable was the predator-prey system above (section 2.14.). The existence of obligate predators (or parasitoids) both lowers the critical release rate of sterile insects and also destabilizes the system, so that it is likely to collapse even when the sterile release rate is lower than the critical rate. Unfortunately there appears to be no experimental evidence to test these ideas.

2.17. Integration of Control Methods

Since the SIT works best at low pest densities (section 2.11.), it is common practice to reduce the population with insecticide prior to the release of sterile insects. This brings the population down to a level at which the number of sterile insects (required to be produced by a rearing facility) is manageable. It might also be possible to combine contemporaneously the action of the SIT with other control methods to share the required mortality among two or more imposed sources, each one then having to impose only a modest level of mortality, and each one perhaps operating best under conditions not favourable to the others (Barclay 1992b; Mangan, this volume). On the other hand, certain combinations might interfere with each other and thus prove unsuitable (Barclay 1987c).

Knipling (1964, 1979) examined several combinations of various control methods with the SIT, using simple numerical intra-generational models. These include combinations of sterile releases with insecticides, sterilants, pheromones, parasitoids or predators. Barclay (1980b, 1987a, b) and Barclay and van den Driessche (1989) also examined some of these combinations using more general inter-generational algebraic models. They found that the results of the combined use of sterile insects and other control methods became less clear when other biotic interactions were included.

2.17.1. SIT with Application of Insecticide

It might be thought that insecticides and the SIT are incompatible since the insecticide would kill sterile, as well as fertile, insects. However, Knipling (1964, 1979) reasoned that insecticide application would kill both sterile and fertile insects in the same proportion, and thus maintain the overflooding ratio, rendering the two control methods compatible. By numerical examples he showed that these two methods could work well together, and thus reduce both total costs and the need for excessive insecticide application. If the sterile insects were also resistant to insecticide, then the combination would be even more effective.

Barclay (1980a, b) found that, when the pest species was considered in isolation, the application of insecticide coupled with the release of sterile insects increased total mortality. However, the results of the combined use of insecticide and sterile insects became less clear when other biotic interactions were included. For species with two life stages, and when used together with sterile insects, a larvicide appears to be more useful than an adulticide, but if the pest is already under considerable predation, the combination of insecticide and sterile insects might be detrimental.

2.17.2. SIT with Pheromone Traps for Male Annihilation

Knipling (1979) found that the combined use of sterile releases and pheromone traps was less efficient than an equivalent effort put into either method alone. This was because of the interference caused by the killing of sterile males in the pheromone traps. As a variant of this combination, Knipling proposed that releasing pheromone-treated sterile insects could enhance mate-finding, thus increasing the competitive ability of sterile insects, especially at low densities. It might also act as a vehicle for confusion of the wild population. Knipling found that, for insects in which the males produce female-attracting pheromone, such as the boll weevil *Anthonomus grandis grandis* Boheman, the release of pheromone-treated males would substantially increase the effectiveness of the control program, assuming that the applied pheromone did not deteriorate badly. Knipling also considered the situation in which females produce male-attracting pheromone, and he modelled the release of pheromone-treated sterile females alone. These would probably be most effective if they were free-living rather than contained in traps. He again found that this method was much more effective than the use of untreated sterile females, and that control might be possible using pheromone-treated sterile females where the release of only non-treated sterile females would be hopelessly inadequate.

Hamada and Miyai (1985), using a continuous model, modelled the combination of the simultaneous release of sterile insects and pheromone trapping for male annihilation (Box 8). They found that the two methods in combination required less effort for each control method than when using either method alone. Their recommendation was to use male annihilation first and then sterile releases, although the model did not specifically explore that scenario.

Barclay and van den Driessche (1989) also modelled this combination, and found that the two methods combine synergistically, especially when the fecundity and daily survivorship are both high. When the fecundity and survivorship are low, the synergism disappears. For parameter values approximating those of tsetse flies *Glossina* spp., synergism is reduced.

Box 8. Combination of Sterile Releases and Pheromone Trapping

Miyai's model consisted of four differential equations: $dM / dt = F(a-bF) - cM - kM$; $dV / dt = F(a-bF) - cV - \alpha[\min(M+S, V)]$; $dF / dt = \alpha[\min(M+S, V)] M / (M+S) - cF$; $dS / dt = R - cS - kS$; where M, V, F and S are the numbers of males, virgin females, fertilized females, and sterile insects, respectively. The parameters are: a is a density-independent fecundity, b is a density-dependent fecundity, c is a death rate, k is the rate of trapping of males, α is the mating efficiency, and R is the sterile insect release rate.

Knipling (1979) described the interaction of methyl eugenol for male annihilation used concurrently with the release of both sterile males and females, and found no interference and a high degree of synergism. Since the development of resistance to methyl eugenol has been demonstrated (Shelly 1997), it might also be possible to incorporate the use of sterile insects of the resistant strain, and thus increase effectiveness even more.

In the case where the attractant is non-sex-specific, such as with food baits, Barclay and van den Driessche (1989) showed that the two methods combine synergistically, especially when sterile insects are fed before release, and fecundity and survivorship are high. For parameter values approximating those of tsetse flies, there is still some synergism.

2.17.3.   SIT with Release of Parasitoids

This combination has the advantage that parasitoids work well at high host densities, while the SIT works best at low pest densities. Knipling (1979, 1998) considered the release of both sterile males and females, and also *Trichogramma* sp., an egg parasitoid. His tables showed clearly that these two methods were synergistic. A recent field study found that the two methods in combination were more efficient than either method alone (Bloem et al. 1998). In addition, the release of sterile females provides an egg resource for egg parasitoids, further augmenting the parasitoid population. Many parasitoids attack the larvae, so that the release of sterile females would not directly assist these parasitoids. If both sterile insects and parasitoids are released inundatively (Carpenter et al., this volume), then each should become more efficient as the density declines, offering a powerful source of synergism.

Barclay (1987b) modelled the interaction of the inundative release of parasitoids and sterile insects using several variations on the usual host-parasitoid equations. This combination shows a high degree of synergism in all the models investigated, and appears to be close to ideal (Fig. 8). The main problems to be anticipated probably involve dispersal and phenology.

2.17.4.   SIT with Sanitary Measures and Oviposition Traps

If sanitation destroys oviposition habitat and traps destroy oviposited eggs, then there should be a complementary effect between the two, albeit moderated by density-dependence. If sterile insects are also released, then the system has three sources of population reduction, none of which interferes with any other. Knipling

(1979) calculated that these three should result in a highly efficient combination for control.
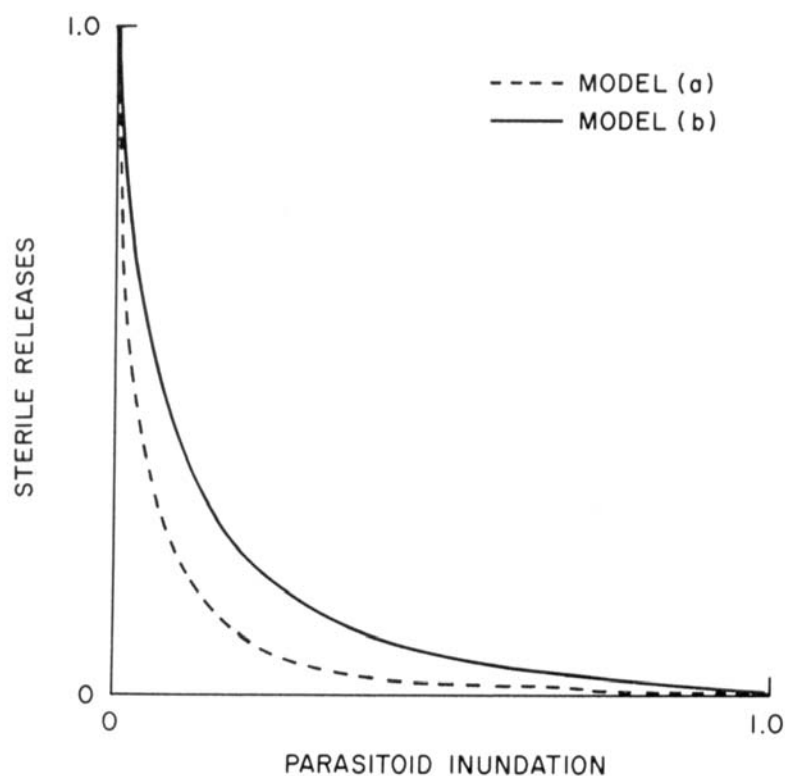
Figure 8. Critical release rates of sterile insects and parasitoids. All points on each curve are just sufficient to eradicate the pests. Density-dependence is in the hosts in model (a), and in the parasitoids in model (b). (Figure from Barclay 1987c, reproduced with permission.)

2.18. Optimization of Programme Releasing Sterile Insects

Optimization inevitably involves economics. Although that is somewhat outside the scope of this chapter, a beginning has been made on this topic. Geier (1969) used demographic models incorporating density-dependence to analyse the efficiency of programmes that release sterile insects, and to derive optimal strategies for control. Barclay and Li (1991) used a general treatment of combinations of pest control to determine optimal proportions of each control method. Atzeni et al. (1992) examined the situation for the Old World screwworm fly *Chrysomya bezzania* (Villeneuve), and included buffer width, male competitiveness, and population

aggregation in their analysis. Anaman et al. (1994) performed a cost/benefit analysis with *C. bezzania,* and incorporated beef losses into the equation.

2.19. *Development of Resistance*

There has been a marked tendency for insects to develop resistance to insecticides or other control methods. It is conceivable that a wild pest population could develop resistance to the use of sterile releases as a means of control (Lance and McInnis, this volume; Whitten and Mahon, this volume). This resistance might involve behavioural mechanisms that would preclude the sterile-fertile matings (Barclay 1990). Selection for resistance to several pest control methods operating together has been modelled by Barclay (1996), and it appears that selection for resistance to a particular control method is a linear function of the amount of mortality being inflicted by that control method. Itô and Yamamura (this volume) develop further the subject of resistance.

2.20. *Educational and Instructional Modelling*

There have been a number of computer simulations of the SIT, for the purpose of instruction, in both the technique and in insect population dynamics generally. At least two have been documented and are available to the public. Both represent various elaborations on the original model of Knipling.

Sawyer et al. (1987) described a simulation that includes spatial heterogeneity, aggregation, immigration, random effects, and reduced sterile male competitiveness. This model was adapted and named "Curaçao" by Arneson (1996) to run on Microsoft Windows (3x or 95), and is available for downloading on the website. Instructions for running the programme are included on the website. The user can specify the various options, and then compare runs to draw conclusions.

Weidhaas (2001) constructed a similar model written in Visual Basic called "Sterility", which runs on a personal computer with DOS capabilities. It allows incomplete sterility in males, reduced sterile-male competitiveness, different sex ratios, and determination of the growth rate of the population, and runs for 12 generations to assess the results. It also has the capability to compute costs of the control programme.

3. PARAMETER ESTIMATION FOR THE MODELS

Knowledge of several parameters is crucial to the success of any programme that releases sterile insects. With reference to the models outlined above, the basic parameters that will always be of interest are: F, the population size; λ, the potential rate of population increase each generation; q_m and q_f, the proportions of the released males and females that remain fertile; and c, the competitive ability of sterile males relative to the wild fertile male population. Some of the estimations can be done using standard population biology methods. The population size can be crudely estimated from trap catches. Hargrove (1990) used mark-recapture techniques to

estimate the size of tsetse fly populations (Weidhaas 1973). The rate of increase, λ, would normally be determined using oviposition rates. The residual fertilities, q_m and q_f, could be determined by caging sterile males with fertile females, and fertile males with sterile females, either in groups or pairs, and noting the resulting fertile egg production. Competitive ability of sterile males, c, could then be determined from laboratory, field cage or small-scale field experiments, where immigration could be assumed to be negligible, using the equation involving competitive ability, and then solving for c. The information on λ, q_m and q_f must be determined first, or the equation becomes confounded. Alternatively, Meats (1998) used release and recapture techniques to estimate the quality of released sterile insects. Immigration into the control area could then be determined using either mark-recapture or the equation involving immigration and solving for v. Plant and Cunningham (1991) detailed procedures for estimating the dispersal of Mediterranean fruit flies *Ceratitis capitata* (Wiedemann), and estimates of immigration could be obtained from considerations of dispersal.

The determination of density-dependence is problematic, because there are many models and none of them is particularly mechanistic. Thus rates of oviposition and subsequent survivorship would have to be monitored at various densities to derive a function to describe the depressing effects at various levels. In many wild populations, even just detecting the existence of density-dependence is difficult, much less the quantification of depressing effects. However, in view of the potential assistance to the SIT, an estimation of the effects of density-dependence is worthwhile. Itô and Yamamura (this volume) develop further the subject of parameter estimation.

4. ASSESSMENT OF SIT MODELLING

4.1. Uses of Models

Models can be used to predict and explain the behaviour of a population. This information guides research, generates hypotheses, and aids teaching. Most models of the SIT have so far have been aimed mainly at predicting the behaviour of pest populations under various constraints, such as incomplete sterility, lack of competitive ability of sterile males, the immigration of wild insects into a control area, etc. One of the best uses of models is to generate ideas or hypotheses that are capable of experimental testing. Thus, ideally, modelling should go hand-in-hand with field and laboratory experiments to verify or falsify a model's predictions.

4.2. Advantages and Limitations of Modelling

The models of the SIT constructed thus far fall generally into three groups: (1) models that investigate processes that determine the proportion of eggs laid that are sterile, (2) models involving population dynamics and other population level phenomena, and (3) models that investigate the interactions of the SIT with other control methods, although it might be argued that the last two really belong together.

The first category, including residual sterility, reduced sterile competitive ability, mating patterns, and immigration, is of crucial importance in planning and executing a programme that releases sterile insects. Unless one can accurately predict the level of sterility in eggs produced by the wild females, the programme is liable to fail. In addition, it is here that models are most likely to give realistic answers, as these processes rely mostly on determinable proportions or coefficients, rather than somewhat nebulous population processes.

Models of more general aspects of population dynamics involve many hidden factors, such as the strength of density-dependence, the functional responses of predators, synchrony of pest and predator phenologies, the degree of pest population aggregation, the extent to which sterile insects assume the same spatial patterns as the wild insects, etc. These are not easy to determine, and the models in the second and third categories must be taken as heuristic, rather than quantitatively predictive. They provide insights into the kinds of responses to expect, but quantitative accuracy must await species-specific simulations based on accurate and detailed biological and ecological information regarding the whole system.

4.3. Transient versus Equilibrium Models

Many analytic models of the SIT are solved for equilibrium, and the results of the parameters on the equilibrium are noted. In real life, populations are almost always changing. Analysis of the equilibrium behaviour certainly has much to say about the effects of the parameters on the transient behaviour as well as on the equilibrium, but a proper analysis should include the effects of the parameters on the dynamics of transient behaviour. The problem is that there is an infinite number of trajectories that any population can follow, and to encapsulate the behaviour of these in digestible form is no small task. One criterion that can be used is that of stability of the system, in its various forms. Stability characteristics can be related to parameter ranges, and certain characteristics of the resulting transient behaviour can be inferred from them.

4.4. Future Directions and Information Needs

The models reviewed in this chapter cover most of the relevant topics in the dynamics of the SIT. However, it is only a good beginning, and there is much left to do. Models that have a more realistic ecological basis will be required to suggest new hypotheses and to give more accurate predictions of behaviour. One area still largely untapped is metapopulation models — models including patches with migration among patches, local extinctions and re-establishments. A start has been made with the models including immigration, heterogeneity, and diffusion. The next step is to tie these together into a meaningful whole.

Another area, which will yield useful information, is the construction of species-specific models for the SIT, including all relevant factors. Many species-specific models have been constructed, but many appear to have inadequate detailed ecological information. In addition, the area of behavioural ecology will probably emerge as being especially relevant.

Testing models, experimentally and in the field, is in its infancy. Information is needed on the effects of pest density-dependent regulation on the efficiency of the SIT, the effects of predators and parasites on the dynamics of the SIT, and the effects of ecosystem resilience. The simultaneous use of other control methods with the SIT is still largely hypothetical, and this potentially useful area needs considerable investigation.

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PART III

TECHNICAL COMPONENTS OF THE STERILE INSECT TECHNIQUE

CHAPTER 3.1.

ROLE OF POPULATION AND BEHAVIOURAL ECOLOGY IN THE STERILE INSECT TECHNIQUE

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SUMMARY

The principles of population and behavioural ecology in relation to the application of the sterile insect technique (SIT) for eradication of a pest are explained. These include: (1) a logistic population model for estimation of the population fluctuation of target animals and the number of sterile males to be released for successful eradication, (2) mark-recapture estimations of density and mortality rate of the target population, especially for remote areas, where repeated releases and recaptures are difficult, (3) models of dispersal to assess dispersal distance of target animals, and (4) equations for estimating the decrease of sexual competitiveness of mass-reared strains under field conditions. The method to estimate dispersal distance curves when attraction areas of traps are overlapping, and changes in mate-choice of wild females resulting from inadvertent selection when the SIT is applied, are explained. The necessity of field estimation of sexual competitiveness of released sterile males is also emphasized.

1. INTRODUCTION

In a paper, amongst a set of three papers (Knipling 1955, Baumhover et al. 1955, Lindquist 1955) which included reports of the first success of the sterile insect technique (SIT) to eradicate insect pests, Knipling presented a table showing an example of model simulation for explaining the effect of sterile male releases. The model is

$$N_{g+1} = N_g RQ$$

where N_g and N_{g+1} are numbers of females at the gth and $(g+1)$th generation, and R and Q are rates of change in the population size per generation and the proportion of normal females (females which can lay hatchable eggs), respectively. R was first assumed to be constant, that is, density-dependency was neglected, but this model still shows that the SIT is a way to control insects, based completely on population ecology theory.

However, the early success in eradicating the New World screwworm *Cochliomyia hominivorax* (Coquerel), in the area-wide integrated pest management (AW-IPM) programme in Florida in 1959, was not always replicated in subsequent AW-IPM programmes integrating the SIT. Government officials began to think that the SIT was an established technique, and animal and plant health workers engaged in programmes releasing sterile insects began to plan and execute large-scale eradication programmes without basic ecological and behavioural studies. Many progammes carried out so far were made without first estimating the number of wild females, simulating the process based on a population model incorporating the SIT, and evaluating in the field the mating competitiveness of the released sterile males. Thus in many cases, in which many sterile males were released but eradication failed, it was not possible to know the major reason for failure, in spite of overflooding the wild population with sterile males, e.g. ratios of the number of sterile to wild males were 112:1 in a Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) programme in Nicaragua (Rhode et al. 1971), and 311:1 in another *C. capitata* programme on Procida Island (Cirio and de Murtas 1974). Without a full understanding of the population ecology, the planning, implementation, and evaluation of programmes that release sterile insects become very difficult (Barclay, this volume).

2. THEORETICAL POPULATION DYNAMICS

2.1. Logistic Model

The most basic model of population increase is the Hale-Malthus model:

$$dN_t/dt = rN_t \tag{1}$$

or integrating equation 1,

$$N_t = N_0 e^{rt} \tag{2}$$

where N_t, N_0, r and e are number of individuals at time t, number at the beginning of increase, intrinsic rate of increase, and the base of natural logarithms (= 2.71828...), respectively. If population increase with discrete generations, as seen in many insects, is considered, equation 1 can be written as

$$N_{g+1} = N_g R \tag{3}$$

or

$$N_g = N_0 R^g \tag{4}$$

the equation used by Knipling (1955), where $\ln R = r$ (Begon and Mortimer 1981).
In equations 1 and 3, the population size increases indefinitely, but the large N_t or N_g may result in a smaller rate of increase, due to density-dependency, and the population may reach an upper limit. The most widely used model of density-dependent population increase is the logistic model,

$$dN_t/dt = N_t(r - hN_t) \tag{5}$$

where h is the suppressive effect of existence of an individual on the intrinsic rate of increase. Here r/h is the upper limit of increase, and writing $r/h = K$,

$$\frac{dN_t}{dt} = rN_t \frac{K - N_t}{K} \tag{6}$$

or by integration,

$$N_t = \frac{K}{1 + e^{a - rt}} \tag{7}$$

where a is a constant.
To establish a population model of the melon fly *Bactrocera cucurbitae* (Coquillett) under control by the SIT, Itô (1977) used a discrete expression of

equation 7 to calculate generation-based increase of a logistic population (Fujita and Utida 1953). We have the following relation from equation 7 of g generation: $e^{a-rg} = (K/N_g) - 1$. By substituting this relation for equation 7 of $(g + 1)$ generation, we obtain

$$R_g = N_{g+1}\big/N_g = \frac{K}{1+e^{a-rg}e^{-r}}\bigg/N_g = e^r\big/(1+N_g B) \qquad (8)$$

where $B = (e^r - 1)/K$. In place of Itô's procedure, we can use a logistic difference model, such as

$$N_{g+1} = \frac{N_g R}{(1+cN_g)^b} \qquad (9)$$

where b and c are constants (Hassell 1975, Begon et al. 1996), in place of equation 4 for constant increase. Fig. 1 shows examples of Hale-Malthusian (dashed) and logistic increase of density (N_g).
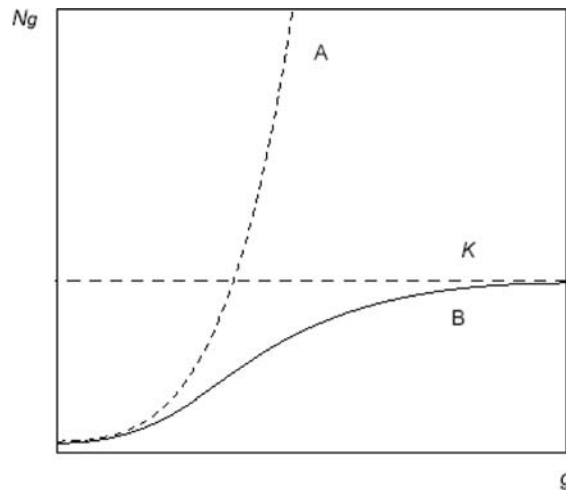
Figure 1. Exponential (A) and sigmoidal (B) increase in the population.
The equation giving a sigmoidal increase is the logistic model.

2.2. *Dynamics of Populations Under Control by SIT*

In equations 8 or 9, the population size of the next generation can be expressed as

$$N_{g+1} = N_g R_g \tag{10}$$

and when the SIT is applied

$$N_{g+1} = N_g R_g H_g \tag{11}$$

where H_g is the proportion of fertile (hatchable) eggs, and this value indicates the suppressive effect of sterile males on population increase. Thus H_g is considered to be a function of the ratio of the number of sterile males, N_s, to fertile (normal) males, N_f, in the field, that is,

$$H_g = f(N_{s(g)}/N_{f(g)}) \tag{12}$$

If the number of sterile males released in each generation is the same,

$$H_g = f(N_s/N_{f(g)}) \tag{12'}$$

how can f be determined? To establish a model of the SIT process for the melon fly on Kume Island, Okinawa, Itô (1977) adopted a Poisson distribution (mean number of matings = 1.61) for the frequency of matings per wild female. The probability that a female would mate with normal and/or sterile males was approximated by the binomial distribution. Based on this Poisson-binomial model, a curve showing the relationship between the expected hatchability of eggs and the N_s/N_f ratio was obtained (Fig. 2). The H_g values read from this graph are incorporated into equation 11 where R_g is derived from equation 8.

For the melon fly on Kume Island, Itô used the following values: $N_0 = 125\ 000$, $K = 2\ 700\ 000$, $r = 1.2$ (3.3 times increase per generation when N_0 is near 0), and $a = 3.971$. R_g changes in response to N_g, but based on the observed tendency that the number of melon flies decreases twice per year, in summer and winter in almost every year, Itô used the following R values for 4 months (assuming 12 generations per year): $R_4 = R_5 = 0.5$, $R_{10} = 0.2$ and $R_{11} = 0.238$. By these decreases the population returned to the minimum density (125 000). Calculation of N_g for the untreated period (before the SIT) gave a series of bimodal curves in which 125 000 and 2 621 568 were the annual minima and maxima, respectively (curve A of Fig. 3). H_g values estimated from Fig. 2, using N_g ($N_{f(g)}$ in equation 12) and a constant N_s, were used for simulation of the SIT process.
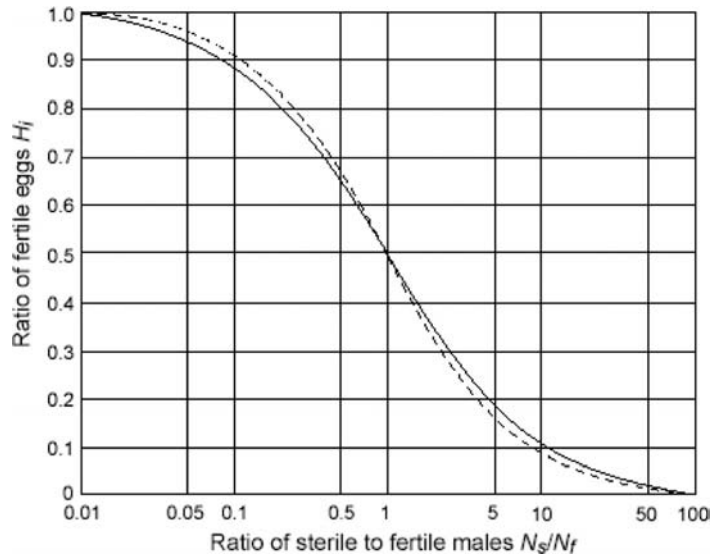
Figure 2. Relationship between the ratio of sterile to fertile males, and the ratio of fertile to sterile eggs, in a Poisson-binomial model (solid line). The broken line depicts the relationship when the insects mate only once. (Figure from Itô 1977, reproduced with permission.)

Curves B, C, and D of Fig. 3 are the results of simulation (initial ratios of sterile to normal males are shown in the legend). Fig. 3 indicates that, although eradication was not possible when the ratio of the number of sterile males released per month was one half of the minimum density of wild males ($N_0 = 125\,000$ and $N_s = 62\,500$), eradication can be attained only during 2 years when the number of released males is the same as the minimum density (curve C). Note that the ratio of sterile males to wild ones is less than unity when a decrease of wild insects has occurred.

When Itô published this result, it was unexpected since it shows that if the sexual competitiveness (hereafter mating competitiveness + sperm competitiveness) of released sterile males is the same as that of wild males, the release of much smaller numbers of sterile males than previously anticipated (e.g. 10/1 in Knipling 1955) can lead to success in an eradication programme. In the same year, using a similar logistic model for mosquito populations, Haile and Weidhaas (1977) obtained a similar result.

Once the curve of H_g against N_s/N_f is obtained, this curve can be used to estimate the sexual competitiveness of sterile males under field conditions. If the ratio of fertile eggs known from observation of eggs laid by wild females from the target population fell within the area below the curve in Fig. 2, this can be due to a reduction of sexual competitiveness of the released males.

The required ratio of sterile to wild males is a function of r or R. Itô and Kawamoto (1979) attempted to simulate the population processes based on Itô's model by substituting different values of r. Their results show that a ratio of 10 sterile males to 1 wild male could result in the eradication of the target species within only 12

generations even when the rate of increase per generation, R, was 20 ($r = 3$).

These results show that the quality (sexual competitiveness) of mass-reared and sterilized males is much more important than the overflooding ratio.

Barclay and Mackauer (1980), Barclay (1982), and Itô et al. (1989) provided detailed explanations of differential equation models of the SIT.
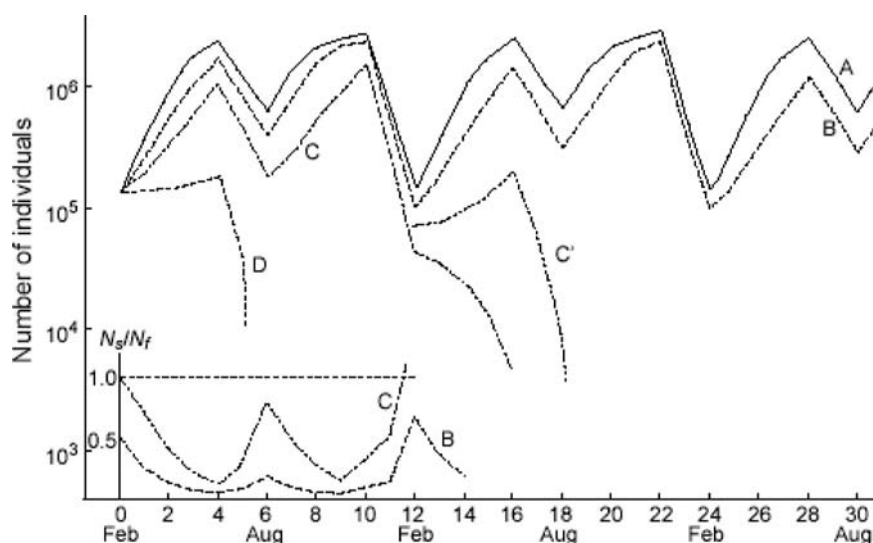
Figure 3. Fluctuations in the number of flies in the model population without the SIT (A) and populations with the SIT. The initial ratios of sterile to normal males are 0.5/1 (number of sterile males = 62 500) in B, 1/1 (number of sterile males = 125 000) in C, and 2/1 (number of sterile males = 250 000) in D, respectively. C' is the trend when winter mortality was halved in treatment C. Seasonal trends in the ratio of sterile to fertile (wild) males (N_s / N_f) are shown in the lower section of the figure. (Figure from Itô 1977, reproduced with permission.)

3. ESTIMATION OF POPULATION DENSITY AND MORTALITY BY MARK-RECAPTURE EXPERIMENTS

The previous section indicated that the number of sterile males to be released must be the same as, or more than, the minimum number of males in the target locality. Thus the density of target populations must be estimated to determine the appropriate number of sterile males for release. Since direct counting is not applicable to adults that move freely, mark-and-recapture methods are the only way to estimate the density of many species that are targets of the SIT. In this section, equations of mark-and-recapture methods are briefly explained. Krebs (1989) provided a detailed explanation of equations and ways of using them, and useful computer programmes for calculation.

3.1. Petersen Method

The Petersen method is the simplest way to use the mark-and-recapture method because it is based on a single episode of releasing marked animals and a second single episode of recapturing individuals. The equation is

$$\hat{N}_P = M_1 n_2 / m_2 \tag{13}$$

where $\hat{N}_P$, M_1, n_2 and m_2 are the estimated total population, the number of individuals marked and released on the first day (day 1), the number of individuals caught on day 2, and the number of marked individuals recaptured on day 2, respectively. Variance of $\hat{N}_P$ can be estimated by

$$V(\hat{N}_P) = \frac{M_1(M_1 - m_2)n_2(n_2 - m_2)}{m_2^3} \tag{14}$$

In a release experiment to estimate population size for the SIT, usually laboratory-reared insects are released. This is especially necessary at the final stage of the SIT, because at this stage the number of wild males is very small. The estimation of the number of wild individuals (unmarked individuals) is of greater interest than the total number of individuals. An estimate of the number of wild individuals, which is denoted by $\hat{U}_P$, can be obtained by

$$\hat{U}_P = M_1 u_2 / m_2 \tag{15}$$

where $u_2 = n_2 - m_2$. The variance is given by

$$V(\hat{U}_P) = \frac{\hat{U}_P(\hat{U}_P - u_2)(u_2 + m_2)}{u_2 m_2} \tag{16}$$

The following five assumptions are used for the Petersen method: (1) the population is closed, so the total number is constant, (2) all animals have the same chance of getting caught in the first sample, (3) marking individuals does not affect their catchability, (4) animals do not lose their marking(s) between the two sampling periods (Box 1), and (5) all marked individuals are reported on discovery in the second sample. Even when these assumptions hold, the Petersen method tends to overestimate the actual population, especially in small samples. For small samples (e.g. $m_2 < 10$), the use of the following two modified equations is recommended (for equations for variance, see Southwood 1978, and Itô and Murai 1978):

Box 1. Method to Estimate Loss of Marking(s)

The fourth assumption in the Petersen method, that animals do not lose marking, is a prerequisite for every model of mark-recapture estimation. Seber (1982) described a method to estimate the loss of marking.

Mark all the n_1 individuals in the first sample (or all the laboratory-reared animals released on day 1) in two ways, *A* and *B* (by two colours or two points on the body). Then

π_x = Probability that a marking of type *x* is lost by the time of the second sample (*x* = *A, B*),

π_{AB} = Probability that both markings are lost,

m_x = Number of marked animals caught on day 2, with mark *x* only,

m_{AB} = Number of marked animals on day 2 with both marks, and

m_2' = True number of recaptures on day 2 ($m_2' + u_2 = n_2$).

Then the maximum likelihood estimates of m_2', π_A and π_B are:

$$\pi_A = m_B / (m_B + m_{AB}),$$
$$\pi_B = m_A / (m_A + m_{AB}) \text{ and}$$
$$m_2' = (m_A + m_{AB})(m_B + m_{AB}) / m_{AB}$$
$$= c(m_A + m_B + m_{AB})$$

This means that the observed recapture ($m_A + m_B + m_{AB} = m_2$) must be corrected by a factor

$c\ [= 1/(1 - \pi_A)(1 - \pi_B)$] to give an estimate of the actual number of recaptures m_2'.

For large samples,

$$\hat{N}' = n_1 n_2 / m_2'$$

and, if laboratory-reared animals are released,

$$\hat{N}' = n_1 u_2 / m_2'$$

with

$$\hat{V} = \frac{N^3}{n_1 n_2} \pi_A \pi_B \left[\frac{1}{(1 - \pi_A)(1 - \pi_B)} \right]$$
$$+ \frac{N^3}{n_1 n_2} \left[1 + \frac{2N}{n_1 n_2} + 6 \left(\frac{N}{n_1 n_2} \right)^2 \right]$$

When $n_1 = 500$, $n_2 = 149$, $m_{AB} = 7$, $m_A = 1$ and $m_B = 2$,

$\pi_A = 2/(2 + 7) = 0.222$, $\pi_B = 1/(1 + 7) = 0.125$

$m_2' = [(1+7) \times (2 + 7)]/7 = 10.286$

As $m_A + m_B + m_{AB} = 10$, $m_2' - m_2 < 1$, suggesting that the rate of loss of marking is negligibly small. Here

$\hat{N}' = (500 \times 149)/10 = 7450$ and

$\hat{V} = 6869641$, and s. d. = 2621

$$\text{A:} \qquad \hat{N}_{P'} = \left[\frac{(M_1 + 1)(n_2 + 1)}{m_2 + 1} \right] - 1 \qquad\qquad (17)$$

$$\text{B:} \qquad \hat{N}_{P''} = \frac{M_1(n_2 + 1)}{m_2 + 1} \qquad\qquad (18)$$

If estimated N_P is very large as compared with M_1 and n_2 (e.g. $N_P > 10M_1$ or $10n_2$), equation 18 is recommended, but if N_P is relatively small and/or n_2 animals are killed during sampling (e.g. by trapping), equation 17 is recommended (Southwood 1978, Itô and Murai 1978).

The assumption of a closed population is usually not satisfied. For long-lived animals, mark and recapture during a short period may permit neglecting mortality, but the longevity of adult insects is often relatively short. However, Itô (1976) showed that the Petersen method gives the true population density on day 1 (before death) if only mortality exists, and gives the density on day 2 (after recruitment) if only recruitment exists. If both exist, the true density on day 1 is $N_P/(1 + B)$ or $N_P/(1 + B/S)$, where S and B are rates of survival and recruitment, respectively (Table 1). If there is no recruitment, the Petersen method gives a good estimate of density even when mortality and/or emigration exist. For a method to determine the existence of recruitment, see Seber (1982).

Table 1. Effects of mortality and/or emigration and dilution (emergence and/or immigration) on the Petersen estimates

	N_2	M_2	n_2	m_2	N_P
(1) Mortality/ emigration occurs but no dilution	SN_1	SM_1	pSN_1	pSM_1	$(pSN_1M_1)/(pSM_1)$ $= N_1$
(2) Dilution occurs but no mortality/ emigration	$N_1(1+B)$	M_1	$pN_1(1+B)$	pM_1	$[pN_1(1+B)M_1]/pM_1$ $= N_1(1+B)$
(3) Both mortality/ emigration and dilution occur	$N_1(S+B)$	SM_1	$pN_1(S+B)$	pSM_1	$[pN_1(S+B)M_1]/pSM_1$ $= N_1(1+B/S)$
(4) Same as in (3) but in reverse order	$SN_1(1+B)$	SM_1	$pSN_1(1+B)$	pSM_1	$[pSN_1(1+B)M_1]/pSM_1$ $= N_1(1+B)$

N_2, M_2, n_2 and m_2 are the numbers of individuals living in the target area, those marked and released, individuals caught on day 2, and recaptured on day 2, respectively.

N_P, S, B and p are Petersen estimates (of day 1), rates of survival and dilution from day 1 to day 2, and rate of capture, respectively.

3.2. Yamamura Method

Although the Petersen method is the simplest method of estimation, the estimates are subject to large biases because the method ignores the mortality that must exist in the field. Yamamura et al. (1992) proposed the second simplest procedure with field mortality, where: (1) individuals reared in the laboratory are marked and released, and (2) traps are used for the recapture census, and all captured individuals are removed from the field population. This method requires one release procedure and two capture censuses. Thus one more sampling census must be added to the Petersen method. There are four assumptions: (1) the wild population size is constant during the two consecutive sampling censuses (even if many wild individuals are lost by emigration from the study area or by artificial removal, the wild population size returns to the original level through immigration from the surrounding area), and (2) the proportion of marked individuals that survive and remain in the population between the two successive censuses, i.e. the rate of remaining, is constant. The last two assumptions are the same as (3) and (4) of the Petersen method.

If the marked individuals are released on day 1, and recaptured on day 2 and day 3, then the maximum likelihood estimates of the survival rate, S_Y, and the wild population size, U_Y, are given by

$$\hat{S}_Y = \frac{u_2 m_3}{m_2 u_3} + \frac{m_2}{M_1} \tag{19}$$

$$\hat{U}_Y = \hat{S}_Y M_1 u_2 / m_2 \tag{20}$$

3.3. Jolly-Seber Method

Most populations are constantly changing in size because birth (emergence for adult populations), death, immigration, and emigration are not always balanced. Although the wild population is assumed to be nearly constant in two successive census periods in the Yamamura method, this is not always the case. The Jolly-Seber method is applicable to such a changing population. Two or more releases are required to apply the Jolly-Seber method. Recapture censuses are also required two or more times, the first recapture being conducted just before the second release. Estimates are obtained by using the following equations (for small samples, see Seber 1982 or Krebs 1989):

$$\hat{M}_i = \left(R_i Z_i / r_i \right) + m_i \qquad (i = 2, 3, \dots, s{-}2)$$

$$\hat{U}_{J(i)} = \hat{M}_i u_i / m_i \tag{21}$$

$$\hat{N}_{J(i)} = \hat{M}_i n_i / m_i$$

$$\hat{S}_{J(i)} = \hat{M}_{i+1} / (\hat{M}_i - m_i + R_i)$$

$$\hat{B}_{J(i)} = \hat{N}_{J(i+1)} - \hat{S}_{J(i)}(\hat{N}_{J(i)} - n_i + R_i)$$

where

$\hat{M}_i$ = Estimated number of marked individuals living in the area just before the sample *i*.

$\hat{N}_{J(i)}$ = Total number of individuals just before the sampling at time *i*.

$\hat{U}_{J(i)}$ = Number of unmarked individuals just before the sampling at time *i*.

$\hat{S}_{J(i)}$ = Survival rate during *i* and *i*+1.

$\hat{B}_{J(i)}$ = Number of individuals that entered the population during *i* and *i*+1.

n_i = Total number of animals caught in sample *i* (= $m_i + u_i$).

m_i = Number of marked animals caught in sample *i*.

u_i = Number of unmarked animals caught in sample *i*.

R_i = Total number of animals released after sample *i*.

r_i = Number of the R_i individuals released at sample *i* and caught again in some later sample.

Z_i = Number of individuals marked *before* sample *i*, not caught in sample *i*, but caught in some sample after time *i*. Let m_{hj} be the number of marked animals caught in sample *j* last caught in sample *h* ($1 \leq h \leq j-1$). Then we obtain

$$Z_i = \sum\nolimits_{j=i+1}^{s} c_{i-1,j} \qquad \text{where} \qquad c_{i-1,j} = \sum\nolimits_{h=1}^{i-1} m_{hj}$$

An example calculation is shown in Box 2. The method of calculation for larger samples, and special tables to be used for these, are shown in Seber (1982) using good numerical examples including a method to estimate variances. The variance formula for $\hat{U}_{J(i)}$ is given by Yamamura et al. (1992). Assumption (3) of the Petersen method is especially important for the Jolly-Seber method. Krebs (1989) described many methods to assess whether this assumption is satisfied or not.

3.4. Hamada Method (Modified Jackson Positive Method)

The above-mentioned methods were listed in ascending order of the required amount of work. The Petersen method requires one release and one capture census, the Yamamura method one release and two capture censuses, and the Jolly-Seber method two releases and two capture censuses. The difference in requirements between the Yamamura and the Jolly-Seber methods seems to be especially large, since the addition of one more release usually requires more work than that of one more capture census. Since AW-IPM programmes releasing sterile insects are often carried out in remote areas, the addition of a release is especially laborious. Therefore in many cases one release is conducted, accompanied by three or more subsequent capture censuses, resulting in a series of regression estimates (see Jackson (1939) method).

Box 2. *Jolly-Seber Three-Point Method*

As a special case of the Jolly-Seber method, an example of estimating population parameters based on two releases and two recaptures is shown. Let us consider a typical case of mass-marking experiment in which individuals reared in the laboratory are marked and released. All captured individuals are killed. On day 1, individuals are released with a red mark. On day 2, individuals are released with a blue mark. The following is the result of a release experiment of *Spodoptera litura* (F.) conducted by Wakamura et al. (1992).

Day	n_i	R_i	U_i	Number of recaptured individuals	
				Red marked	Blue marked
1		1934			
2	409	1968	26	383	
3	633		24	181	428

The Jolly-Seber method yields

$$\hat{M}_2 = R_2 Z_2 / r_2 + m_2 = (1968 \times 181 / 428) + 383 = 1215$$

$$\hat{S}_{J(1)} = \hat{M}_2 / R_1 = 1215 / 1934 = 0.63$$

$$\hat{U}_{J(2)} = \hat{M}_2 u_2 / m_2 = 1215 \times 26 / 383 = 83$$

For the Yamamura method, we obtain

$$\hat{S}_Y = (u_2 m_3)/(m_2 u_3) + m_2 / M_1$$
$$= (26 \times 181)/(383 \times 24) + 383 / 1934 = 0.71$$

$$\hat{U}_Y = \hat{S}_Y M_1 u_2 / m_2 = 0.71 \times 1934 \times 26 / 383 = 93$$

For the Petersen method, we obtain

$$\hat{U}_p = M_1 u_2 / m_2 = 1934 \times 26 / 383 = 131$$

The estimate obtained by the Petersen method is much larger than that obtained by other methods. The Yamamura method generally yields results similar to those of the Jolly-Seber method if marked individuals are sufficiently mixed with wild individuals.

Jackson (1939) presented equations to estimate the density and survival rate in an open population by a single release and multiple recapture censuses (Jackson positive method). As a first step an index y_i is calculated using the following equation:

$$y_i = 10^4 m_i / M_0 n_i \tag{22}$$

where M_0 is the number of individuals marked and released on the first day (day 0; here day 0 is used to show the first day in place of day 1 as in the preceding methods, for simplicity of explanation of the regression method), y_i is a standardized number of marked insects to be recaptured on day i, assuming that 100 marked individuals are released on day 0 and 100 individuals are randomly caught on day i. Other symbols are the same as in the preceding equations. If total insect density is almost constant, and if marked individuals are returned to the field after being recaptured, by plotting y_i against i (Fig. 4) a survivorship curve of marked individuals in the field is obtained.
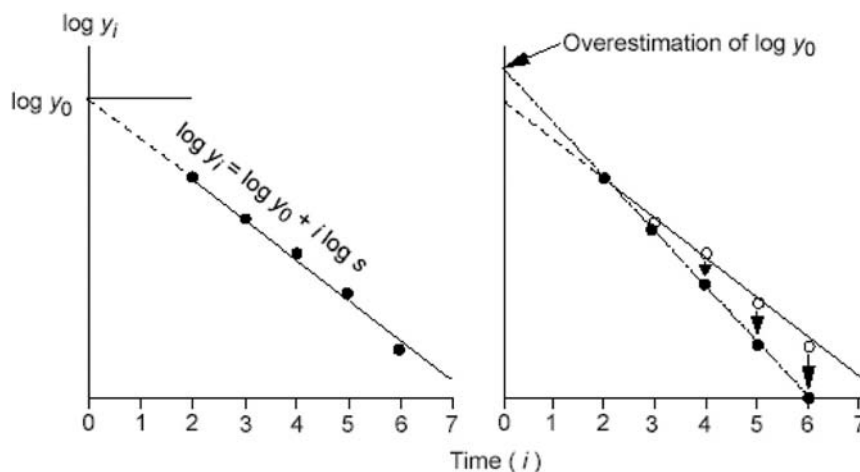
Figure 4. Comparison of Jackson positive and Itô methods using hypothetical values.
Assuming no trapping mortality, change in y_i *should parallel the survivorship curve of*
marked insects and, when daily survival rate is constant, the curve of $\log y_i$ *should be linear*
against i *(left). However, if there is trapping mortality,* y_i *should have smaller than expected*
values with time, and the linear regression should yield an overestimated value of $\log y_0$
(right), which in turn results in an underestimation of N_{J+}. *(Figure adapted from Itô et al.*
1989.)

If the survival rate is constant, the survival rate can be estimated using a linear regression:

$$\log y_i = \log y_0 + i \log S \tag{23}$$

where S is the survival rate per unit time (as $0 \le S \le 1$, $\log S$ is always negative), y_0 is a constant representing the expected number of recaptures on the assumption that 100 marked individuals released on day 0 are instantaneously intermingled into the wild population, and that 100 specimens are randomly caught before either mortality or recruitment occurs. Then an estimate of the total number of individuals on day 0 using

$$\hat{N}_{J+} = 10^4 / y_0 \tag{24}$$

is obtained together with the survival rate S from the slope of the regression line.

In the Jackson positive method, recaptured individuals should be returned to the original population. Since, in programmes integrating the SIT, recaptures are made with traps, the survival rate decreases as the number of recaptures increases, resulting

in an overestimated y_0 (Fig. 4). Thus N_{J+} will be underestimated. Itô (1973) devised a modified equation to reduce some of this bias by using a modified index y'_i, such as

$$y'_i = 10^4 m_i / M'_{0(i)} n_i \qquad (25)$$

where

$$\hat{M}'_{0(i)} = M_0 - \sum_{j=1}^{i-1} m_j$$

then

$$\log y'_i = \log y'_0 + i \log S \quad \text{and} \quad \hat{N}_I = 10^4 / y'_0$$

Hamada (1976) found that the Itô method gives an overestimated value when the number of released laboratory-reared marked males is much larger than the wild population size. He showed that a direct estimation of the number of wild males with the following equation gives the least (but not zero) stable negative bias (usually below 20%):

$$z_i = 10^4 m_i / M'_{0(i)} u_i \quad \text{and} \quad \hat{U}_H = 10^4 / z'_0 \qquad (26)$$

where $u_i = n_i - m_i$, and $\log z_i = \log z_0 + i \log \hat{S}$. The Hamada method has been applied widely to estimate the wild melon fly density in Okinawa. The Hamada method is based on a similar assumption as in the Yamamura method — the number of wild individuals is kept constant during the capture period. If the number of recapture censuses is two, the Yamamura method is preferable since it is the maximum likelihood estimate for this situation. Table 2 gives estimated densities and survival rates of male melon flies using the Petersen and Hamada methods, showing the relative stability of the estimated values.

3.5. Jackson Negative Method

Jackson (1939) also presented another model (Jackson negative method) to estimate the population size based on multiple-release single-recapture data. Marked individuals are released on several occasions (on days $-i$, $(-i + 1)$, ... , -1) with different markings, and thereafter a single random catch is made on day 0. Here

$$y_{-i} = 10^4 m_{-i,0} / M_{-i} n_0 \qquad (27)$$

where $m_{-i,0}$, the number of individuals released on day $-i$ and recaptured on day 0, is expected to increase with time. If the survival rate of marked individuals is constant,

one can estimate y_0 by the linear regression of log y_{-i} on i, that is,
log y_i = log y_0 + i log S. Then, as in the positive method, N_{J-} is

$$N_{J-} = 10^4/y_0 \tag{28}$$

When laboratory-reared individuals are released, the following equations are applicable:

$$y'_{-i} = 10^4 m_{-i,0} / M_{-i} u_0, \quad \text{and} \quad U_{J-} = 10^4 / y'_0 \tag{29}$$

Reisen et al. (1979) and Koyama et al. (1982) noted that the trapping mortality, which induces a negative bias in the Jackson positive method, does not cause bias in density estimates obtained by the negative method because recapture is made only once for any group of released individuals. This method was used to estimate melon fly density in mountainous parts of Okinawa. Multiple releases of marked flies were made from a helicopter, followed by a single recapture by the many persons who checked traps.

Table 2. Comparison of estimates of the density of male melon flies with the Petersen and Hamada methods (data from Tanaka et al. 1978)

Station	Date of release	$\hat{U}_P$ /ha	$\hat{U}_H$ /ha	$\hat{S}$ /day[1]
1	June 28	136	85	0.81
	July 12	173	87	0.83
2	July 22	166	109	0.74
	August 9	251	180	0.77

$\hat{U}_P$ and $\hat{U}_H$ are the numbers of wild males estimated by the Petersen and Hamada methods, respectively. For the Petersen method, the numbers of males caught on the 4th (station 1) and 2nd (station 2) days after release are used as n_2 and m_2 (equation 13). Numbers of recaptures in the Hamada method are four in station 1, and five in station 2.

[1] Estimated by the Hamada method, using linear regression of log z_i and log $\hat{S}$ in equation 23

4. ESTIMATION OF DISPERSAL DISTANCE BY MARK-RECAPTURE EXPERIMENTS

The immigration of wild insects into an area treated with sterile insects is one of the most important causes of failure of some AW-IPM programmes integrating the SIT (Lance and McInnis, this volume). If wild males have sufficient dispersal ability, the male sterile:fertile ratio ($N_s/N_{f(g)}$ in equation 12') will decrease, causing higher

fertility in wild females. Estimation of the dispersal range is also important for estimating the $N_s/N_{f(g)}$ ratio from field data. If the dispersal range is small, the $N_s/N_{f(g)}$ ratio should be estimated using samples obtained from the small area, whereas if the dispersal range is large, samples obtained from the large area should be combined. Furthermore, if the range of dispersal is known, an optimal spatial design for the release of sterile males can be constructed. If the dispersal range is small, sterile males should be released at many spatial points to increase uniformly the male sterile:fertile ratio. In this section several techniques to estimate the dispersal range of individuals are described.

4.1. Diffusion Equation

A two-dimensional simple diffusion equation will be the simplest theoretical model that can be applicable to the two-dimensional dispersal of marked individuals. Assuming that the movement of marked individuals is Brownian random motion (the rate of which is invariant in time and space), the number of marked individuals at time t at coordinate (x, y), which is denoted by $m(x, y, t)$, is described by a partial differential equation (Okubo 1980, Shigesada and Kawasaki 1997):

$$\frac{\partial m(x, y, t)}{\partial t} = D\left(\frac{\partial^2 m}{\partial x^2} + \frac{\partial^2 m}{\partial y^2}\right) \tag{30}$$

where D is the diffusion coefficient that measures the dispersal rate with units (distance^2/time). When M_0 individuals are released at time 0 from the origin (0, 0), the solution is given by

$$m(x, y, t) = \frac{M_0}{4\pi Dt}\exp\left[-\frac{(x^2 + y^2)}{4Dt}\right] \tag{31}$$

which is a two-dimensional normal distribution with mean zero, correlation coefficient zero, and variance $2Dt$ in each dimension. If the distance from the origin is denoted by $r = \sqrt{x^2 + y^2}$, then equation 31 is rewritten in a simpler form

$$m(r, t) = \frac{M_0}{4\pi Dt}\exp\left[-\frac{r^2}{4Dt}\right] \qquad (r \geq 0) \tag{32}$$

The proportion of individuals in a circle of radius d at time t, which is denoted by $F(d, t)$, is given by

$$F(d,t) = \frac{1}{M_0} \int_0^d 2\pi r \cdot m(r,t) dr = 1 - \exp\left[-\frac{d^2}{4Dt} \right] \tag{33}$$

Thus the proportion of individuals in a circle of radius $\sqrt{4Dt}$ is $1 - \exp(-1) = 0.63$, and that in a circle of radius $2\sqrt{4Dt}$ is $1 - \exp(-4) = 0.98$. A rearrangement of the above equation yields

$$\log_e[1 - F(d, t)] = -d^2/(4Dt) \tag{34}$$

Therefore, if the distribution of marked individuals is observed at time t, an estimate of D can be obtained by plotting the observed $\log_e[1 - F(d, t)]$ against d^2 and by estimating the slope $-1/(4Dt)$ using a liner regression with intercept zero (Broadbent and Kendall 1953). If the relation is not linear, it can be judged that the dispersal is not a random diffusion with a constant D (e.g. Inoue 1978). The expectation of the square of the distance is given by

$$E[r^2] = \frac{1}{M_0} \int_0^\infty 2\pi r \cdot r^2 \cdot m(r,t) dr = 4Dt \tag{35}$$

Hence the moment estimate of D is given by the observed mean square of the distance divided by $4t$. If estimates of the mean square distance for several t are available, the common D can be estimated by plotting the mean square distance against t and by estimating the slope $4D$ using the linear regression with intercept zero.

4.2. *Distribution of Cumulative Recaptures*

Traps are frequently used to capture marked individuals (IAEA 2003). If traps are set for a sufficiently short period around t, the observed distribution of captured individuals can be used for a sample distribution of dispersing individuals at time t. If traps are set for a longer period, the observed distribution cannot be used for a distribution of a specific time. Note that a trap also performs some integration of density over space as well as over time. Such a spatial integration may cause some difficulties in the estimation of dispersal distance; one such difficulty is discussed in Box 3.

Box 3. Overlap of Attraction Areas of Traps

In a study of the dispersal of marked male sweetpotato weevils *Cylas formicarius* (F.), and using plastic funnel traps with 1 mg sex pheromone, Miyatake et al. (2000) placed traps in eight directions, and at distances of 10, 20, 50, 100, and 200 m, from the release point. However Yasuda and Sugie (1990) estimated that the radius of the circular attraction area of a trap (area of 30% recapture) is about 50 m. According to Fig. 52 in Yasuda (1998), the radius of 60% recapture is less than 30 m. (The distribution of the attraction rate around a trap might be a normal distribution or a distribution with larger kurtosis. Therefore, how can the radius of the "attraction area" be determined?) If we use a trap site of 10 or 20 m from the release point, the attraction areas of traps placed by Miyatake et al. (2000) at sites near the release point are overlapping, and many males might be attracted by two or more traps. Thus such traps might attract more individuals when they are in an isolated place, leading to an overestimation of the dispersal range.

   If traps were distributed in a lattice pattern, the effect of the overlap of traps can be reduced to a negligible level. If individually marked insects are released from many points of the lattice, reduction of the effect is better. Therefore it is recommended that a lattice-pattern arrangement of traps be used. However, since many data are taken if traps are arranged along four or eight directions to estimate the dispersal range, a method that reduces the effect of overlap of attraction areas is described hereafter.

   Fig. A shows an example of the effect of overlap of attraction areas. Individuals in the striped area would be attracted to two traps. Therefore, we must calculate the area of half of the striped area (double stripes) and subtract this from the area of the circle. In Fig. A, the distance between the two traps, and the radius of the attraction area, are $2a$ and R, respectively. As $\theta = \cos^{-1}(a/R)$, θ can be calculated when R is known. The area of the triangle ABC of Fig. A is $\left[2\sqrt{R^2 - a^2} \times a\right]/2$. Then half of the striped area can be calculated by $\left[\pi R^2 \times 2\theta/(2\pi)\right] - \left[\sqrt{R^2 - a^2} \times a\right]$. This area must be subtracted from the attraction area of a trap, πR^2, to obtain the true attraction area.

   When this procedure is carried out for data from Miyatake et al. (2000), a smaller value for dispersal distance is obtained (Fig. B). Although Miyatake et al. (2000) set traps along eight directions on circles of which the radii were 10, 20, 50, 100, and 200 m from the single release point, data for 10 m are omitted because the attraction areas of three or more traps overlap at 10 m points.
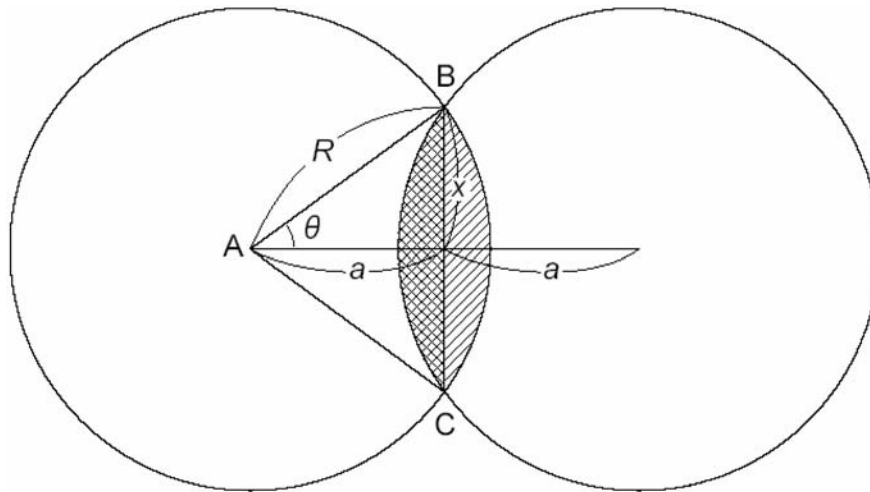
Figure A. Method to estimate overlapping area (striped area) of attraction of two traps.
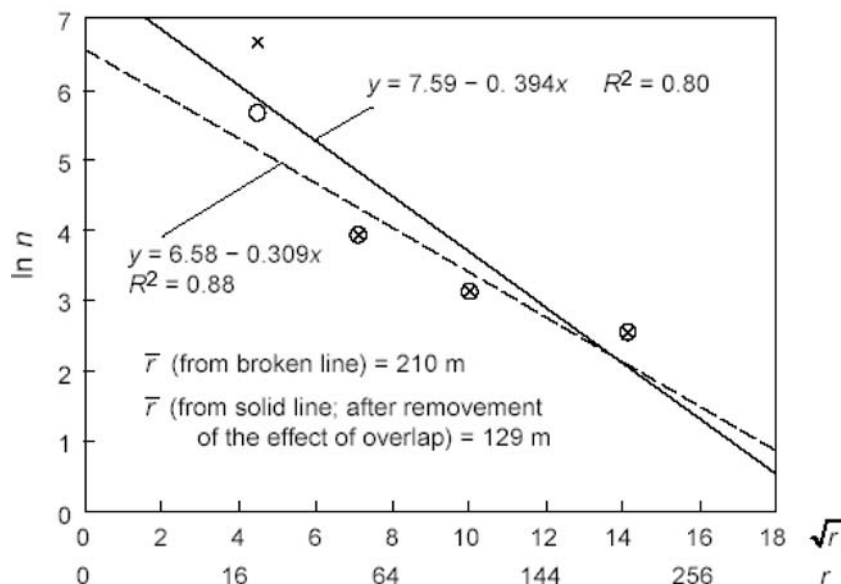
Figure B. Recapture rate of sweetpotato weevils in traps at different distances (r) from release point (circles and broken line), and removal of effect of attraction-area overlap (radius = 10 m, crosses and solid line).

In several experiments, marked individuals have been trapped until most of them died or left the study area. In these cases estimates of the distribution of the cumulative number of recaptured individuals in each trap can be obtained. The theoretical distribution of the cumulative recaptures can be described by relatively simple equations under several assumptions. Let δ be instantaneous natural mortality (or disappearance rate), and α trap efficiency. Assuming that the number of recaptures is sufficiently small relative to the number of total release, M_0, so that the mortality δ is not influenced by the mortality caused by trapping, then the cumulative number of recaptures at a trap placed at a distance r is given by

$$C(r) = \frac{\alpha M_0}{2\pi D} K_0\left(\sqrt{\frac{\delta}{D}}\, r\right) \tag{36}$$

where K_0 is a zero order modified Bessel function of the second kind (Broadbent and Kendall 1953, Williams 1961). Turchin and Thoeny (1993) used an approximation for equation 36

$$C(r) \approx \frac{A}{\sqrt{r}} \exp\left(-r\sqrt{\frac{\delta}{D}}\right) \tag{37}$$

where A is a constant. This equation can be described by a linear form,

$$\log_e[C(r)] + \frac{1}{2}\log_e(r) \approx \log_e(A) - r\sqrt{\frac{\delta}{D}} \tag{38}$$

therefore $\sqrt{\delta/D}$ can be estimated by plotting the observed $\log_e[C(r)] + \frac{1}{2}\log_e(r)$

against r, and by estimating the slope by using a linear regression method, although such an estimation procedure is not preferred from a statistical point of view. Like Inoue's (1978) method using equation 34, equation 38 can be used to judge the randomness of dispersal. If the relation is not linear, then dispersal is not a simple random diffusion with a constant D (Cronin et al. 2000). Note that δ and D cannot be estimated separately since the accumulation of recaptures eliminates information on the velocity of dispersal in this estimation procedure.

There is a dilemma in applying these models. If trap efficiency is high, these models cannot be applied since δ is influenced by the mortality caused by traps, whereas if trap efficiency is low, the spatial distribution cannot be estimated with sufficient precision. This dilemma can be solved by a uniform placement of traps. If traps are placed uniformly in a lattice pattern, the mortality caused by traps is constant, and hence δ will be kept constant irrespective of trap efficiency.

4.3. Empirical Distributions

The models described above, being based on clear assumptions such as random diffusion and constant mortality, do not always fit the data sufficiently well. The heterogeneity among individuals used to calculate the diffusion coefficient, along with the spatial and temporal heterogeneity, may be one of the causes of such discrepancy. A promising approach for incorporating the heterogeneity of the diffusion coefficient is to assume that the population consists of two groups with different D, as seen in Inoue (1978) and Cronin et al. (2000). An actual population may sometimes consist of many groups that have different tendencies of dispersal. At present, however, there is no simple theoretical model to describe such complicated situations. Therefore, in such cases, empirical equations will still be useful for describing the dispersal of individuals.

Taylor (1980) and Turchin (1998) pointed out the usefulness of an empirical equation to describe the number of recaptures, $\phi(r)$, in a trap placed at a distance r:

$$\phi(r) = \lambda r^{-\varepsilon} \exp\left[-(r/\beta)^{\gamma}\right] \tag{39}$$

where β , λ , ε , and γ are constants. Several empirical equations are given by special cases of equation 39. If $\varepsilon = 0$ and $\gamma = 2$, we obtain a half-normal distribution (Itô and Miyashita 1965):

$$\log_e[\phi(r)] = a - br^2 \tag{40}$$

where $a = \log_e(\lambda)$ and $b = 1/\beta^2$. If $\varepsilon = 0$ and $\gamma = 1$, we obtain an exponential distribution (Kettle 1952):

$$\log_e[\phi(r)] = a - br \tag{41}$$

where $a = \log_e(\lambda)$ and $b = 1/\beta$. If $\varepsilon = 0$ and $\gamma = 0.5$, we obtain the equation used by Wallace (1966):

$$\log_e[\phi(r)] = a - b\sqrt{r} \tag{42}$$

where $a = \log_e(\lambda)$ and $b = 1/\sqrt{\beta}$. Several theoretical distributions are also described by equation 39. The instantaneous distribution of dispersing individuals, equation 32, corresponds to the case of $\gamma = 2$ and $\varepsilon = 0$. The cumulative distribution under random dispersal, equation 37, corresponds to the case of $\gamma = 1$ and $\varepsilon = 0.5$ of equation 39. Taylor (1978) compared the descriptive ability of these empirical equations, and showed that Wallace's equation (equation 42) is most preferred. Plant and Cunningham (1991), in analysing the dispersal of sterile Mediterranean fruit flies, also concluded that equation 42 is most preferred.

The parameters such as a or b of the above equations cannot readily be interpreted in biological terms. Several statistics will be more useful than the parameters themselves for describing the dispersal ability of individuals. Hawkes (1972) suggested that "mean dispersal distance" be used. For equation 39, the statistic is calculated by

$$\bar{r} = \frac{\int_0^\infty 2\pi r^2 \phi(r)dr}{\int_0^\infty 2\pi r\phi(r)dr} = \beta\Gamma\left(\frac{3-\varepsilon}{\gamma}\right)\Big/\Gamma\left(\frac{2-\varepsilon}{\gamma}\right) \tag{43}$$

where Γ indicates the gamma function. In the case of equation 42, for example, we obtain $\bar{r} = 20/b^2$ from equation 43. The median dispersal distance, $r_{0.5}$, i.e. the radius of a circle that encloses 50% of the individuals, will be another useful statistic for the description of the dispersal ability of individuals (Turchin and Thoeny 1993). This

statistic is obtained by numerically solving the equation

$$\frac{\int_0^{r_{0.5}} 2\pi r\phi(r)dr}{\int_0^{\infty} 2\pi r\phi(r)dr} = 0.5 \tag{44}$$

Fig. 5 is an example of fitting equation 42 to data from male sweetpotato weevils. For simplicity, linear regression was used to estimate b, 0.342. Hence the estimate of mean dispersal distance is obtained by $20/0.342^2 = 171$ m. The median dispersal distance was estimated by a numerical calculation to be 115.2 m. The mean dispersal distance is much larger than the median dispersal distance, since the form of $\phi(r)$ is highly leptokurtic, i.e. L-shaped. In summarizing the characteristics of the dispersal curve, the median dispersal distance will generally be preferred over the mean dispersal distance.

5. BEHAVIOURAL ECOLOGY: SEXUAL COMPETITIVENESS OF RELEASED STERILE MALES IN FIELD

The quality of sterile males to be released is the most important element in the success of an AW-IPM programme integrating the SIT. Although quality includes survival rate, dispersal ability, and other aspects that relate to the vigour of released males, the most attention must be paid to the decline of sexual competitiveness (Calkins and Parker, this volume; Lance and McInnis, this volume; Vreysen, this volume).

Even when the survival rate or dispersal ability of mass-reared and sterilized males is lower than those of wild males, an increase in the frequency of mass releases or the number of release points can compensate for these deficiencies. However, if long-term mass-rearing creates a strain in which released males have a different courtship behaviour (pattern of vibration, courtship sound, etc.) than that of the wild males and therefore are not accepted by wild females, an increase in the number of released sterile males cannot compensate for this deficiency.

This is a subject of behavioural ecology or sociobiology. Although sexual behaviour and behavioural changes in mass-reared strains have been studied in several species that are targets of the SIT (e.g. Prokopy and Hendrichs 1979, Sivinski et al. 1989), probably the only AW-IPM programme releasing sterile insects, which incorporated behavioural ecology as an important aspect throughout implementation, has been the melon fly eradication programme in Okinawa, which was completely successful (Yamagishi et al. 1993).
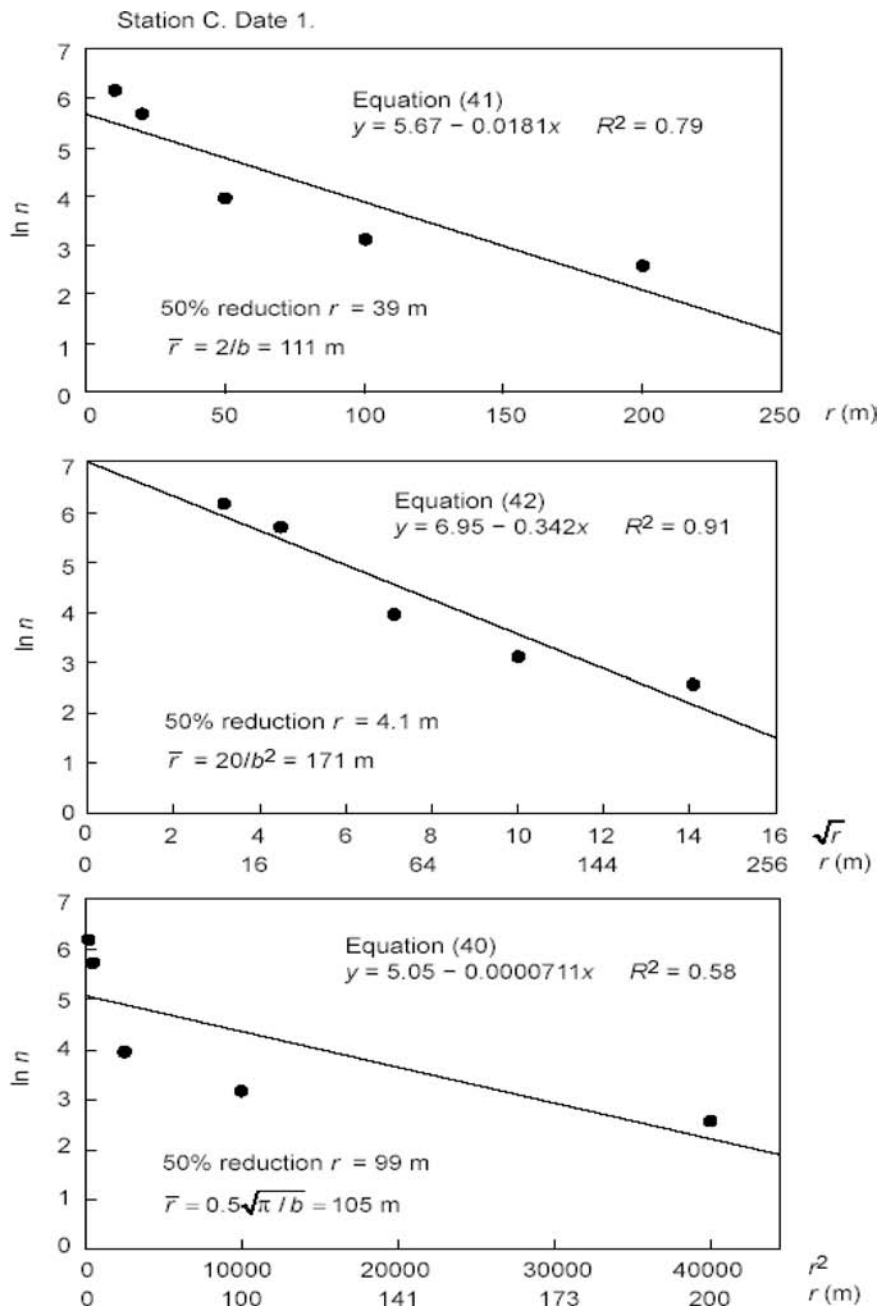
Figure 5. Distance-density curve of number of marked adult sweetpotato weevils recaptured in traps. Equation 42 is fitted by linear regression. (Data from Miyatake et al. 2000.)

5.1. Effects of Long-Term Mass-Rearing More Important than Effects of Sterilization

The effects of sterilization, e.g. by γ-radiation, are often regarded as an important source of the decline of vigour and/or sexual competitiveness of males (Bakri et al., this volume). However, experiences accumulated during the Okinawa melon fly programme suggest that changes in sexual behaviour of released males due to long-term mass-rearing are much more important than the effects of sterilization (Itô et al. 1993; Calkins and Parker, this volume). The inadvertent selection of strains with rapid development and early fecundity produces reduced longevity and other genetic changes in the selected strain (Miyatake 1996, 1998; Shimizu et al. 1997). The high-density rearing of adults in small cages can lead to the selection of strains in which reared males do not perform the sexual behaviours of wild males in the field.

5.2. Competitiveness Must be Measured in Field

In many AW-IPM programmes integrating the SIT, sexual competitiveness has been measured in the laboratory, e.g. observing wild females mating with marked wild males and mass-reared and/or sterilized males in a cage (Fried 1971; Calkins and Parker, this volume). However, conditions in a laboratory cage are different from those in the field (Lance and McInnis, this volume; Vreysen, this volume). In a programme to eradicate the melon fly from Kume-zima, Okinawa (1973–1977), Iwahashi et al. (1983) measured sexual competitiveness in the field. They collected wild females from Kume-zima, the target area, and Okinawa-Hontô, the control (non-SIT) area, and examined the hatch rates of eggs laid by those females.

Haisch (1970) presented the following equation for the laboratory examination of competitiveness, c:

$$\hat{c} = \frac{H_n - H_c}{H_c - H_s} \cdot \frac{w}{1-w} \tag{45}$$

where
w = proportion of males of wild strain among all males,
H_n = percentage egg hatch in matings between normal (wild) males and females of wild strain,
H_c = percentage egg hatch in competitive matings,
H_s = percentage egg hatch in matings between sterile males and normal females.

To use this equation in the field, Iwahashi et al. (1983) substituted percentage hatch of eggs laid by females collected in the target and control areas for H_n and H_c, respectively. H_s is 0 in the Okinawa melon fly programme. For comparison of c values while applying the SIT, or between two or more SIT areas, an estimation of variance is necessary. Iwahashi et al. (1983) presented an equation for estimating the variance of c (Box 4).

Box 4. Variance of Haisch Index of Sexual Competitiveness of Mass-Reared/Sterilized Males

In the final stage of an eradication programme that releases sterile insects, when most males collected are sterile males, $1 - w$ becomes near zero. In this stage, H_c may also become small. As c values become quite sensitive to small changes in $1 - w$ and H_c, estimation of variance is necessary. The following equation from Iwahashi et al. (1983) is recommended:

$$V(\hat{c}) = \left[\frac{w}{H_c(1-w)} \right]^2 \times$$

$$\left[\frac{H_n(1-H_n)}{N_n} + \frac{H_n^2(1-H_c)}{H_c N_c} + \frac{(H_n - H_c)^2}{w(1-w)N_w} \right]$$

Here N_n and N_c are the numbers of eggs examined in the control area and the release area, respectively. N_w is the number of flies examined in the release area. For other symbols, see explanation of equation 45.

Competitiveness decreased from about 80% in the 5th generation after the beginning of mass-rearing to 20% in the 18th generation, during the final stage of the Kume-zima programme (Fig. 6, Upper). Even at this time, high sexual competitiveness in laboratory cages was still observed (see closed triangles in Fig. 6, Upper).

Soemori et al. (1980) described experiments that suggest an explanation for such a discrepancy between laboratory and field data. They released individually marked flies into cages or rooms of different sizes and recorded the matings. Fig. 6 (Lower) shows the percentage of males that mated in relation to the volume of space available per male in the experimental area. Males of the wild strain could not mate well in a small space, whereas males of the laboratory strain performed best in these conditions.

5.3. Inadvertent Selection of Mate-Choice When SIT Applied

In a field cage, Hibino and Iwahashi (1988) compared the mating success of males of wild and mass-reared strains. One of their results, using flies of a wild strain of Okinawa Hontô (O-males and O-females), is shown in Fig. 7 (Upper). Firstly, even when courted by wild males, wild females accepted copulation in only 4 of 37 courtship trials, showing strong mate-choice by females. Secondly, it is noted that O-females never accepted (0/46) courtship from mass-reared (R) males. Other experiments showed similar results (Itô et al. 1993). When Hibino and Iwahashi (1988) carried out these experiments, melon flies on Okinawa Hontô, as a result of the application of the SIT, were near extinction. Therefore O-females had been subjected to strong selection pressure by the released sterile males.

Hibino and Iwahashi (1991) carried out similar experiments using wild flies taken from Ishigaki-zima, where a programme releasing sterile insects had not yet begun. Fig. 7 (Lower) shows that females of Ishigaki wild flies (I-females) accepted courtship from R-males (4/57) as well as from I-males (3/51).
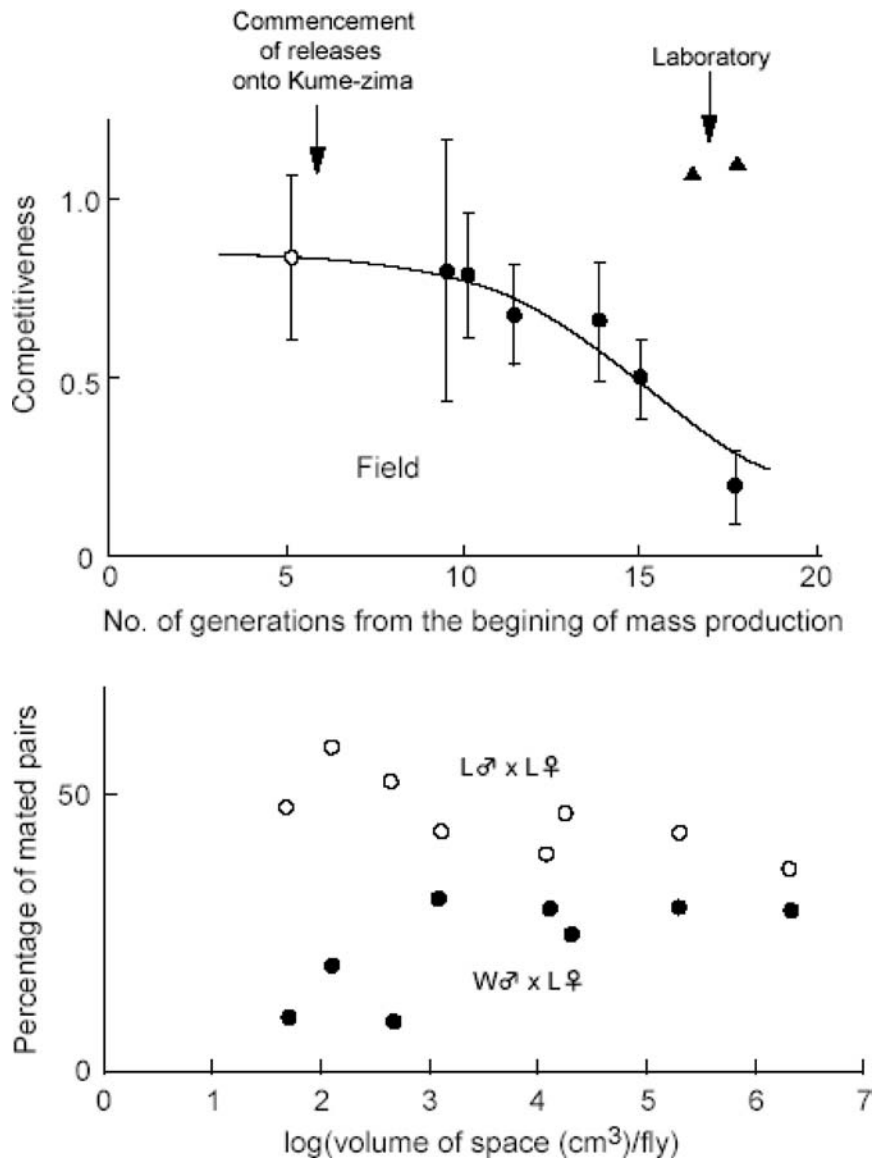
Figure 6. Upper: Sexual competitiveness of mass-reared and sterilized male melon flies measured in the field (adapted from Iwahashi et al. 1983). Closed circles are for data from Kume-zima, open circle is for data from another islet Kudaka-zima. Solid triangles are competitiveness values measured in laboratory cages. Vertical lines show standard deviations. (Box 4 shows the equation for variance.) Lower: Relationship between size of cage per fly and percentage of successful mating of males of mass-reared (open circles, 33–34 generations) and wild (closed circles) strains when caged together with mass-reared females. (Figure from Soemori et al. 1980, reproduced with permission.)
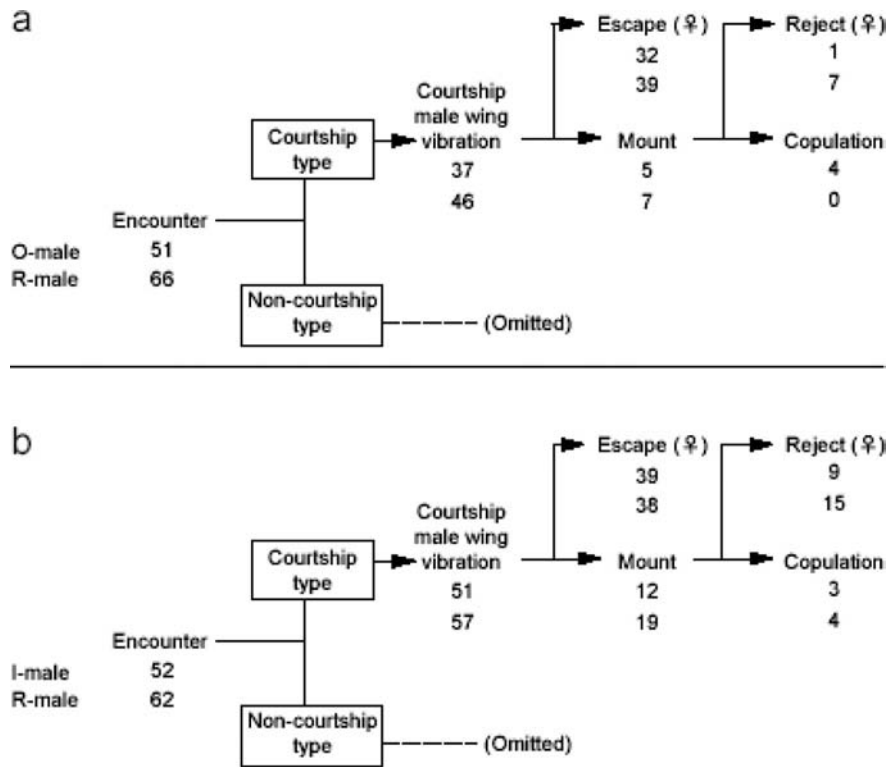
Figure 7. Upper (a): Mate-choice by wild female melon flies collected on Okinawa Hontô (O-females) for wild males (O-males) or males of mass-reared strain (R-males). Arrows indicate the direction in which the behavioural sequence proceeds. Upper numerals indicate the frequencies of the transitions when O-females encountered O-males, lower numerals indicate cases when O-females encountered R-males (adapted from Hibino and Iwahashi 1988). Lower (b): Mate-choice by wild female melon flies collected on a non-SIT island Ishigaki-zima (I-females) for Ishigaki wild males (I-males) or mass-reared males (R-males). (Figure from Hibino and Iwahashi 1991, reproduced with permission.)

There are two possible explanations for the difference in behaviour between flies on the two islands: (1) O-flies and I-flies had genetically different courtship and acceptance characters, and the courtship character of R-males was more similar to that of I-males, and (2) the "SIT-resistance hypothesis" — the wild female population was initially heterogeneous and contained individuals which accepted a broad range of male courtship characters (Lance and McInnis, this volume; Whitten and Mahon, this volume). However, females that accepted sterilized R-male courtship could not produce progeny. Thus, under strong selection pressure from the SIT, a female genotype that accepted the courtship of mass-reared males may have become extinct. Next Hibino and Iwahashi (1991) conducted an experiment on mate choice of

O-females between O- and I-males. O-females then accepted courtship by I-males (1/19) as well as by O-males (1/14), indicating that the second explanation is correct, thus demonstrating for the first time the evolution of mate-choice in insects.

5.4. How Can Spread of SIT-Resistant Strain be Overcome?

How can the problem of an increase in the number of females that do not accept courtship from mass-reared males be overcome? An answer is provided by the development of the logistic population model (e.g. equation 8).

Tsubaki and Bunroongsook (1990) conducted a simulation experiment to estimate the effect of the change in mate-choice, using a logistic model (equation 8) incorporating two strains. They showed that the effect of a reduction in mating competitiveness of mass-reared and sterilized males is much more important than the effect of a change in female mate-choice. Their simulation also indicated that releases of two or three times more sterile males than would be released in a non-mate-choice model can eradicate a target population that has a change in female mate-choice. In the Okinawa melon fly AW-IPM programme integrating the SIT, an increase in the number of released sterile males over that first planned for release (based on an estimate of the wild fly density) resulted in complete eradication of the population.

The incorporation of recent ideas in behavioural ecology, as well as in population ecology, into the SIT is indispensable for its success. It is recommended that Krebs and Davies (1993) be consulted on the basic concepts of this subject.

6. ACKNOWLEDGEMENTS

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CHAPTER 3.2.

MASS-REARING FOR STERILE INSECT RELEASE

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SUMMARY

As the sterile insect technique (SIT) relies upon released sterile male insects efficiently competing with wild males to mate with wild females, it follows that mass-rearing of insects is one of the principal steps in the process. Mass-rearing for the SIT presents both problems and opportunities due to the increased scale involved compared with rearing insects for most other purposes. This chapter discusses facility design, environmental concerns, strain management, quality control, automation, diet, sex separation, marking, and storage in relation to rearing for the SIT.

1. INTRODUCTION

The sterile insect technique (SIT) depends upon inducing a high proportion of sterile matings in a natural population that reduces reproduction to a level below population maintenance. Therefore, the production of insects, in sufficient number and of adequate quality to achieve this aim, is one of the principal requirements for the success of the technique. Further, since the integration of the SIT into an area-wide integrated pest management (AW-IPM) programme competes economically with other control techniques, the production of insects must be timely and cost effective. Large numbers of insects are required for AW-IPM programmes, and therefore it is possible to take advantage of economies of scale in the rearing.

There are numerous published accounts of the rearing of many insect species including general reviews (Smith 1966, King and Leppla 1984, Singh and Moore 1985, Anderson and Leppla 1992, and Ochieng'-Odero 1994a), and detailed studies for many individual species and groups (e.g. Zethner 1980, Brown 1984, Stewart 1984, Bigler 1986, Vargas 1989, Gerberg et al. 1994, Nordlund 1999, Mahon and Ahmad 2000, Yamagishi and Kakinohana 2000). Insects are reared for many reasons — bioassays, physiological research, rearing parasitoids, postharvest treatment testing, etc. (Singh and Ashby 1985), where rearing is rarely an end in itself. For these purposes, the cost of rearing is not critical, e.g. the diets tend to be all inclusive, rather than minimal, and once a diet is developed that is able to maintain an adequate colony, little or no further work is done on it.

For the SIT, a rather different approach is needed, with due attention being paid to all the factors affecting quality, fecundity, and cost. Even though Singh (1985) listed more than 1300 species that have been reared on artificial diet in the laboratory for part or all of their life cycle, relatively few species have been mass-reared for the SIT (IDIDAS 2004). The details of the rearing protocol for any one species will not be discussed here, but examples to illustrate key points and issues common to the successful application of the SIT will be given.

2. ECONOMICS

Mumford (this volume) discusses the economics of the SIT. However, that discussion focuses on benefit/cost analyses of overall programmes, and rearing economics appear only as a component of overall cost.

One of the main characteristics that distinguishes rearing for the SIT from other insect rearing is scale, e.g. the El Pino factory in Guatemala produces more than 2000 million sterile male Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) per week. Scale brings with it problems of labour supply, automation, and diet supply, which are dealt with later in this chapter, but there is also an issue of the relative efficiency and economics of scale. In general, cost per unit reduces as scale increases, and this can be seen in the efficiency of the Guatemala facility (Hendrichs et al, 2002). At the same time, if rearing is optimized for numbers without regard to quality, there is a risk of reduced quality (Calkins and Parker, this volume). The

scale at which a particular programme becomes cost effective will depend on the species involved and the cost and efficiency of alternate means of control.

Unfortunately, few published data are available on the actual cost of rearing, separate from whole programme costs. The figures that are available indicate that costs have fallen dramatically, but the figures derive from different situations that are not directly comparable, and most of the change is attributable to improvements in rearing technology rather than scale. The cost of male Mediterranean fruit fly pupae has been reduced to less than USD 300 per million in the Guatemala facility, but this cost depends on the utilization efficiency of the available capacity (Fig. 1) (Enkerlin 2003), indicating that utilization efficiency is at least as important as scale per se. Caceres et al. (2004) compared mass-rearing costs of Mediterranean fruit fly sexing and non-sexing strains; here the different characteristics of the *tsl* sexing strain lead to a small increase in rearing costs (which may be reversed with future developments), but significantly reduced post production programme costs. In Tanzania, with increased production, the production cost of the tsetse fly *Glossina austeni* Newstead was reduced from about USD 1 per male insect to less than USD 0.10; again, however, this is largely due to changes in procedures.
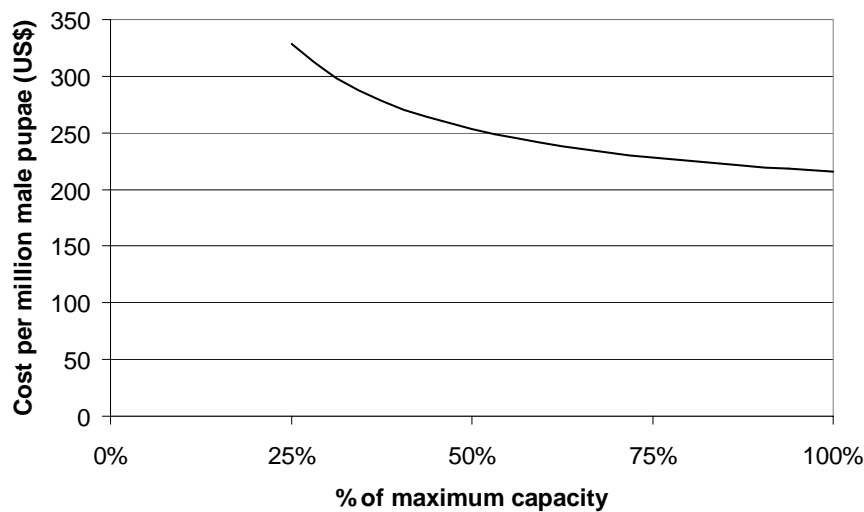
Figure 1. Variation in Mediterranean fruit fly production cost (USD) with capacity utilization at El Pino, Guatemala. (Figure adapted from Vollmerhausen 2001.)

3. FACILITY DESIGN AND LOCATION

Facility design is a critical aspect of the rearing process. Poor design will lead to inefficient utilization of space and energy, increased problems with contamination, and increased risk of escape of fertile insects. Leppla and Ashley (1978), Griffin

(1984), Fisher and Leppla (1985), and Dowell et al. (this volume) discuss general facility design. Specific aspects of biosecurity are discussed in USDA/APHIS/PPQ (1995), Kahn and Mathur (1999), Leppla and Eden (1999), and ACME (2003). Harrell et al. (1979), Owens (1984), Wolf (1984), Goodenough and Parnell (1985), and Oborny (1998) discuss environmental control. Tween (1987) discusses a modular approach to constructing a rearing facility that has been adopted in Guatemala for the Mediterranean fruit fly facility. The modular approach has the advantage of simplifying design and expansion, but it also means that there is little economy from increased scale as each additional module costs the same as previous ones, the only saving coming from the common areas. However, it has the advantage of allowing the facility to be easily scaled to the required size, so as to maintain the utilization efficiency (Fig. 1); if demand falls, complete modules can be closed without affecting production in the remaining modules, or converted to rearing another insect.

The criteria for determining the optimum location for a mass-rearing facility must take several factors into account, both technical (Phillimore 2002; IAEA/FAO 2004; Dowell et al., this volume) and managerial (Dyck, Reyes Flores et al., this volume). The principal factors, not in order of priority, are:

- Logistical access — ease of delivery of rearing supplies and equipment
- Geological stability — earthquake risk, hurricanes, flooding, etc.
- Political stability
- Acceptance of the facility by the local community
- Local government requirements or restrictions
- Access to water and utilities, reliability of supplies
- Construction and maintenance costs
- Availability of support and repair services
- Labour costs and availability
- Waste disposal
- Access to a suitable airport for rapid delivery of insects
- Distance from release area
- Quarantine considerations — can the insect survive in the surroundings?
- Proposed progression in case eradication is the objective — will the facility become stranded behind the eradication front?

The relative importance of a given factor will depend on the programme, and on factors such as the degree of automation utilized. For highly sophisticated systems, access to support and maintenance for the equipment may be critical, as may electricity supply reliability, but for a less automated system these may be less important. Some of the factors can be accommodated within the programme itself, e.g. installing independent generation capacity and a water supply. Even though not always possible, locating the facility in an area where the insect cannot survive and establish, perhaps due to desert conditions or cold winters, greatly eases the quarantine considerations, and reduces costs. The problem of being stranded behind the eradication front might be addressed by developing a relocatable, containerized rearing system.

4. ESCAPES AND ENVIRONMENTAL CONCERNS

A problem unique to the SIT is that the pest insect itself is being reared. For other large-scale rearing, e.g. classical or augmentative biological control, the reared insect is not the pest itself, so any escape from the rearing process is unlikely to pose a threat. (An exception is where the pest must be reared as a host for a parasitoid or predator, but living hosts are increasingly being replaced by factitious hosts or artificial diets). However, when the pest is being reared, any escape of fertile material poses a risk. Usually the rearing is done in an area where the pest is already present, but if an eradication programme is successful, the rearing facility can become stranded behind the eradication front and poses a substantial reinfestation risk. This happened to both the Mexican facility producing the New World screwworm *Cochliomyia hominivorax* (Coquerel) and the Japanese facility producing the melon fly *Bactrocera cucurbitae* (Coquillet). Stringent containment procedures are required (see references above), usually coupled with preventive sterile insect releases and other control measures in the vicinity of the rearing facility. Following screwworm eradication in the country, the screwworm facility in Mexico has operated successfully for many years, employing a combination of stringent security, containment, and prophylactic sterile male releases. An alternative is to site the facility in an area where the insect cannot, due to local environmental conditions, establish a self-sustaining population, e.g. the facility under construction in Addis Ababa, Ethiopia, to rear the tsetse fly *Glossina pallidipes* Austen.

The mass-rearing process for the SIT potentially poses some environmental problems in relation to waste disposal, and preventing the accidental escape of fertile insects with the spent diet and wastewater. These waste products must be treated to ensure that no living insects in any stage remain — for the diet by steam treatment or extrusion, and for wastewater by filtering, heat treatment or a combination. Diets should be readily biodegradable to reduce environmental concern over waste disposal, or even to allow their re-utilization for other purposes, such as feeding fish or livestock. Some of the bulking agents used in diets do not readily degrade, and these should be avoided, not least because they can lead to significant waste disposal charges (Chaudhury and Alvarez 1999). Facilities are also increasingly being required to install costly wastewater treatment plants.

Probably the biggest environmental concern comes from the sterilization process, which involves a radioactive source. Standard procedures for the use of such sources are available and, if followed, minimize the risk. Sterilization itself is discussed by Bakri et al. (this volume).

Insect mass-rearing can pose a significant health hazard through inhalant and contact allergies (Wirtz 1984, Wolf 1985, Bellas 1990, Kfir 1994, Myers and Barnard 2002). Allergic reactions to mould spores, mites, and pheromones also occur. Preventing allergic reactions involves recognition and documentation of the problem (Wirtz 1980), and correction of the problem through appropriate air-handling and filtering (Owens 1984, Wolf 1984, Froehlich 1995), coupled with protective clothing and filter masks or respirators. The environmental conditions in the rearing facility may also be changed by manipulation of the diet (Vargas et al.

1984). In addition to the health hazard caused by spores and mites, they impact the rearing directly by reducing diet quality or causing disease in the insects (see below).

5. STRAIN MANAGEMENT

Strain management ensures that a strain continues to perform the function required of it, i.e. it remains sexually compatible with wild insects and does not gradually deviate from wild behaviour. The strain must also maintain fecundity, and potential problems with contaminant organisms must be suppressed or eliminated. Fisher (1984), Schwalbe and Forrester (1984), and Singh and Ashby (1985) discussed strain management.

One of the most common concerns is genetic diversity (heterozygosity) of the strain, and this can be an important consideration during colony establishment. The strain must be colonized with a sufficient number of individuals from a wide genetic background. There has been much discussion, with a wide divergence of opinion, as to how many are sufficient — from many thousands to just a few tens or hundreds of females (Mackauer 1972, 1976 for parasitic wasps; Bartlett 1985 and Nunney 2002 for the SIT). It is generally agreed that the size of the founder population should depend on the degree of heterozygosity of the wild population. Usually it is stated that the founder material should come from a wide geographic range, but this is not necessarily beneficial; crossing strains from different geographic locations can break up adaptive gene combinations and render the strain less fit than the parents (Mackauer 1976). Individual fecundity will also influence founder numbers. In a slow-breeding group, such as tsetse flies, each individual has to be carefully nurtured, and no one individual's or small group's progeny can dominate the next generation. The logistics of collection also play a major role in how the field population is sampled.

Insects newly collected from the field rarely thrive in the laboratory, and the first few generations usually suffer high mortality, with the colony stabilizing after about five generations (Bartlett 1984). This process involves the rapid selection of individuals better adapted to the laboratory rearing conditions, resulting in a rapid decline in heterozygosity. There is considerable concern that these changes will result in insects significantly different from the wild population, and therefore non-competitive, although as yet it has not been possible to show unequivocally that a reduction in heterozygosity per se leads to a reduction in competitiveness. Leppla et al. (1983) showed that this adaptation process was not influenced by gradually introducing mass-rearing conditions. Adverse changes resulting from long-term mass-rearing are not uncommon, with the development of strains lacking the necessary courtship behaviour and responses to pheromones, host odours, environmental cues, flight range, and even vision (Boller 1972, Miyatake 1998, Cayol et al. 2002). During long-term mass-rearing, due to the accumulation of random mutations, heterozygosity may gradually be regained (Bartlett 1984), but the newly acquired heterozygosity will not match that of the field population. Heterozygosity can also be encouraged deliberately (Joslyn 1984).

Potential changes in competitiveness during mass-rearing are best detected by careful quality control monitoring (FAO/IAEA/USDA 2003). A range of parameters is monitored routinely, including mating competitiveness and flight ability (Calkins and Parker, this volume). If adverse changes are observed, it may be possible to deliberately select for desirable characteristics (Collins 1984, McInnis et al. 2002), although there are some dangers associated with this approach.

A recent development in mass-rearing the Mediterranean fruit fly has a potential application to colony management in almost any insect-rearing programme. The concept, called the filter rearing system (FRS), involves maintaining a small colony at a low density, or even under semi-natural conditions, and therefore assumedly a low-selection pressure (Fig. 2) (Cáceres et al. 2000, Fisher and Cáceres 2000). Surplus insects from this low-density mother stock or clean stream are fed into a high-density amplification chain, leading up to the final release numbers. The important feature is that no individuals are ever fed from the amplification stages back to the mother stock. The low-density rearing conditions of the filter can be supplemented with any further conditions deemed desirable, e.g. host plant, mating competition or pheromone response, and non-performing individuals eliminated. Any undesirable traits selected for in the high-density amplification stages have only three or four generations to accumulate before release, and do not affect the mother stock. A further advantage is that, if it is desired to replace the strain with a new one, the new mother stock can be set up in parallel with the old one, and amplification easily switched from one to the other. Filter rearing is similar to the concept used by Bigler (1986) to rear *Trichogramma maidis* Pintureau and Voegele.

6. PRODUCTION, PROCESS, AND PRODUCT CONTROL

The concept of quality control can be divided into three areas: production control (monitoring all rearing operations in terms of personnel, materials, equipment, schedules, environment, etc.), process control (sampling immature insect stages to predict quality and determine sources of variability), and product control (both at the output from a rearing facility and in the field), according to the normal industrial definitions (Leppla 1994). All three aspects are equally important in quality control, but most work has been done on product quality control (Calkins and Parker, this volume). Field quality control is a particularly neglected area (Vreysen, this volume).

In general, methods of rearing for the SIT are developed from those used to rear insects for other purposes, but on a much larger scale. The techniques are similar, but the issues of quality control require a different approach. The larger scale means larger rearing facilities, and for larger facilities production control becomes potentially more difficult. There are also greater opportunities to automate, reducing labour costs, variability, and the likelihood of microbial contamination from the staff employed in the facility. Environmental control systems are very important, and have to be designed taking into account the amount of insect biomass producing heat (Oborny 1998). These are also amenable to increased automation, with networked monitoring of temperature, humidity, and light as is done in the codling moth *Cydia pomonella* (L.) rearing facility in Osoyoos, Canada. In an interesting approach to
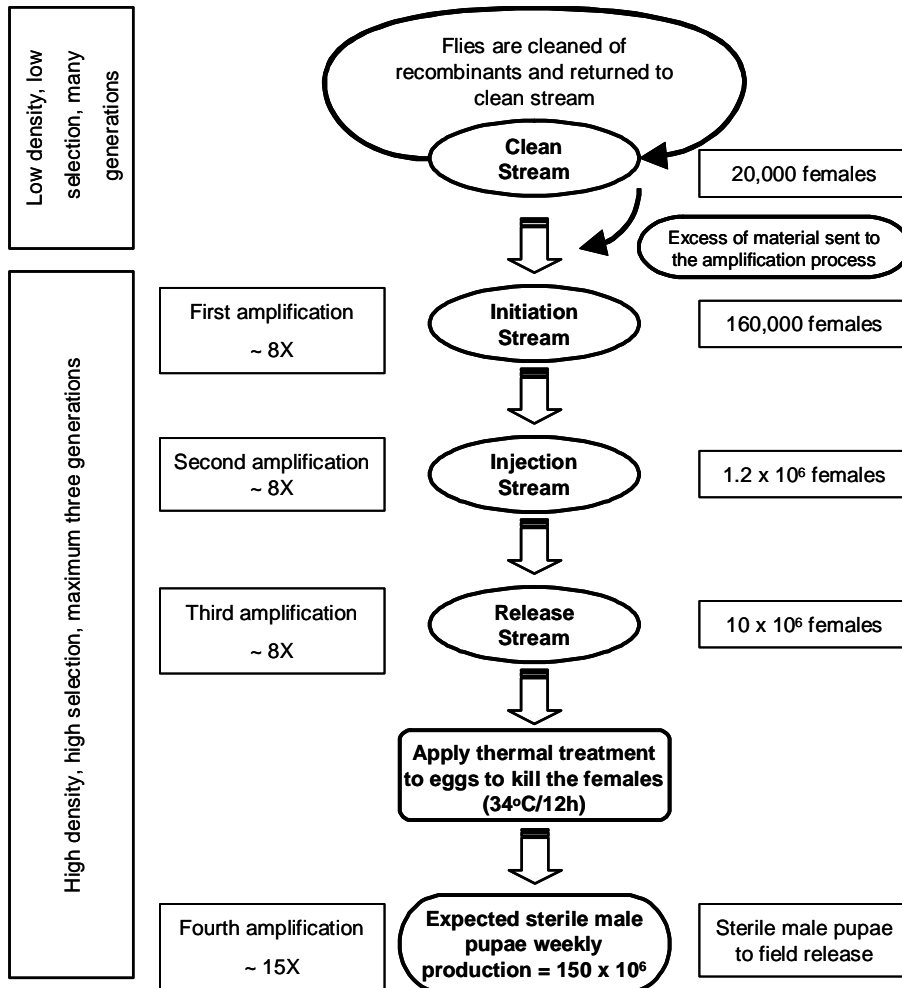
Figure 2. Continuous filter rearing system for producing Mediterranean fruit fly sterile males in Guatemala. (Figure adapted from Cáceres et al. 2000.)

process control, air space volatiles were monitored for changes that would indicate changes in the rearing parameters (Hedin et al. 1975).

The specific nature of the SIT requires that the product, i.e. reared insects, mate with the wild population, and mating behaviour tests are critical. Recently several of the techniques used to measure these behaviours under semi-natural conditions in tephritid fruit flies (FAO/IAEA/USDA 2003) have been adapted for tsetse flies (Mutika et al. 2001). However, monitoring itself does not correct problems. Each rearing facility should develop standard operating procedures (SOPs) for rearing

operations, quality control operations, and finally responses to adverse quality control findings.

Problems with other organisms that consume diet, parasitize or prey on the insects, or spread disease, may have a significant impact on the colony. Although rather common, few actual cases have been described in the literature (Sandhu and Varma 1980, Rawlins et al. 1982, Hanks et al. 1992). Changes in resistance to the various pesticides used to control them means that each facility will have to find a solution to its own problem.

Data management is a vital component of large-scale mass-rearing. Akey et al. (1984) reviewed data processing, and modern computer systems have further simplified data collection and analysis. As rearing becomes commercialized, customers will require up-to-date information on insect status and quality control, and this information could be made available via the Internet.

Finally, a factor not unique to the SIT, but more prominent with increased centralization of rearing, is the issue of transboundary shipment. To date, there is no explicit regulatory framework for the transboundary shipment of sterile insects, but this issue is currently being addressed (Enkerlin and Quinlan 2004). In this context, the existence and careful documentation of a comprehensive quality control system will be important (FAO/IAEA/USDA 2003).

7. AUTOMATION OF REARING

The scale of rearing for the SIT naturally lends itself to some degree of automation. Rearing systems vary greatly in their sophistication, from largely manual (tsetse fly, New World screwworm) to highly sophisticated (melon fly) systems. The balance between labour and automation depends on many factors, but the chief one is the cost of labour. In areas with low-labour costs, the drive towards automation will be reduced, but in high-wage areas, e.g. Japan, automation is essential to reduce costs. Other reasons to automate include: (1) reduction in human error, with increased product performance and consistency, (2) reduction in microbial contamination from personnel, and (3) increased space utilization efficiency, resulting in lower costs — for building and for energy for environmental control.

Another aspect of automation is in climate-control systems. Air-handling is essential to control temperature and humidity, reduce airborne particulates and microbial contamination of the colony, and reduce medical problems of the staff. Klassen (1978), Sikorowski et al. (1983), Harrell and Gantt (1984), Opiyo et al. (1999), Smith (1999), Smith and Nordlund (1999), and Opiyo et al. (2000) reviewed aspects of mass-rearing automation. For almost all stages of the process, descriptions of automated systems are available — egg collection (Leppla et al. 1974, Carlyle et al. 1975, Pearson et al. 2002), diet preparation and dispensing (Grisdale 1973, Gantt and King 1981), larval holding and pupal harvesting (Hartley et al. 1982), and environmental-control systems (Oborny 1998, Peng and Ohura 2000).

8. DIET

Diet is probably the single most important component of rearing and, with labour, constitutes the main cost. Therefore, it is in the interest of insect-rearing programmes to improve the performance or reduce the cost of the diet. A balance has to be achieved between cost and performance of the insects; in the long run, a cheap diet that produces less competitive insects may prove more expensive. Cohen (2003) recently reviewed all aspects of diet development.

Diets for many insects have been described in the literature (Smith 1966, King and Leppla 1984, Singh and Moore 1985, Gerberg et al. 1994, Ochieng'-Odero 1994b), with a gradual progression from natural-host material towards synthetic and defined diets (Singh 1984, Moore 1985, Shimoji and Yamagishi 2004). A natural-host diet is limited by the availability of the host, which may be seasonal or limited in distribution, and variable in quality. It tends to be expensive, but should provide a complete diet and avoid changes in host-location behaviour. Semi-synthetic, synthetic, and defined diets offer the convenience of shelf storage and consistent product, but risk being deficient in one or more factors. Often natural-host material needs to be incorporated into the diet for initial colonization, but then can gradually be removed as the colony adapts. Generalist herbivore insects tend to be the easiest to feed, with specialist herbivores and predators being more difficult. Singh (1985) worked on a range of diets suitable for several to many species, even across different orders. However, even though general diets are unlikely to provide optimum nutrition and cost, they do act as a good starting point for developing diets for newly colonized species.

The first step in reducing the cost of a diet that was not designed specifically for the species is to remove unnecessary components. The next stage is to identify local agricultural products and industrial by-products (e.g. brewers' yeast, sugar cane bagasse) or standard commercial products that are cheap (Chaudhury et al. 1998, Chaudhury et al. 2000, Cohen 2000, Shimoji and Yamagishi 2004), and provide the necessary nutrition and physical properties. Utilization of local components will often also make the supply more secure.

It is important to ensure the quality of diet ingredients before purchase or use, e.g. by monitoring the physical and chemical variability of the ingredients, bacterial load, and any insecticide residues. Poor quality control of diet ingredients is a major problem in mass-rearing, and therefore requires most attention, including specifying chemical and biological assays that the ingredients must pass. In the end, a cheaper ingredient may in fact be more expensive due to frequent "crashes" in production caused by low-quality ingredients.

In AW-IPM programmes that integrate the release of sterilized insects, the availability of an irradiator means that diet components can be decontaminated. In rearing tsetse flies, the bacterial load of the blood diet is critical, and can be reduced through irradiation (Ochieng'-Odero 1994b). Bacterial contamination of wheat bran used in an uncooked diet for rearing the carob moth *Ectomyelois ceratoniae* (Zeller) can also be reduced by irradiation.

Minor components of the diet frequently contribute a disproportionate amount to the cost. For example, many diets are gelled with agar, but agar is very expensive, and several alternatives as partial or complete replacements of agar have been investigated, e.g. Leppla 1976, Spencer et al. 1976, Harris et al. 1984, Taylor et al. 1991, Honda et al. 1996, and Chaudhury and Alvarez 1999. Such minor (by weight) components are important in controlling both the consistency and water retention of the diet; water is a crucial component that influences all stages of egg, larval, and pupal development

Mechanical handling and processing of diet is also possible, e.g. in the pink bollworm *Pectinophora gossypiella* (Saunders) rearing facility in Phoenix, AZ, USA, diet is processed and sterilized in a twin-screw extruder (Edwards et al. 1996, Miller et al. 1996). The application of agricultural engineering and food processing techniques promises to produce significant further improvements.

Additives to diets can have a profound effect on the performance of insects. Supplementing boll weevil *Anthonomus grandis grandis* Boheman diet with beta-carotene increases dispersal and trap response (Reinecke 1991). Feeding methyl eugenol to adults of the oriental fruit fly *Bactrocera dorsalis* Hendel reduces the time to sexual maturity and the catch of sterile males in methyl-eugenol-baited traps (Shelly and Dewire 1994). Other minor components, that can be added to the adult diet or applied directly to modify the behaviour of released insects, are being identified (Papadopoulos et al. 2001; McInnis et al. 2002; Shelly et al. 2002; Lance and McInnis, this volume). However, the effect of adding probiotic bacteria to adult diet is still unclear (Niyazi 2004).

Control of disease in the colony and of microorganism contamination of the diet are intimately linked. The identification and control of microbiological contaminants have been reviewed by Sikorowski (1983), Goodwin (1984), Sikorowski and Goodwin (1985), and Sikorowski and Lawrence (1994). Apart from immediate mortality, microbial contamination can lead to changes in development, body composition, and susceptibility to insecticides (Sikorowski and Goodwin 1985). Control begins with preventing infection by appropriate handling techniques and sterilization of ingredients, as well as surface sterilization of eggs or pupae (Leppla et al. 1974). A wide range of antimicrobial compounds for insect diets has been tested (Gifawesen et al. 1975, Ludemann et al. 1979, Hartley et al. 1982, Funke 1983, Bathon 1997), but many are unsuitable, and none is suitable for all insect species. Alverson and Cohen (2002) found that some of the most common ones have a significant negative impact on rearing *Lygus hesperus* Knight. Other means of microbial control include irradiation and various forms of heat treatment.

Future diet development will concentrate on utilization efficiency, ease of storage and usage, and cost. Defined diets may become important, but the highly refined ingredients tend to be very expensive, and so semi-defined diets utilizing much cheaper local raw materials are more likely to be useful. At present, research on the Mediterranean fruit fly diet is investigating liquid diets, which increase utilization efficiency and ease disposal problems, and offer the possibility of recycling the diet by removing waste products and replacing only the nutrients that have been consumed (Fay and Wornoayporn 2002).

9. SEX SEPARATION

In the SIT, released sterile males mating with wild females produce the only sterilizing effect. If sterile females are also released, they can have a minor positive effect by distracting wild fertile males and acting as a "sperm sink". However, the simultaneous release of both sexes is usually less economical, and also less effective, than the release of only males, since there may be a tendency towards assortative mating (Robinson et al. 1999). In haematophagous disease vectors, the females are usually the vectors and must be removed prior to release.

In most insects, the sexes can be separated on the basis of external morphology (Klassen, this volume), but this may be possible only in the pupal or adult stages, and may be difficult to automate. For example, in programmes that release sterile male tsetse flies, teneral adult flies are usually hand-sorted in a chiller to separate the sexes, a very slow and laborious procedure. Research showed that a computer-based optical recognition system was too slow and too prone to error to be useful, but recent work on separating pupae according to sex by near-infrared spectroscopy shows promise. In many insects, e.g. some mosquitoes (Gerberg et al. 1994) and Lepidoptera, sexual dimorphism in size (apparent in the pupal stage) can be used to separate the sexes, but the often considerable overlap in size renders the sorting inefficient. In some insect groups, pupae show genitalia characters that identify the sex, but these are difficult to observe, require hand-sorting, and have not been automated.

The sexes often show a variation in developmental rate, and this can sometimes be used to separate them. Female tsetse flies emerge first and, by manipulating temperature conditions during pupal development, sex separation based on the timing of adult emergence is possible (Opiyo et al. 1999, 2000); the efficiency was more than 99%, and eliminated the laborious hand-sorting work in a chiller.

Various methods to separate the sexes, based on Mendelian genetics or engineered sex-linked mutations, have been proposed (Robinson and Franz 1999, Robinson et al. 1999, Marec et al. 2005). These systems, together with other genetic aspects, are discussed by Franz (this volume).

10. MARKING

To mark or not to mark sterile insects for release, that is the question. It is usually assumed that marking is essential for field monitoring of sterile to wild ratios so as to follow programme progress, but the hugely successful New World screwworm eradication programme (Klassen and Curtis, this volume; Vargas-Terán et al., this volume) has never used any form of marking. Progress is monitored by measuring egg-hatch rates, and such a method may work for other insects as well (Vreysen, this volume). No one method of marking is universally applicable, and all may have negative effects on the insects.

Methods of marking were recently reviewed by Hagler and Jackson (2001); the various individual marking systems, including numbering and coding systems, are clearly not appropriate for production levels of hundreds of millions per week.

Methods appropriate to mass-rearing include dye marking (that can be observed directly or with a simple UV system), chemical marking that requires some form of instrumentation for detection, and genetic markers.

Dye marking falls into two categories, internal dyes fed through the larval or adult diet, and external dyes. Internal dyes are usually oil-soluble; considerable testing of dyes has been done (Vail et al. 1966, Hendricks et al. 1970, Graham and Mangum 1971, Hendricks 1971). It was found that a dye suitable for one species is not necessarily appropriate for even a closely related species. The ease of administering the dye in the diet makes this system very attractive, but the disadvantages are that the dyes can be toxic or cause behavioural or other changes (Schroeder et al. 1974) and may not persist in later stages, and it is relatively difficult to identify a series of different markers. Nevertheless, the dye Calco Red has been used extensively for marking Lepidoptera, e.g. pink bollworm and codling moth.

Almost as extensively, external dye marking has frequently been used in AW-IPM programmes that release sterile insects. Usually this involves a fluorescent dye that is dusted or sprayed onto the insects (Chang 1946, Taft and Agee 1962, Stern and Mueller 1968, Schroeder and Mitchell 1981, Reinecke 1990, Enkerlin et al. 1996), but oil-soluble dyes have also been used for external marking (Steiner 1965, Schroeder and Mitchell 1981). The procedure is simple to apply; several different colours and dyes can be used. There are no published accounts showing the impact of externally applied dyes on the behaviour or longevity of insects, but it has been reported that excessive dye may reduce the response of codling moth males to calling females (Logan and Proverbs 1975), and there is evidence that reducing the quantity of dye used can significantly increase survival and quality in fruit flies. The disadvantages are that an additional processing step is involved, the dyes may affect insect quality, the marker may not be as reliable as an internal one, and the dyes can pose a health hazard.

An alternative to dye marking is using elemental markers (reviews by Akey 1991 and Akey et al. 1991). Three forms of detecting elemental marking utilize radioisotopes, neutron activation, or one of several forms of atomic spectroscopy (Akey and Burns 1991). Elemental fingerprinting may also be included here — detecting the ratios of unsupplemented elements that will vary between field populations and mass-reared insects. Due to environmental concerns, radioactive-isotope marking has fallen out of favour. Neutron activation of rare elements is a very sensitive technique, in principle able to detect picogram quantities (Curtis et al. 1973, Hamann and Iwannek 1979), but the need for a fast-neutron source makes the technique extremely expensive and impractical. Atomic absorption spectroscopy of stable isotopes offers a much more accessible technique (Akey 1991, Stimmann 1991, Van Steenwyk 1991, Fernandes et al. 1997). Different forms of spectroscopy can be used to identify the marker with varying levels of sensitivity (Akey and Burns 1991). For a programme that releases sterile insects, the cost of false positives is potentially so high that the additional equipment and procedure may well be justified, particularly in the later stages of eradication, and elemental marking could provide a valuable second means of identification to back up dye marking.

An interesting recent development in chemical marking is the use of non-insect protein markers (Hagler and Cohen 1990, Hagler et al. 1992, Hagler and Miller 2002). The technique has been tested using rabbit IgG, fed in the diet or applied externally, and detected by sandwich enzyme-linked immunosorbent assay. The technique looks promising, requires only simple laboratory equipment, and rapid application should be possible. A big advantage is that the mark is completely invisible, and should have no biological effect on insects.

To distinguish released insects from wild ones, naturally occurring phenotypic mutations can be used in a similar manner to dyes, as long as the mutation is sufficiently rare in the wild population (Bartlett 1982). However, almost certainly, most mutations have some deleterious effect. For the SIT, the disadvantage is often not so great that their use is prevented, and a few such mutations have been investigated or used (Fay and Craig 1969, Schroeder and Mitchell 1981). The main problem with mutations is that, since they cannot be transferred between species by conventional genetics, they must be identified for each species to be marked.

Most mass-reared insect colonies have a limited genetic basis, resulting in limited genetic diversity. This can be exploited to distinguish colony insects from the target population, by identifying natural DNA markers specific to the colony or target insects. Mitochondrial DNA markers were used in South Africa to distinguish wild from released sterile insects (Barnes et al. 2004), but for identification this technique requires the use of polymerase chain reaction (PCR) amplification.

Once again, genetic engineering offers the prospect of new marking techniques (Niyazi et al. 2005; Robinson and Hendrichs, this volume). In principle, any transgenic insect carries an identifiable marker (the transgenic construct), but for identification this may require PCR amplification. It would be easier to use a gene that encodes a visible product. At present, the most popular genes are green fluorescent protein (GFP) and its derivatives (Peloquin et al. 2000), and DsRed (Alphey 2002). Such transformations, if carefully constructed, should avoid most of the disadvantages of natural mutation markers.

For any marking technique, quality control of the marking — detecting and rapidly correcting any failure of marking — is important (Enkerlin et al. 1996, Kohama et al. 2003). The misidentification of a released sterile insect as a fertile insect can have huge financial consequences for an AW-IPM eradication programme, justifying both the commitment of significant resources to, and great care in, the marking.

11. STORAGE

Successful field operations using the SIT require a timely and predictable supply of sterile insects, and this can present logistical problems. There is usually a premium on the release of sterile insects at the beginning of the target population build-up, before the population level becomes too large, but colony rearing may be constrained by diapause or hibernal quiescence. Also, for a seasonal pest, to save on costs the rearing must be reduced to a maintenance level during the winter, but this is an inefficient use of the facility. In an ideal situation, the rearing would be continuous,

with some means to stockpile the insects over winter in preparation for release in the spring. For these reasons, the ability to store insects, and to manipulate quiescence or diapause, becomes very valuable. For shorter timescales, storage for a few days may be useful to synchronize insects for periodic release, and storage in a quiescent state for a period of hours may be desirable for delivery to the release site and for the release process itself. Leopold (1998, 2000) reviewed cold storage and cryopreservation of insects for each of these timescales.

Insects with an obligate or facultative diapause may be of better quality than non-diapause individuals (Bloem 1997, Bloem et al. 1998, 2000) and, assuming diapause termination can be controlled, form a convenient way of storing insects. Denlinger (2002) reviewed the regulation of diapause. Obligatory diapause in a one-generation-per-year species, e.g. western cherry fruit fly *Rhagoletis indifferens* Curran (Vankirk and AliNiazee 1982), may make continuous rearing impossible. The main aim is to find a means to induce and then break diapause early to permit the production of sterile insects in advance of the field population. The duration of induction, diapause, and breaking of diapause may last many months, and present a formidable obstacle to successful incorporation into the rearing system. The obligate duration of some of the cues may be circumvented by the application of hormones, and certain volatile organic solvents can influence the termination of diapause.

The exposure of insects to low temperature often results in damage. Denlinger and Lee (1998) reviewed the physiology of cold damage. Below 0°C the main threat to an insect is ice formation, but a range of hysteresis proteins, cryoprotectants, and stress proteins, and the elimination or masking of nucleating proteins and bacteria in freeze-susceptible species, lower the super cooling temperature below that likely to be experienced. Without freezing, osmotic stress is minimized. In freeze-tolerant species, nucleating proteins induce rapid freezing at higher temperatures, and osmotic stress is countered mainly by cryoprotectants.

Diapausing insects often exhibit inherent cold tolerance. Also, in many non-diapause insects, it is possible to induce cold tolerance by exposing the insects to heat or less extreme cold before storage, producing so-called "rapid cold hardening" in a matter of minutes or hours (Lee et al. 1987, Denlinger et al. 1992, Rinehart et al. 2000). Cold or heat shock induces the production of specific stress proteins, commonly called heat-shock proteins (HSPs), which among other effects are thought to prevent protein denaturation (Parsell and Lindquist 1993). The rapid production of HSPs makes them useful for short-term storage, shipment, and release, but their effect can be longer term, and can be re-induced by brief warming to higher storage temperatures (Chen and Denlinger 1992). In the future, it may be possible to genetically engineer desirable traits to enhance the storage potential of a mass-reared strain (Robinson and Franz 1999).

Finally, during colonization and rearing, insects are subject to selection pressure of varying intensity that can result in rapid genetic drift and loss of heterozygosity. Cryopreservation provides a method for: (1) the long-term storage of genetic material with specific desirable traits, and (2) the storage of strains where diapause or conventional cold storage is not possible (Leopold 1998, 2000). Even in situations

where it is possible, cryopreservation may reduce the labour and cost of maintaining many strains.

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CHAPTER 3.3.

STERILIZING INSECTS WITH IONIZING RADIATION

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SUMMARY

Exposure to ionizing radiation is currently the method of choice for rendering insects reproductively sterile for area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT). Gamma radiation from isotopic sources (cobalt-60 or caesium-137) is most often used, but high-energy electrons and X-rays are other practical options. Insect irradiation is safe and reliable when established safety and quality-assurance guidelines are followed. The key processing parameter is absorbed dose, which must be tightly controlled to ensure that treated insects are sufficiently sterile in their reproductive cells and yet able to compete for mates with wild insects. To that end, accurate dosimetry (measurement of absorbed dose) is critical. Irradiation data generated since the 1950s, covering over 300 arthropod species, indicate that the dose needed for sterilization of arthropods varies from less than 5 Gy for blaberid cockroaches to 300 Gy or more for some arctiid and pyralid moths. Factors such as oxygen level, and insect age and stage during irradiation, and many others, influence both the absorbed dose required for sterilization and the viability of irradiated insects. Consideration of these factors in the design of irradiation protocols can help to find a balance between the sterility and competitiveness of insects produced for programmes that release sterile insects. Many programmes apply "precautionary" radiation doses to increase the security margin of sterilization, but this overdosing often lowers competitiveness to the point where the overall induced sterility in the wild population is reduced significantly.

1. INTRODUCTION

The potential of ionizing radiation to interact with materials has numerous applications in industry, medicine, and agriculture. Ionizing radiation breaks down molecules, causing various effects in irradiated material. Radiation can cause polymerization of plastics, and can kill pathogens and micro-organisms, leading to applications in food processing and the sterilization of health-care products. In organisms, composed of differentiated and undifferentiated cells, mitotically active cells, such as stem and germ cells, are the most radiation-sensitive cells. In the case of the sterile insect technique (SIT), radiation can make an insect reproductively sterile by damaging the chromosomes of gonial cells, specifically causing germ-cell chromosome fragmentation (dominant lethal mutations, translocations, and other chromosomal aberrations) that leads to the production of imbalanced gametes and subsequently the inhibition of mitosis and death of fertilized eggs or embryos (Klassen, this volume; Robinson, this volume). In adult insects, midgut stem cells, which undergo continuing mitotic divisions, are particularly sensitive to ionizing irradiation, and the irradiation of certain species may cause a significant reduction in lifespan and increased mortality (Sakurai et al. 2000). Nevertheless, the successful sterilization of certain insect species, without a reduction in their lifespan, may indicate that cell replacement in the midgut is either not affected or is not of major importance to viability (Riemann and Flint 1967). Somatic cells, generally differentiated cells that have lost their ability to divide, are less sensitive to radiation than stem cells. Thus a lethal effect requires a higher radiation dose than a reproductive sterilization effect. The impact of radiation on somatic cells is expressed as the development of abnormalities, a reduction in lifespan, flight ability, mating propensity, and nutrition, and ultimately the death of the insect.

Radiation sterilization of insects is a relatively straightforward process, with reliable quality control procedures. The key parameter is the radiation absorbed dose which is expressed in Système International d'Unités (SI) units as gray (Gy) (1 Gy = 100 rad), where 1 Gy is equivalent to 1 joule (J) of absorbed energy in 1 kg of a specified material (1 Gy = 1 J/kg). As long as the dose is delivered correctly, efficacy of the irradiation process is guaranteed. Other advantages of using radiation to sterilize insects include: (1) temperature rise during the process is insignificant, (2) sterile insects can be released immediately after processing, (3) irradiation does not add residues that could be harmful to human health or the environment, and (4) radiation can pass through packaging material, allowing insects to be irradiated after having been packaged.

In the 1950s and 1960s, numerous mutagenic chemicals were tested as alternatives to radiation to induce sterility in insects (Knipling 1979; Klassen, this volume; Lance and McInnis, this volume). Chemosterilants were added to rearing diets, applied topically to insects, or even deployed in attractant-baited devices in the field. The efficacies of irradiating and chemosterilizing insects for population control were, in general, similar (Guerra et al. 1972, Flint et al. 1975, Moursy et al. 1988). However, today, chemosterilants are not used for sterilizing mass-reared insects. Most chemosterilants are carcinogenic, mutagenic, and/or teratogenic, leading to environmental and human-health issues such as the integrity of ecological food chains, waste disposal, e.g. spent insect diet, and worker safety (Hayes 1968,

Bracken and Dondale 1972, Bartlett and Staten 1996). Insect resistance to chemosterilants is an additional concern (Klassen and Matsumura 1966). Exposure to ionizing radiation is now the principal method of inducing sterility for area-wide integrated pest management (AW-IPM) programmes that release sterile insects.

2. RADIATION SOURCES

The suitability of a radiation type for the SIT depends on properties, such as relative biological effectiveness (RBE), penetrability, availability, safety, and cost. The RBE of radiation is defined as the ratio of the dose of 200–250 kV X-rays required to produce a specific biological effect to the dose of radiation required to produce the same effect. The RBE of radiation for the induction of chromosome aberrations depends on its linear energy transfer (LET — the energy imparted to a medium by a charged particle of a specified energy, per unit distance). Radiation with a higher LET is more effective in inducing sterility, and most likely would yield insects that are more competitive (North 1975). However, a higher LET also means that penetration is limited. For example, alpha particles have a high value of LET, but can penetrate only a fraction of a millimetre into a container of insects, which makes them unsuitable to sterilize insects for release in AW-IPM programmes. Neutrons are more effective than gamma rays or X-rays in sterilizing insects (Hooper 1971, North 1975, Offori and Czock 1975). However, neutrons can induce radioactivity in irradiated materials, which, along with the immobility of nuclear reactors (the usual source of neutrons), makes their use impractical for most programmes.

Considering this, the types of radiation that can be used practically in programmes that release sterile insects include gamma rays, high-energy electrons, and X-rays (Bushland and Hopkins 1951, 1953; Baumhover et al. 1955; Lindquist 1955). All have similar effects on materials (since they have a similar RBE), and in particular on insects. For certain insect life stages and radiation doses, several studies found no significant difference between electrons and gamma rays in their lethal effects (Hooper 1971, Adem et al. 1978, Watters 1979, Dohino et al. 1994).

To maintain the fitness of irradiated insects, and for the safety of workers, the induction of radioactivity in irradiated materials, such as canisters and insects, must be avoided. This is achieved by ensuring that energy used for the SIT is less than 5 million electron volts (MeV) for photons (gamma rays or X-rays), and 10 MeV for electrons (Elias and Cohen 1977, Codex Alimentarius 1983, FAO/IAEA/WHO 1999, IAEA 2002a). Thus, gamma rays from cobalt-60 (^{60}Co) (photon energies are 1.17 and 1.33 MeV) and caesium-137 (^{137}Cs) (0.66 MeV), electrons generated by accelerators with energy less than 10 MeV, and X-rays generated from electron beams with energy below 5 MeV, are acceptable for sterilizing insects.

2.1. Radioisotopes

Currently, the most commonly used radiation for the SIT is gamma radiation from the radioisotopes ^{60}Co and ^{137}Cs. These isotopes have long half-lives, and the energy of their gamma rays is relatively high (Table 1). To provide the same throughput, caesium sources, because of the difference in photon energy, require about four

times more activity than cobalt sources. Cobalt-60 is produced by placing small cylinders of natural cobalt (which is 100% ^{59}Co) into a nuclear reactor, where the ^{59}Co atoms absorb neutrons and are converted into ^{60}Co. These cylinders are removed from the reactor after 1 or 2 years, and are further encapsulated in corrosion-resistant stainless steel to produce source pencils. Caesium-137 is produced from the fission of uranium and plutonium, and must be chemically separated from other fission products and actinides present in used nuclear fuel. This process is very elaborate, and thus the use of caesium is declining for radiation processing, including in AW-IPM programmes that apply the SIT.

Table 1. Comparison of properties of Co-60 and Cs-137

Property	Co-60	Cs-137
Production mode	Neutron absorption in nuclear reactors	Chemical separation from spent nuclear fuel, e.g. uranium
Half-life	5.271 years	30.07 years
Photon energy	1.17 and 1.33 MeV (in equal proportions)	0.66 MeV
50% dose-decrease (depth in water)	23 cm	21 cm

2.2. Electron Beam

In the near future, the use of high-energy (5–10 MeV) electrons to sterilize insects will likely increase. Such electrons are generated by an electron accelerator, which does not involve any radioactive materials. Electrons are introduced into an accelerating structure from an injector, where they are accelerated to the designed high energy that can be derived from a variety of sources depending on the type of accelerator. An electron accelerator yields a narrow and intense electron beam, and thus the dose rate can be up to 1000 times greater than from a gamma irradiator.

2.3. X-Rays

When a beam of electrons strikes material with a high atomic number, e.g. tungsten, X-rays are generated. X-rays, like gamma rays, are electromagnetic radiation. Radiation generated in this manner (by the rapid deceleration of a charged particle) is also known as "Bremsstrahlung" (braking radiation). While gamma rays from radioisotopes have discrete energies, "Bremsstrahlung" has a broad energy spectrum with a maximum equal to the energy of the incident electrons. Gamma rays from ^{60}Co or ^{137}Cs, and X-rays, penetrate irradiated materials more deeply than electrons.

For example, for ^{60}Co gamma rays, dose decreases to half at a depth of about 23 cm in water, but for 10-MeV electrons, the useful depth is only about 4 cm.

3. RADIATION TECHNOLOGY AND STERILIZATION PROCESS

3.1. Irradiation Units

The design of an irradiation unit affects the dose distribution and the attainable dose range. A unit may be designed either for a specific application (or product) or for multiple applications, depending on local considerations and user requirements. The basic components of an irradiation unit (gamma-ray or electron) include:

- Radiation source (radioisotope gamma source or accelerator) and the associated control systems, sometimes referred to as an "irradiator"
- System for transporting the product, e.g. insects, or in some cases the source, to and from the position at which irradiation occurs
- Shielding to protect workers and the surrounding environment from radiation

The irradiation unit should include a dosimetry laboratory, and a product-handling system with areas designated for receiving and for segregated pre- and post-irradiation storage.

3.1.1. Gamma Irradiators

The radiation source consists typically of several source pencils of either cobalt or caesium. The dose rate is predetermined by the current activity of the source, and the operator controls the absorbed dose delivered to the insects by adjusting the time that they are exposed to radiation (an exception — in some large-scale irradiators, several dose rates can be obtained by raising different subsets of the source pencils into the irradiation room). The only variation in the source output is the known reduction in activity (strength) caused by radioactive decay, which can have a significant impact on the programme (financial as well as scheduling) if not taken into account. The activity of a cobalt source, for example, decreases about 12% annually. The irradiator operator compensates for this loss of activity by incrementally increasing irradiation time (approximately 1% each month) to maintain the same dose to the insects. Since irradiation times eventually become impractically long, sources need to be replenished at regular intervals, depending on the initial activity of the source and the operational requirements.

Typically there are two types of gamma irradiators used in programmes that release sterile insects — self-contained dry-storage irradiators, and large-scale panoramic irradiators.

Self-Contained Dry-Storage Irradiators. At present, most sterilization of insects is accomplished using gamma rays from self-contained irradiators (Fig. 1). These devices house the radiation source within a protective shield of lead, or other appropriate high-atomic number material, and they usually have a mechanism to rotate or lower the canister of insects from the loading position to the irradiation

position. These canisters, which are reusable and generally made of steel, aluminium, or plastic, hold packaging containers of insects during irradiation. To irradiate, a canister is placed in the irradiation chamber while it is in the loading (shielded) position and, depending on the current dose rate of the irradiator, the timer is set to deliver the pre-selected dose. On the push of a button, the chamber is automatically moved to the irradiation position. In most self-contained irradiators, the irradiation position is in the centre of an annular (circular) array of long parallel pencils that contain the encapsulated radiation source. With this design, the dose is relatively uniform within the irradiation chamber (section 3.3.2.). An alternate method of achieving a relatively uniform dose is to rotate the canister of insects on a turntable. The axis of rotation is parallel to the source pencils, which are usually vertical. The canister stays in the irradiation position for the set time interval, and then automatically returns to the unloading (shielded) position at the end of the treatment. Self-contained dry-storage irradiators provide a high dose rate but a small irradiation volume (1 to 4 litres), and are suitable for research as well as small-scale programmes that apply the SIT.

Figure 1. In preparation for irradiation, a canister of insects is placed in the irradiation chamber (while it is in the shielded position) of a self-contained gamma irradiator. Depending on the dose rate of the day, the timer on the control panel (lower right) is set to give the desired dose.

Large-Scale Panoramic Irradiators. For large-volume irradiation, panoramic irradiators are more suitable. The source consists of either several ^{60}Co rods (pencils) arranged in a plane or a single rod that can be raised/lowered into a large irradiation room. When retracted from this room, the source is shielded either by water (wet storage), or by lead or other appropriate high-atomic-number material (dry storage). Since isotopic sources emit gamma rays isotropically (in all directions), they may be surrounded by canisters of insects to increase the energy utilization efficiency, and several canisters can be irradiated simultaneously.

Many large-scale irradiators run in a continuous-operation mode, in which canisters of insects are carried on a conveyor around a central source. The canisters may pass by the source several times to increase dose uniformity in the canisters as well as energy utilization. The speed of the conveyor is selected so that the insects receive the intended dose. The source is moved to the storage position only when the irradiator is not in use. An alternate method is batch operation, where several canisters of insects are placed in the irradiation room while the source is in its storage position. The source is then moved into the irradiation room for the length of time required to achieve the desired absorbed dose. To improve dose uniformity, each canister may be rotated on its own axis during irradiation using turntables.

3.1.2. Electron and X-Ray Irradiators

Accelerator-generated radiation has two modes, electrons and X-rays produced from these electrons. The two principal electron-beam characteristics are beam (electron) energy, in MeV, and the average current, in milliamperes (mA). The beam energy determines the penetration of electrons in a material (thus dictating the useful size of the canister for irradiation), and the average beam current affects absorbed-dose rate (thus determining throughput, e.g. the number of canisters treated per hour). Unlike gamma radiation, electron beams are rather focused (for both modes), and typically conveyors are used to move canisters of insects continuously through the beam. Since X-rays penetrate deeper than the electrons, from which they are generated, larger canisters of insects can be used when using the X-ray mode.

3.1.3. Selection of Irradiator

Since gamma rays and electrons have similar sterilizing effects, the choice of source for SIT irradiation is based on other considerations, such as penetration, cost, product throughput (DIR-SIT in IDIDAS (2004)), expertise available at the site, and environmental and safety factors. The shallow penetration of electrons restricts the size of the canister used for irradiation. In addition, gamma irradiators are usually simpler to operate and less expensive than accelerators, at least within the range of power required for SIT applications. Electron accelerators, however, may have more public acceptance because they produce no radiation when switched off, and there are no transportation or radioactive waste issues (Cavalloro and Delrio 1974, Piedade-Guerreiro and Gomes da Silva 1983, Cleland and Pageau 1985, Smittle 1993, EBFRF 2004, FDACS 2004, LAF 2004). The power emitted by a gamma-ray source containing 100 kCi of ^{60}Co is roughly equivalent to that of a 1.5 kW electron accelerator. The power capacity of currently available commercial accelerators with

5–10 MeV electrons is usually much greater than this, making them unsuitable for dedicated SIT use. X-ray irradiators have the advantages of both gamma irradiators (high penetrability) and accelerators (no radiation when switched off). However the efficiency of converting electrons into X-rays is about 7% for 5 MeV electrons; thus 93% of the electron beam power is "wasted" in heating the converter target material (Farrell et al. 1983). Based on all of these factors, almost all current insect sterilization programmes have chosen to use gamma irradiators (Table 2).

3.2. Radiation Safety

It is essential that written descriptions of specific safety procedures, for all activities at an irradiation unit, are prepared. Before using a radiation source, workers must be given detailed training on relevant national legislation and regulations, and on safety procedures for the installation and use of a radiation source (IAEA 1992, IAEA 1996a, IAEA 2003).

Irradiators are designed to keep the radiation exposure and dose to workers "as low as reasonably achievable" (ALARA), and within preset levels. These dose limits are based on the recommendations of several agencies of the United Nations (UN), including the International Atomic Energy Agency (IAEA), Food and Agriculture Organization of the United Nations (FAO), and World Health Organization (WHO) (IAEA 1996a). Appropriate safety methods and procedures have been developed for each type of irradiator, and when operated correctly with the appropriate safeguards, they are safe and easy to use. Irradiators are usually licensed by national atomic energy authorities which set certain requirements, such as restricting access to certain areas and to authorized persons, a periodic survey of the radiation field in the vicinity where workers could be present, the use of personal radiation dosimeters, and the availability of radiation survey meters. These requirements are specifically aimed at protecting all workers from radiation. In addition, irradiators incorporate interlocks that prevent unintentional access to areas with high radiation fields. Cases of accidental exposure to ^{60}Co gamma rays are usually reported by the IAEA (IAEA 1996b, Gonzalez 1999), and data from such historic cases are useful for probabilistic risk assessment. When the useful life of a gamma source is over, the irradiator or the source pencils are usually returned to the supplier for storage, reuse, recycling, or disposal. This is now becoming an elaborate procedure.

During handling of insects, especially adult Lepidoptera, irradiator operators may be exposed to insect allergens, and additional safety measures may be required to minimize the risk of allergy and health hazards (Parker, this volume).

Table 2. Examples of insect mass-rearing facilities, and the types of irradiators used for reproductive sterilization (more extensive list found in IDIDAS (2004))

Location of facility	Insect reared	Dose (Gy)[1]	Initial activity (kCi)	Irradiator model (MANUFACTURER)	Source
Argentina	*Ceratitis capitata*[4]	110	20	IMCO-20[2]	Co-60
Canada	*Cydia pomonella*[5]	150	24	Gammacell® 220[2] (NORDION)	Co-60
Chile	*Ceratitis capitata*	120	16	Gammacell® 220[2] (NORDION)	Co-60
Guatemala	*Ceratitis capitata*	100–145	11	Gammacell® 220 E[2] (2 units) (NORDION)	Co-60
			12	Gammacell® 220 R[2] (J. L. SHEPHERD)	Co-60
			42	Husman 521A[2] (ISOMEDIX)	Cs-137
			46	Husman 521[2] (ISOMEDIX)	Cs-137
Mexico	*Anastrepha ludens*[6]	80	35	JS-7400[3] (NORDION)	Co-60
	Anastrepha obliqua[7]	80			
	Ceratitis capitata	100			
Mexico	*Cochliomyia hominivorax*[8]	80	47	Husman 520[2] (3 units) (ISOMEDIX)	Cs-137
Philippines	*Bactrocera philippinensis*[9]	64–104	30	GB 651 PT[3] (NORDION)	Co-60
Portugal	*Ceratitis capitata*	100	20	Gammacell® 220[2] (NORDION)	Co-60
South Africa	*Ceratitis capitata*	90	10	(LOCAL MANUFACTURER)[3]	Co-60
Thailand	*Bactrocera dorsalis*[10]	90	24	Gammacell® 220[2] (NORDION)	Co-60
USA (Hawaii) CDFA[11]/USDA	*Ceratitis capitata*	140	47	Husman 521[2] (2 units) (ISOMEDIX)	Cs-137
USA (Hawaii) ARS/USDA[12]	*Ceratitis capitata*	120	24	Gammacell® 220[2] (NORDION)	Co-60
USA (Texas)	*Anastrepha ludens*	70	38	Husman 521[2] (ISOMEDIX)	Cs-137

[1] Sterility-inducing dose in hypoxia (except *Cydia pomonella*)
[2] Self-contained dry-storage irradiator
[3] Panoramic irradiator
[4] *C. capitata* (Wiedemann)
[5] *C. pomonella* (L.)
[6] *A. ludens* (Loew)

[7] *A. obliqua* (Macquart)
[8] *C. hominivorax* (Coquerel)
[9] *B. philippinensis* Drew and Hancock
[10] *B. dorsalis* Hendel
[11] California Department of Food and Agriculture
[12] Agricultural Research Service, United States Department of Agriculture

3.3. Measurement and Distribution of Absorbed Dose

3.3.1. Radiation Dosimetry
For the success of a programme using the SIT, the absorbed dose delivered to the insects needs to be accurately quantified and controlled. Also, if contractual arrangements or national regulations prescribe specific doses, the programme will require adequate means to demonstrate compliance. Therefore the programmes need to have an established dosimetry system to accurately measure absorbed dose and estimate the associated confidence interval, a process known as dosimetry (ISO/ASTM 2004a). Dosimetry is performed using dosimeters — devices that, when irradiated, exhibit a quantifiable change in some property, e.g. colour, that can be related to the absorbed dose. A dosimetry system includes dosimeters (that are placed into the canister), measuring instruments (to read the change in the dosimeters) along with their associated reference standards, and procedures for using them (ISO/ASTM 2004b).

Dosimeters are commonly used in sterile insect production for such tasks as absorbed-dose mapping (section 3.3.2.), process control (section 3.5.3.), and qualification of the irradiator (section 3.5.2.). Several dosimeters are suitable for routine dosimetry at SIT facilities (ISO/ASTM 2004a). Many sterile insect production facilities use radiochromic film systems because they are relatively affordable and are simple to use (avoiding extensive training) (IAEA 2004). Procedures for calibrating routine dosimetry systems, and for determining radiation fields in irradiators used for insect sterilization, are described in ISO/ASTM standards (section 3.5.1.) (ISO/ASTM 2004a, 2004b, 2004c, 2004d), which are updated periodically, and in IAEA technical reports (IAEA 2002b). Reference-standard dosimeters are used to calibrate the routine dosimetry system and radiation fields, e.g. determining the dose rate at a reference position in a self-contained gamma irradiator. Sterile insect production facilities use reference-standard dosimeters for both of these purposes. Accredited dosimetry laboratories typically provide these dosimeters and make the readings, resulting in measurements that are "traceable" to national or international standards.

3.3.2. Absorbed-Dose Mapping
Ideally, it would be desirable to irradiate all insects in a container (or a canister) at the same dose. In practice, because of the characteristic of radiation interaction with matter, there is a systematic pattern of dose variation within the canister, and therefore not all insects receive the same dose. Dose distribution within the canister is determined by "dose mapping", which typically is conducted by placing several dosimeters at known locations throughout the canister. Dose mapping provides operators of SIT irradiators with information on the dose within the canister, including areas of maximum and minimum dose, the dose uniformity ratio (maximum dose/minimum dose), and areas where the dose rate is relatively uniform (Fig. 2). Techniques for dose mapping are described in detail in ISO/ASTM (2004a) and Walker et al. (1997).
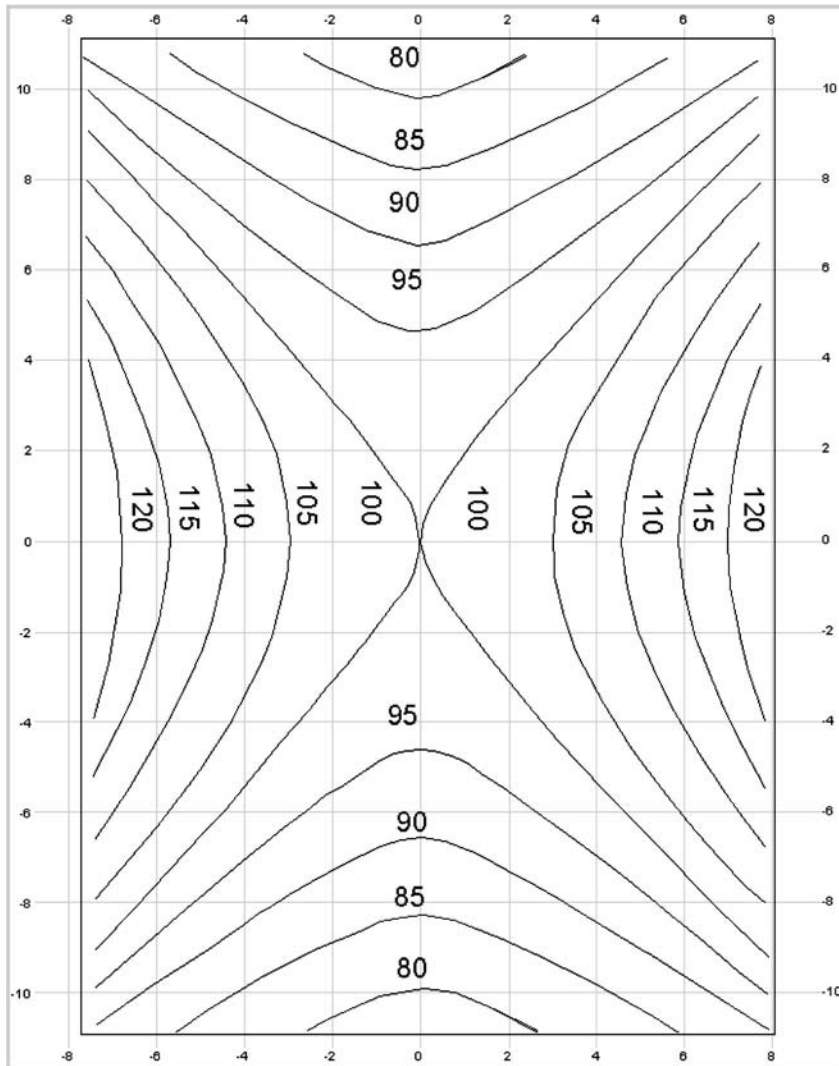
Figure 2. Example of isodose curves in the irradiation chamber of a Gammacell® 220.
Values are normalized to 100 in the centre of the gamma field. The field is most uniform
in the centre. Grid is at 2-cm intervals from the centre of the chamber. (Figure from MDS
Nordion, reproduced with permission.)

3.4. Radiation Sterilization of Insects

3.4.1. Selecting Sterilizing Dose

The absorbed dose that is used to induce sterility is of prime importance to programmes that release sterile insects. As it increases, sterility increases, but insect quality and competitiveness will decrease (Calkins and Parker, this volume; Lance and McInnis, this volume). Insects that receive too low a dose are not sufficiently sterile, and those that receive too high a dose will be non-competitive, reducing the effectiveness of the programme. Quite often, full (100%) sterility may not be the most favourable condition for a programme, and thus process optimization is necessary to balance sterility level and competitiveness, taking into consideration factors that could affect the radiation sensitivity of insects (section 4) and programme requirements. If quarantine security is a consideration, 100% sterility may be required for any released females. Males, however, tend to be less radiosensitive, and, in many species, eliminating a residual egg hatch of 1% (or less) from fertile females mated to irradiated males (even though many of these eggs do not survive other stages) requires doses that substantially reduce the ability of males to compete with, and thus induce sterility into, wild populations (Fisher 1997, Toledo et al. 2004).

In reality, because of the unavoidable dose variability within a canister (as mentioned above), sterile insect production facilities define an acceptable range of doses given to the insects. Most often, programmes or regulatory officials specify a minimum dose that all insects must receive to ensure sufficient sterility. Due to dose variability, most insects actually receive a dose that is somewhat higher than that minimum. An alternate approach is to specify an optimum (or central target) dose, and set this as the average or median dose within the irradiated volume of insects. In either case, the dose uniformity ratio should be small; the goal is to sterilize all insects sufficiently without treating large proportions with doses that are high enough to substantially reduce competitiveness. Unit operators can often adjust process parameters to achieve a more uniform dose distribution (section 3.5.2.).

Induced lethal mutations may exert lethality at any stage of development. Quite often, for reasons of simplicity and convenience, the induction of detrimental lethal mutations is made based solely on egg hatchability. However lethal mutations occur at all developmental stages. Therefore researchers should measure dose effects all along this developmental continuum, or the actual survivorship from egg to adult, to give a true picture of induced sterility. As a result, 99 or 100% sterility in the egg stage is not essential, nor desirable, if it drastically reduces the competitiveness and vigour of the sterile insect.

An informed decision on treatment dose requires accurate data on how factors such as dose, insect stage and age, and various process parameters affect levels of sterility and insect quality. For programmes that apply the SIT, the accuracy and value of such data depend on the use of standardized dosimetry systems, procedures, and reporting methods (ISO/ASTM 2004c). Published data on the radiation biology of the same or similar species can provide guidance, but, in many cases, are of limited value because dosimetric procedures, dose-measurement traceability, dose distribution, and other pertinent information are often not reported. In addition, the

details of insect-handling procedures, and, perhaps, strain-related differences, can influence radiation sensitivity (section 4.).

3.4.2. *Preparing Insects for Irradiation*

Stage/Age of Insects. The selection of the insect development stage and age that will be irradiated is based on knowledge of the timing of maturity of insect reproductive organs (section 4.2.), handling suitability during irradiation and subsequent shipping, and sensitivity to somatic damage. For many holometabolous species (having complete metamorphosis), a good time for irradiation is late in the pupal stage, or early in the adult stage, when germ tissues have formed (Anwar et al. 1971, Ohinata et al. 1971, 1977, 1978). For example, tephritid flies are usually irradiated 1 or 2 days prior to adult emergence (pupae kept at about 25°C). Flies that are irradiated earlier in the pupal stage will tend to be of lower quality (in terms of mating propensity, flight, and sex pheromone production), an indication that somatic tissues were adversely affected (Fletcher and Giannakakis 1973). However, when tephritid pupae are irradiated too close to adult emergence, females can already have some developed oocytes that, in spite of having been irradiated, can become viable eggs (Williamson et al. 1985). Ideally, the development and maturity stage should show an external physical indicator that acts as a quick and reliable identification tool, such as pupal eye-pigment colour in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Ruhm and Calkins 1981, Seo et al. 1987).

In the pentatomid bug *Nezara viridula* (L.), sexual maturity and mating occur 5–17 days after adult emergence. Fourth- and fifth-instar nymphs are most frequently selected for irradiation because they are less radioresistant than adults, and male and female reproductive systems are already well developed at this stage (Kiritani 1963, Mitchell and Mau 1969). However Williamson et al. (1985) found in the Mediterranean fruit fly that, due to advanced egg development in the ovaries, irradiation at 1 day before emergence or later resulted in some fertility.

Packaging for Irradiation. Insects for AW-IPM programmes that integrate the SIT are usually irradiated within primary packaging containers that are subsequently transferred, unopened, to an emergence facility where the adult insects are prepared for release. These containers provide protection to the sterile insects, and guard against their escape. They also prevent tampering. A variety of packaging containers has been used, e.g. 2- and 4-litre polyethylene bags, unwaxed paper cups (with lids), paper boxes, and plastic bottles of up to 15-litre capacity. SIT irradiation protocols may incorporate reusable canisters (typically steel, aluminium or plastic) that hold the primary containers during irradiation. The size and shape of these canisters are usually a function of the size and shape of the irradiation chamber, especially in the case of self-contained irradiators (section 3.5.2.).

If insects are irradiated in a reduced-oxygen atmosphere, as a means of reducing the formation of free radicals (section 4.1.), the packaging container must be airtight. For example, tephritid pupae are sealed, with as little air space as possible, in plastic bags or bottles and then held at cool temperatures (12–20°C) for at least 1 hour

before irradiation. During this period the insects exhaust most of the oxygen remaining within the container. Hypoxia (a deficiency of oxygen reaching the tissues of the body) can also be achieved by saturating the atmosphere within the container with helium or, more commonly, nitrogen, prior to and during irradiation (Ashraf et al. 1975; Ohinata et al. 1977, 1978; Hooper 1989).

3.5. Quality Assurance

3.5.1. Quality Assurance Programmes

Quality assurance (QA) is an important part of any successful AW-IPM programme using the SIT. A QA programme provides various benefits with respect to irradiation procedures, including:

- Success of the process — adequately sterilized insects of good quality can be produced consistently
- Compliance with regulations — a QA programme makes it convenient to audit the process against established standards
- Harmonization — as international trade is growing, it has become more important to ensure dependable uniformity across geographical and political regions
- Public acceptance — when the public realizes that SIT facilities strictly follow set procedures and document the process, it has more confidence in the programme

An effective QA programme includes standard operating procedures (SOPs) for all activities related to packaging, sterilization, and dosimetry. Also, processing equipment that controls key operating parameters (those affecting dose) is periodically tested and/or calibrated to verify that the irradiator is operating properly. This is then documented as part of the record for the QA programme.

For many processes related to insect sterilization using radiation, standards and guidelines are available that can be incorporated into a facility's QA programme. These include dosimetry standards developed by the International Organization of Standardization and the American Society for Testing and Materials (ISO/ASTM 2004a, 2004b, 2004c, 2004d, 2004e), an SOP for using the Gafchromic® film dosimetry system for the SIT (IAEA 2004), and a comprehensive quality-control manual for applying the SIT against fruit flies (FAO/IAEA/USDA 2003).

3.5.2. Irradiator Operation and Configuration

When an irradiator is installed, it is evaluated to ensure that it is working according to the manufacturer's specifications, and to develop baseline data on its performance. These two activities are known as installation qualification and operational qualification, respectively (ISO/ASTM 2004a). Operational qualification includes, among other things, initial dose mapping (section 3.3.2.), and measurement of the dose rate at a reference position, e.g. at the centre of a fully filled canister (section 3.3.1.). The reference dose rate is then used to establish the basic relationship between key operating parameters, such as timer setting or conveyor speed, and absorbed dose. Dosimetry standards recommend repeating periodically

the reference dose-rate measurement, e.g. every 3 years for gamma irradiators (ISO/ASTM 2004a). A caesium-137 (^{137}Cs) source, in particular, may contain impurities (^{134}Cs) that affect the decay rate, and thus, over time, the dose rate. Reference-standard dosimetry and dose mapping are repeated as appropriate following any changes in the irradiator, such as source renewal in gamma irradiators, that could affect the dose rate or dose distribution.

Before insects are sterilized, key process parameters are established as part of performance qualification. For most insect irradiators, the absorbed dose delivered to the insects is controlled by adjusting a single parameter, such as timer setting (irradiation time) or conveyor speed. Values of these parameters depend on the dose specifications (section 3.4.1.) and the reference dose rate on the day of irradiation. Dose mapping is again performed to ensure that all insects within a given canister will receive an appropriate dose. If necessary, process parameters can often be adjusted to improve dose uniformity; common alterations include optimizing the size or shape of the canister, rotating the canister on a turntable during irradiation, using dose attenuators, and using plugs of simulated product, e.g. styrofoam, in the canister or irradiation chamber to exclude insects from areas with unacceptably low or high dose rates. This procedure establishes a canister design and a loading configuration of insects that result in an acceptably uniform dose distribution. The results of this mapping may also be used to establish a reference location for performing routine dosimetry as part of process control (section 3.5.3.).

3.5.3. Process Control

The accidental release of insects that are not irradiated properly could potentially be disastrous (Knipling 1982), especially in programmes like those in California and Florida, USA, where the SIT is used to eradicate extremely small pest populations and/or as a prophylactic measure to prevent the establishment of newly introduced pests. To avoid this problem, programmes that release sterile insects implement various process-control elements to help ensure that all insects are irradiated according to specifications (FAO/IAEA/USDA 2003). In addition to the elements listed below, programmes applying the SIT often monitor relevant process parameters such as information on the preparation and packaging of insects, setting of the irradiator timer, conveyor speed, canister specifications, and position and loading of the canisters. The results of process monitoring are routinely documented as part of the record of the QA programme.

Sterility Testing. Most AW-IPM programmes that integrate the SIT test samples of irradiated insects on a regularly scheduled basis to confirm that specified levels of sterility are being achieved. The quality-control manual for using the SIT against fruit flies suggests that this could be done for every shipment (FAO/IAEA/USDA 2003), comparing the egg hatch from pairings of irradiated and non-irradiated insects with that from crosses of non-irradiated insects. Besides making regularly scheduled sterility tests, unscheduled tests should also be conducted whenever changes are made to any equipment or procedures and before any insects are shipped. However, it takes time to obtain the results of a sterility test, and the results

may be known too late to prevent the release of incorrectly treated insects. Therefore sterility testing must be supplemented with other methods, such as routine dosimetry.

In addition, the competitiveness of the sterile insects needs to be checked with insects of the target population to ensure the efficacy of the programme. Such testing helps to ensure that all procedures are being followed correctly, including rearing, pre-irradiation preparation (e.g. age-based selection of insects), packaging for hypoxia or nitrogen (if used), temperature control, irradiation-dose control, and post-irradiation handling.

Routine Dosimetry. Regular use of routine dosimetry can help to confirm that insects are being irradiated according to programme specifications, and may be required for every shipment (FAO/IAEA/USDA 2003). This is usually done by placing dosimeters on packaging containers or canisters at a specific location (which may be the reference location identified through dose mapping performed during performance qualification (section 3.5.2.)), where the dose rate has a known and predictable relationship to the minimum and maximum dose rate within those canisters. Unlike sterility testing, routine dosimetry can identify problems in the irradiation process quickly enough so that improperly sterilized batches of insects can be intercepted prior to release in the field. Although standard dosimetry is faster than sterility testing, a third control described below provides an immediate visual confirmation check that a given container has gone through the irradiation process.

Radiation-Sensitive Indicators. A radiation-sensitive indicator is a material, such as a coated or impregnated adhesive-backed (or adhesive-fronted) substrate, ink, or coating, that undergoes a qualitative visual change when exposed to a specified dose of radiation (ISO/ASTM 2004e). The dose at which the indicator changes should ideally be below, but near, the minimum dose required. Since the degree of colour change is not proportional to the dose, these indicators cannot substitute for dosimeters.

Indicators are used as aids in tracking whether or not specific containers have been irradiated. The quality-control manual (FAO/IAEA/USDA 2003) suggests that an indicator should be attached to each packaging container of insects to help ensure (along with product segregation protocols and other procedural methods) that non-irradiated insects are not unintentionally released in the field. Also, indicators could potentially be used to assist in tracking multiple passes of containers through an irradiator when the sterilizing dose is fractionated into several smaller doses (section 4.1.).

Indicators that are exposed to excessive humidity, high temperature or UV radiation, e.g. sunlight, before or after irradiation may give erroneous readings; hence they are useful only within an irradiation unit where these conditions are controlled.

Recommended dosimetric procedures, including routine dosimetry and the use of indicators for programmes releasing sterile insects, are described in published

standards and guides, e.g. ISO/ASTM 2004a, 2004d, 2004e; FAO/IAEA/USDA 2003.

4. FACTORS MODIFYING INSECT RADIATION SENSITIVITY

The sensitivity of arthropods to radiation depends on many parameters. Radiation sensitivity varies widely among species (section 6.), but environmental conditions, and the biological state of the organism at the time of irradiation, also can have significant influences. These latter factors should be optimized so that sterilized insects are of the highest possible quality.

4.1. Environmental and Physical Factors

4.1.1. Ambient Atmosphere
Oxygen levels affect the sensitivity of insects to radiation (Baldwin and Chant 1971, Economopoulos 1977, Ohinata et al. 1977, Rananavare et al. 1991, Fisher 1997). Damage induced by radiation is typically lower in an oxygen-reduced environment (hypoxia) than in air, so usually higher doses are needed to produce comparable reproductive sterility. However, because the magnitude of this protective effect tends to be greater for somatic damage than sterility, the use of oxygen-reduced atmospheres is a common strategy to improve sterile insect competitiveness without sacrificing sterility (Calkins and Parker, this volume; Lance and McInnis, this volume). Methods for inducing hypoxia are described in section 3.4.2.

The increased radiation damage in a high-oxygen environment is a general phenomenon in radiobiology. For the protective effect of low oxygen to be seen, the tissues must be anoxic or hypoxic during irradiation; exposure to oxygen before or after is without effect. Ionizing radiation initiates a chain of oxidative reactions along the radiation path in the tissues and the formation of free radicals, which in the absence of oxygen might be neutralized by combining with hydrogen radicals resulting in no net damage. In the presence of oxygen, damaging peroxy-radicals may be formed, and the organic molecules, including the germ cell chromosomes, are irreversibly altered. It must be noted that high-LET radiation (e.g. alphas, neutrons) is less affected by the presence or absence of oxygen than low-LET radiation (X-rays and gamma radiation). This may be because high-LET radiation causes several ionizations within one macromolecule, damaging it beyond repair (Pizzarello and Witcofski 1967).

4.1.2. Dose Rate
The adverse effects of radiation appear, in general, to be lessened by reducing the rate at which the sterilizing dose is delivered to the insects. This can be done by using a lower dose rate, and longer irradiation time, for a single irradiation (Yanders 1959, Nair and Subramanyam 1963, Hooper 1975, Mayas 1975, Ilao 1977). An alternate approach to conserve insect quality is dose fractionation, where the sterilizing dose is delivered over time in a series of smaller irradiations (North 1975, LaChance and Graham 1984, Haynes 1993, Tamhankar and Shantharam 2001).

However, because of its impracticality, current AW-IPM programmes applying the SIT do not follow this procedure.

4.1.3. Temperature
There are some data to suggest that irradiation at reduced temperatures tends to increase the resistance of arthropods to radiation (Rananavare et al. 1991). Cool temperatures, to a certain limit, and hypoxia, also reduce the metabolic rate, and therefore the development rate, of insects during irradiation.

4.2. Biological Factors

4.2.1. Cell Stage and Characteristics
The most radiosensitive cells are those (1) with a high mitotic rate, (2) with a long mitotic future (i.e. under normal circumstances, they will undergo many divisions), and (3) which are of a primitive type. These generalizations, with some exceptions, have become known as the Law of Bergonie and Tribondeau (Casarett 1968). In this regard, germ cells are the most radiosensitive, and show different killing and sterilization susceptibility according to their development stage.

It is generally accepted that chromosomal damage (structural and numerical anomalies) is the cause of dominant lethal mutations. Dominant lethal mutations occurring in a germ cell do not cause dysfunction of the gamete, but are lethal to the fertilized egg or developing embryo (Robinson, this volume). The earlier stages of spermatogenesis (spermatocytes and spermatogonia) are generally more radiosensitive than later stages (spermatids and spermatozoa) (Proverbs 1969). Dey and Manna (1983) found that chromosomes in spermatogonial metaphase and anaphase I were more sensitive to X-rays than those in other stages. Germ-cell sensitivity in female insects is, however, complicated by the presence of nurse cells that are most susceptible to injury during mitosis (LaChance and Leverich 1962).

The dose required to inhibit mitosis is reported to be inversely proportional to the number of chromosomes, and correlates with the average interphase chromosome volume. The larger the nuclear volume, apparently the greater is the sensitivity. Similar relationships were determined in animals and plants, and used to predict their sensitivity to chronic irradiation (Sparrow et al. 1963, Sparrow et al. 1967, Casarett 1968, Whicker and Schultz 1982, Jacquet and Leonard 1983). Furthermore, radiosensitivity appears to be influenced by additional parameters including cell repopulation capacity, tissue and organ regeneration ability, and biological repair (Harrison and Anderson 1996).

Chromosome organization can also affect the response to radiation. Several insect orders (Hemiptera, Lepidoptera, Trichoptera, Odonata and Dermaptera) have holokinetic chromosomes, i.e. properties of the centromere are distributed over the entire chromosome (Kuznetsova and Chubareva 1979). LaChance and Riemann (1973) suggested that, in these taxa, most dominant lethal mutations cause death after blastoderm formation. In other orders, dominant lethal mutations are expressed during the early cleavage divisions.

4.2.2. Developmental Stage and Age

Age and developmental stage are important parameters to be taken into consideration when deciding on radiation process parameters for the SIT. In general, adults are more radioresistant than pupae, which in turn are more resistant than larvae. Similarly, older pupae tend to be more resistant to radiation than younger pupae (Ismail et al. 1987, Ahmed et al. 1990, Hamed and Khattak 1991, Dongre et al. 1997). Also there is a negative relationship between the age of eggs and their sensitivity to treatment (Chand and Sehgal 1978).

4.2.3. Sex

Regarding sterilization or disinfestation, female arthropods are, in general, more radiosensitive than males (Cogburn et al. 1973, Hooper 1989, Hallman 1998), but there are numerous exceptions. For example, males were found to be more radiosensitive than females in the hemipteran families Pyrrhocoidae, Piesmidae, and Pentatomidae (Mau et al. 1967), the American cockroach *Periplaneta americana* (L.) (Wharton and Wharton 1959), certain Coleoptera (section 6.2.), and ixodid ticks (Purnell et al. 1972).

   The wide variation reported among species in relative radiosensitivity of males versus females likely results in part from differences in the maturity of oocytes present when females are irradiated. For example, if Mediterranean fruit fly female pupae are irradiated two or more days before adult emergence, egg production is completely stopped by doses well below those needed to sterilize males. However, on the day before emergence and at later times, females contain increasing numbers of oocytes that mature into viable eggs even if irradiated at doses sufficient to sterilize males (Williamson et al. 1985).

4.2.4. Size and Weight

Early studies (Wharton and Wharton 1957, Willard and Cherry 1975) suggested that species with large adults would tend to be more radiosensitive than those with small adults. Experiments have shown that *Periplaneta americana* is killed or sterilized by radiation doses to which smaller insects such as *Drosophila*, *Habrobracon*, and *Tribolium* are resistant. However, the correlation between size, weight, and radiosensitivity has not proved to be very strong.

4.2.5. Diapause

The effects of diapause on insect sensitivity to radiation appear to vary. Mansour (2003) found that radiation-related reductions in adult emergence were greater following treatment of diapausing than that of non-diapausing larvae of the codling moth *Cydia pomonella* (L.), but other authors reported that diapausing and non-diapausing larvae of other species were equally sensitive to radiation (Ignatowicz 1997, Hallman 2000). Carpenter and Gross (1989) reported no interaction between inherited sterility (IS) and diapause with regard to several traits, although crosses involving moths that emerged from diapaused pupae produced significantly fewer eggs. In contrast, diapausing twospotted spider mites *Tetranychus urticae* Koch

appeared more tolerant to radiation than non-diapausing mites (Lester and Petry 1995).

4.2.6. Nutritional State

Pre- or post-irradiation starvation, or the nutritional state, may influence radiosensitivity (Wharton and Wharton 1959, Stahler and Terzian 1963, Drummond et al. 1966). For example, to achieve 100% sterility, male and female lone star ticks *Amblyomma americanum* (L.) required about 10 Gy before engorgement and 24 Gy after engorgement (Drummond et al. 1966). The data suggested an attenuation of radiation-induced lethality in a blood-fed organism, but the mechanism remains unknown. Beuthner (1975) did not find such differences in *Amblyomma variegatum* (F.), *Hyalomma anatolicum excavatum* Koch or *Rhipicephalus appendiculatus* Neumann.

4.2.7. Additional Factors

An insect's state of hydration, or moisture content, could potentially influence the effects of radiation, but probably this is applicable mostly to commodity disinfestation. Diurnal rhythms apparently can influence the induction of sterility by radiation. Rananavare et al. (1991) found that potato tuberworms *Phthorimaea operculella* (Zeller) irradiated in scotophase were less resistant than those treated in photophase. Finally, genetic differences related to geographical diversity within a species can potentially affect insect radiosensitivity (Fisher 1997, Azizyan 2003, Hallman 2003).

5. ARTHROPOD SPECIES SUBJECTED TO RADIOSTERILIZATION

The International Database on Insect Disinfestation and Sterilization (IDIDAS) (IDIDAS 2004) was developed to collect and share information about radiation doses for disinfestation and reproductive sterilization of arthropods, and to perform a comparative analysis and quality-assurance check on existing data. IDIDAS was based on a literature review and analysis of more than 2750 references that were published during the past five decades. Due to space limitations, references are not included in this chapter, but can easily be obtained from the IDIDAS website.

In the past five decades, at least 217 species of arthropods of economic importance, found in 136 genera, 61 families, 7 insect orders and 2 arachnid orders, have been subjected to irradiation studies for the purposes of research, biological control, or pest management programmes integrating the SIT (Table 3). Of these, 31% are Diptera, 25% Lepidoptera, 24.5% Coleoptera, 9% Hemiptera, 5.5% Acari, 3% Dictyoptera, 1% Araneae, 0.5% Thysanoptera, and 0.5% Orthoptera. Out of 66 entries on Diptera from 15 families and 26 genera, 21 species belong to the Tephritidae, indicating the importance of this group in pest management and international trade. The Culicidae and Pyralidae follow Tephritidae in terms of the number of species radiosterilized.

Potential sources of error in any compilation of records, such as this database, are numerous. One of the main difficulties derives from taxonomy, an evolving science; during the past 50 years the names of many pest species have been revised. Organisms for irradiation drawn from a cultured population should, therefore, be defined for posterity by lodging voucher specimens in an appropriately secure and curated collection. This is particularly important for groups subject to frequent taxonomic changes, such as the Tephritidae.

Table 3. Calculated mean and 95% confidence limits (upper L_2, lower L_1) (Sokal and Rohlf 1995) for radiosterilization doses for insects and related arthropods (data are for in-air irradiation of males treated either as pupae or nymphs, but mosquitoes and apple maggots treated as adults; other factors, e.g. radiation source, temperature, dose rate, and level of sterility achieved, were not necessarily consistent; references for data from IDIDAS (2004))

Order	Family	Number of genera	Number of species	Sterilization dose (Gy)		
				L_2	Mean	L_1
Acari	Acaridae	4	4	305	270	234
	Argasidae	2	2	302	198	93
	Ixodidae	4	7	33	32	31
	Tetranychidae	2	4	273	153	43
Araneae	Eresidae	1	1	150	150	150
	Pholcidae	1	1	20	20	20
Coleoptera	Anobiidae	3	4	71	43	15
	Bostrichidae	2	1	176	132	87
	Bruchidae	3	5	90	80	70
	Cerambycidae	1	1	90	80	70
	Chrysomelidae	2	2	100	54	28
	Coccinellidae	1	1	69	69	69
	Curculionidae	10	12	119	76	33
	Dermestidae	3	5	211	152	93
	Laemophloeidae	1	3	200	200	200
	Lyctidae	1	1	69	69	69
	Scarabaeidae	3	5	75	44	13
	Scolytidae	1	1	65	65	65
	Silvanidae	1	1	117	117	117
	Tenebrionidae	5	11	102	77	52
Dictyoptera	Blaberidae	1	1	5	5	5
	Blattellidae	1	2	32	32	32
	Oxyhaloidae	1	1	140	140	140

Table 3. Continued

Order	Family	Number of genera	Number of species	Sterilization dose (Gy)		
				L_2	Mean	L_1
Diptera	Agromyzidae	1	1	155	155	155
	Anthomyiidae	1	2	45	37	30
	Calliphoridae	3	5	40	30	20
	Chloropidae	1	1	45	45	45
	Culicidae	3	15	116	54	10
	Cuterebridae	1	1	200	150	100
	Drosophilidae	1	1	160	160	160
	Glossinidae	1	7	120	99	60
	Muscidae	4	6	30	26	20
	Oestridae	1	2	50	45	40
	Piophilidae	1	1	100	100	100
	Sarcophagidae	1	1	52	36	19
	Sciaridae	1	1	40	40	40
	Tachinidae	1	1	20	20	20
	Tephritidae	5	21	83	63	44
Hemiptera	Aleyrodidae	2	3	80	70	60
	Aphididae	2	2	10	10	10
	Cicadellidae	1	1	200	180	160
	Coreidae	2	2	80	80	80
	Delphacidae	2	2	50	50	50
	Lygaeidae	1	1	100	100	100
	Pentatomidae	2	3	60	60	50
	Pseudococcidae	2	1	160	160	160
	Pyrrhocoridae	1	1	70	70	70
	Reduviidae	3	3	150	80	10
Lepidoptera	Arctiidae	2	2	400	400	400
	Bombycidae	1	2	250	250	250
	Gelechiidae	3	3	200	200	150
	Lymantriidae	2	2	180	133	80
	Noctuidae	4	13	300	300	300
	Pieridae	1	1	350	350	350
	Plutellidae	1	1	200	200	200
	Pyralidae	11	16	389	260	131
	Sphingidae	1	1	100	100	100
	Thaumetopoeidae	1	1	40	40	40
	Tortricidae	8	12	330	278	226
Orthoptera	Acrididae	1	1	4	4	4
Thysanoptera	Thripidae	2	1	100	100	100

6. RADIATION DOSES FOR ARTHROPOD STERILIZATION

Arthropods are more radioresistant than human and other higher vertebrates (Table 4), but less resistant than viruses, protozoa and bacteria (Ravera 1967, Rice and Baptist 1974, Whicker and Schultz 1982, Blaylock et al. 1996, Harrison and Anderson 1996). One of the main reasons for the higher radioresistance is that arthropods have a discontinuous growth during immature stages, and cells become active only during the moulting process. This is encoded in Dyar's Rule, i.e. insects double their weight at each moult and thus their cells need to divide only once per moulting cycle (Hutchinson et al. 1997, Behera et al. 1999). The high resistance of most adult insects to radiation is attributed to the fact that they are composed of differentiated cells, which do not undergo replacement (Sullivan and Grosch 1953). Such cells are much more resistant to death or damage induced by irradiation than are dividing or undifferentiated cells.

Table 4. Ranges of LD_{50} for acute irradiation of organisms from different taxonomic groups (length of time for survival is usually set at 30 days for mammals, but longer times may be needed for other organisms) (Table from Bakri et al. 2005, reproduced with permission)

Group	Dose (Gy)	Reference
Bacteria, protozoa, viruses	100–10 000	Harrison and Anderson 1996
Insects	30– 1500	Whicker and Schultz 1982
Molluscs	50– 500	Ravera 1967
Higher plants	1.5–>130	Harrison and Anderson 1996
Fish	4– 100	Harrison and Anderson 1996
Amphibians	7– 22	Harrison and Anderson 1996
Reptiles	3– 40	Harrison and Anderson 1996
Birds	5– 20	Harrison and Anderson 1996
Humans	3	Rice and Baptist 1974

Radiation doses for sterilization (Table 3), as reported in the literature (IDIDAS 2004), were selected using similar criteria, when available, for sterility level (full or as available), gender (male), and atmospheric condition (air). The developmental stage at irradiation was the pupa or nymph, except for mosquitoes and apple maggots *Rhagoletis pomonella* (Walsh) which were irradiated as adults. Other experimental parameters such as temperature, radiation source, dose rate, etc., may differ. Even compiling the data was difficult because of the absence of uniform experimental procedures and dosimetry, and the influence of various parameters. Dose values reported below may also differ from doses that are routinely used to

sterilize members of the reported taxa for the SIT, especially in cases where programmes irradiate insects in oxygen-reduced atmospheres. Therefore, the doses presented should be considered only as guidelines for further investigation and to provide general introductory information (Bakri and Hendrichs 2004, Bakri et al. 2005).

6.1. Arachnidae

6.1.1. Acari
The mean dose to sterilize Acari species ranged from 32 to 270 Gy (Table 3). The fowl tick *Argas persicus* (Oken) (Argasidae), Chilean false red mite *Brevipalpus chilensis* Baker (Tenuipalpidae), grain mite *Acarus siro* L. (Acaridae), and brownlegged grain mite *Aleuroglyphus ovatus* (Troupeau) (Acaridae), are among the most resistant species. Hard ticks (Ixodidae), such as *Amblyomma* spp. and *Boophilus* spp., tend to be more sensitive than soft ticks (Argasidae). The radiation sensitivity of some tick species appears to change depending on whether the tick is engorged with blood or not (section 4.2.). Parthenogenesis occurs in many species of ticks and other arthropods, making the practical application of the SIT rather unlikely (Lance and McInnis, this volume).

6.1.2. Araneae
The only known cases of irradiation of spiders for sterilization were conducted to determine the pattern of sperm precedence (Lance and McInnis, this volume) in multiple-mated females. Kaster and Jakob (1997) used a 20-Gy dose to sterilize males of *Holocnemus pluchei* (Scopoli) (Pholcidae), whereas Schneider and Lubin (1996) applied a 150-Gy dose to *Stegodyphus lineatus* (Latreille) (Eresidae). These species showed last-male precedence and complete sperm mixing, respectively.

6.2. Insecta

6.2.1. Coleoptera
The mean sterilization dose for Coleoptera ranged from 43 to 200 Gy (Table 3). Curculionidae and Tenebrionidae, which represent the major groups of species that have been tested for radiation sterilization, both required a dose of about 76 Gy. The most resistant species belong to Laemophloeidae (200 Gy), and the most sensitive to Anobiidae (43 Gy). Some data in this order suggested a differential response of males and females towards sterilizing doses of radiation. Males may be less resistant than females, as in the case of the Japanese beetle *Popillia japonica* Newman (Ladd et al. 1973) and the beetle *Tribolium madens* (Charpentier) (Brower and Tilton 1973), or more resistant as in the case of the khapra beetle *Trogoderma granarium* Everts (Carney 1959, Nair and Rahalkar 1963).

The effects of gamma radiation on the boll weevil *Anthonomus grandis grandis* Boheman were thoroughly studied with a view to applying the SIT (Earle et al. 1979, Villavaso et al. 1989, Haynes 1993). Males were sterilized by about 80 Gy,

but longevity was poor; egg-laying capacity was reduced at doses of 50 Gy or more, but females continued to produce some fertile eggs until doses approached 200 Gy, a dose which rendered the weevils non-competitive (McKibben et al. 2001). Eventually methods were found to block egg hatch using a chitin synthesis inhibitor (diflubenzuron), and studies were conducted on reducing the negative effects of radiation using improved mass-reared strains, oxygen-reduced atmospheres, and fractionated radiation doses (Earle et al. 1979, Haynes 1993, McKibben et al. 2001). Competitive sterile boll weevils can now be delivered to the field, but the SIT remains more complicated and expensive than current pest management strategies, which employ pheromone traps and chemical control (McKibben et al. 2001).

The detrimental effects of radiation have also been one of the main obstacles in applying the SIT to the West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire). Digestive obstruction following the collapse of the epithelial tissue of the midgut was suggested as the cause of the short lifespan of gamma-irradiated adults (Sakurai et al. 2000). Nevertheless an AW-IPM programme in Kume Island, Japan, to eradicate this weevil using the SIT is making good progress (Shimoji and Miyatake 2002, Shimoji and Yamagishi 2004).

6.2.2. Dictyoptera

Several species of dictyopterans have been used in radiobiological studies exploring biochemical, physiological, and genetic properties (Shivaji and Rastogi 1974). In spite of the pest status of many cockroach species, there have been relatively few investigations related to pest suppression using sterile insects, due largely to potential problems with releasing large numbers of males into natural populations (Ross and Cochran 1963, Berndt 1978, Menon 1978, Ross et al. 1981, Gecheva and Apostolova 1986). In terms of sterility and mortality, cockroaches are among the most radiation-sensitive insects, with less than 5 Gy required in some cases to induce sterility (Wharton and Wharton 1959, Ross and Cochran 1963). Sexual differentiation in radiosensitivity was observed in *Blaberus craniifer* Burmeister, where males were less resistant than females (Gecheva and Apostolova 1986). In Dictyoptera, it is the adult stage that is most frequently used for sterilization with ionizing radiation.

6.2.3. Diptera

Radiation sterilization of dipterans generally requires doses from 20 to 160 Gy (Table 3). Drosophilidae and Agromyzidae are among the most radiation-resistant families of Diptera tested, whereas tachinids are the most sensitive. The late pupal (often pharate adult) stage is preferred for the radiation of most fly species because it is practical to handle and ship pupae, and an acceptable balance between competitiveness and sterility is achieved. In the Culicidae, due to the fragility of other stages, the adult is irradiated.

Tephritidae, the major family in this group that uses the SIT, require, on average, about 63 Gy for sterilization. Tephritids are relatively homogeneous with respect to radiation sensitivity — less than 100 Gy are needed to achieve complete sterility in the five major pest genera (*Anastrepha, Bactrocera, Ceratitis, Dacus, Rhagoletis*),

and this confirms the generic recommendation of a dose in the range of 100–150 Gy to disinfest agricultural commodities for international trade (Hallman 2000). Many AW-IPM programmes applying the SIT against major pest tephritids have used 100–150 Gy for sterilization, well over the family "average" of 63 Gy. In some early programmes (LaChance et al. 1967), this was a "precaution" to increase the security margin for sterilization, but the overdose often has lowered competitiveness to the point where it reduced the overall ability of irradiated flies to induce sterility into the wild population (Toledo et al. 2004). In recent programmes, these higher doses are usually associated with the use of hypoxia to enhance sterile male competitiveness (section 4.2.) (Fisher 1997).

6.2.4. Hemiptera

The mean sterilizing dose in the Hemiptera ranged from 10 to 180 Gy (Table 3), with *Circulifer tenellus* (Baker) (Cicadellidae) females being the most resistant species tested thus far (Ameresekere and Georghiou 1971), and *Brachycorynella asparagi* (Mordvilko) (Aphididae) adults being the most sensitive. In general, adult females required a gamma radiation dose of 50–60 Gy to achieve a high level of sterility. However, higher doses of up to 200 Gy (electrons in this case) were needed to achieve complete sterility in female *Myzus persicae* (Sulzer) (Aphididae) and *Pseudococcus comstocki* (Kuwana) (Pseudococcidae) (Dohino et al. 1997). Adult males typically required a dose between 60 and 150 Gy. For 4th- and 5th-instar nymphs, a lower dose was needed; 75 to 100% sterility was achieved with doses between 5 and 60 Gy. Patterns of relative radiosensitivity between females and males differ among species of Hemiptera (IDIDAS 2004).

Only 19 species belonging to 10 out of 53 families of Hemiptera have been subjected to radiation for sterilization. For several species, the feasibility of releasing sterile males for pest suppression was investigated (Shipp et al. 1966, Baldwin and Chant 1971, Tadic 1972, Dyby and Sailer 1999, Calvitti et al. 2000). Some hemipterans are facultatively parthenogenetic, but Steffan and Kloft (1973) argued that, with proper timing and climate, effective genetic control might still be possible.

6.2.5. Hymenoptera

The Hymenoptera include a number of serious pests, such as Africanized honey bees, and various Formicidae (ants) and sawflies. Since bees and ants are social insects with complex life histories, the application of the SIT has been limited to a few laboratory experiments (Sakamoto and Takahashi 1981). For male honey bees, the sterilizing dose is 80–100 Gy (Lee 1958). Most experimental irradiations of hymenopterans, e.g. the parasitic wasp *Bracon hebetor* (Say), have been conducted in conjunction with relatively basic radiobiological investigations. (For these reasons, the doses for sterilization of this group are not included in Table 3.)

6.2.6. Lepidoptera

Lepidopterans as a group are relatively resistant to radiation; mean doses for sterilization range from 40 to 400 Gy, with *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Thaumetopoeidae) requiring the lowest documented average dose

of 40 Gy (Baccetti and Zocchi 1962), while the arctiid *Diacrisia obliqua* Walker has the highest recorded doses — 300 Gy and 400 Gy for complete sterility of pupae and adults, respectively (Khattak 1998). Successful lepidopteran AW-IPM programmes that integrate the SIT (Bloem et al., this volume) include the codling moth in Canada (Proverbs 1982), and the pink bollworm *Pectinophora gossypiella* (Saunders) in the USA (Henneberry and Clayton 1988).

In contrast to other insect orders, the F_1 progeny of irradiated male lepidopterans are typically more sterile than their parents. The sex ratio in the F_1 generation is biased toward males. Thus substerilized males can sire completely sterile offspring, and this phenomenon has been exploited in a number of programmes (Carpenter et al., this volume).

6.2.7. Orthoptera

Acrididae (Orthoptera), along with Blaberidae (Dictyoptera), are among the most radiosensitive insects known (less than 5 Gy needed for sterilization). This is in agreement with Willard and Cherry (1975) who suggested that large long-lived adults are more radiosensitive than small short-lived adults.

6.2.8. Thysanoptera

No species of Thysanoptera has been investigated for pest suppression directly using the SIT. However, in Japan, radiation sterilization has been investigated as a quarantine treatment to disinfest cut flowers of thysanopteran pests. Doses up to 400 Gy (electron beam) and 100 Gy (gamma rays) were given to suppress the pests *Frankliniella occidentalis* (Pergande) (EPPO 1994) and *Thrips* spp. (Dohino et al. 1996, Hayasi et al. 1999, Bansiddhi 2000), respectively.

7. CONCLUSIONS

Although radiation is a key component of the SIT, it is generally not given the attention it deserves — in terms of procedures for irradiation, dosimetry, and the choice of an appropriate dose to maximize the introduction of sterility into wild females. The development of accurate dose-response curves for the target insect, using precise dosimetry, is a prerequisite of any programme releasing sterile insects. The survey of the available literature presented here shows the wide variation in the response of the different insect species to radiation, and also highlights the need for accurate dosimetry.

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CHAPTER 3.4.

STERILE INSECT QUALITY

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SUMMARY

The sterile insect technique (SIT) depends greatly on the production of good quality sterile male insects that are released into target wild populations. Quality is assured through a system of bioassays of quality parameters that reflect the insect's ability to survive, interact with its environment, and locate, mate and fertilize females of the target population. The system was developed by compartmentalizing the essential survival and mating behaviours of the species involved, and then developing a series of tests to confirm that these behavioural traits are present in the mass-reared insects. The system also has a feedback loop to correct problems in the production portion of the system before they become evident. Nevertheless, regular implementation of field or field-cage tests under semi-natural conditions, where sterile males have to compete with wild males for wild females, is required to provide the ultimate assurance that the sterile insects have the ability to fulfil their mission after release.

1. INTRODUCTION

A major concern of entomologists, and managers of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT), is that released sterile males are adequate to fulfil the mission for which they were produced, i.e. that they compete well with wild males, and successfully mate and inseminate the targeted wild females. The components that are most important in such programmes include dispersal, longevity, location of mating arenas, courtship, mating, and sperm transfer (Koyama et al. 2004).

The ability of entomologists to evaluate and quantify the effectiveness of mass-reared sterilized insects in interactions with wild insects has only existed for 35 years (Boller 2002). Before then, the effectiveness of the released sterile insects was evaluated according to whether or not the SIT worked in the field. If it failed, managers were able only to speculate on what went wrong, or where and when the problem occurred.

In large suppression and eradication programmes using the SIT (Hendrichs et al., this volume), large numbers of insects must be reared for sterilization and release. Often the emphasis is on the numbers, and insect quality is overlooked or marginalized. For many years, the SIT was recognized as a "numbers game", i.e. if a programme began to fail, the remedy was to increase the sterile insect release rate. Only when a programme failed completely was the quality of the sterile insects questioned. This happened in the case of the large New World screwworm *Cochliomyia hominivorax* (Coquerel) programme in the early 1970s in south-western USA (Klassen and Curtis, this volume; Vargas-Terán et al., this volume). The controversy surrounding the possible causes of this failure led to the adoption of regular strain renewal, and the development of a quality control system to continuously monitor the insect colony using a series of behavioural and physiological tests to detect colony changes. This fostered the development of "quality control of mass-reared insects" as a recognized entomological discipline.

This chapter discusses the history of the development of quality control technology, the principles and philosophy of assessing insect quality, and the relative importance of the various parameters used to assess insect quality in the context of mass-rearing for the SIT. Quality control is most developed for various fruit fly species (FAO/IAEA/USDA 2003), and therefore much of the discussion will be in that context. Quality control in rearing biological control organisms is beyond the scope of this chapter, and will not be addressed.

2. HISTORY AND PHILOSOPHY OF QUALITY CONTROL

Scientists have long been aware that insects produced for laboratory purposes should be of a quality appropriate to the experimental system being tested. However, with the advent of mass-rearing and sterilization for the SIT, the quality of mass-produced insects became even more important since appropriate behaviour in the field was now critical to the success of field programmes.

Boller (2002) reviewed the history of the quality control of mass-reared insects. He divided this history into four distinct periods: (1) before 1969 — little concern about behavioural quality in rearing programmes, (2) 1969–1975 — a growing awareness of the ideas and concepts of quality control, (3) 1976–1979 — international collaboration began, and prototypes of quality control systems were initiated, and (4) 1980 to the present — finally the concept, and practical applications in most mass-rearing facilities, were generally accepted (Bernon and Leppla 1994, Bigler 1994).

The publication of an "idea book" on fruit fly quality control by Boller and Chambers (1977) is probably responsible for developing the subject of quality control into what is now recognized as a discipline of economic entomology. These authors were able to bring together the diverging schools of thought on quality assurance, and were able to combine the groups into a global Arthropod Mass Rearing and Quality Control Working Group (AMRQC), International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC), and initiated the first workshops in 1982 and 1984. The proceedings of the Eighth and Ninth Workshops were published in 2002 (Leppla et al. 2002). (Most of the presentations in the last workshops involved biological control organisms, many produced by private companies.)

The fundamentals of quality control are basic to any manufacturing process. Rearing insects to fulfil a specific function is analogous to manufacturing a product. The principles and philosophy of quality control in the realm of manufacturing were established many years ago (Charbonneau and Webster 1978), and these same principles are adaptable today to the quality of mass-reared insects. These principles are described in an excellent book by Feigenbaum (1961), who defined total quality control as:

> an effective system for integrating the quality-development, quality-maintenance and quality-improvement efforts of the various groups in an organization so as to enable production and service at the most economical levels which allow for full consumer satisfaction.

Quality control is divided into three categories: (1) production quality control, where the inputs to rearing are addressed, including diet ingredients, equipment, etc., (2) process quality control, measuring how things are done, such as diet preparation, environmental conditions, infestation rate, larval separation, pupal holding, irradiation dose, etc., and (3) product quality control, where the insects produced are evaluated for effectiveness in completing the purpose for which they are required. This chapter concentrates on product quality control and some aspects of process control, while production and process control are covered by Parker (this volume).

The "control" in quality control is a management tool consisting of: (1) setting quality standards, (2) appraising conformance to these standards, (3) acting when the

standards are breached, and (4) planning for improvements in the standards. The difference between mass-producing insects and rearing small colonies for laboratory experiments is in the product rather than the process. In other words, the "bottom line" of a mass-rearing programme is the performance of the released insects in the field (IAEA 1992).

While the factors affecting product quality are both technological and human, the human factors, involving operators, section leaders, and other personnel, are by far the most important, and the problems most likely to occur are often related to the human factors. The purpose of a quality control programme is to identify the factors leading to low quality during the rearing process, instead of trying to correct low quality after insects have been reared. Such a programme results in improvements in product quality and employee morale, and reductions in production costs and production bottlenecks. It also provides improved inspection methods, definite schedules for preventive maintenance, and a factual basis for standards during the rearing process.

Development of a functioning quality control programme depends on a continuous chain of responsibility for quality through all the workers. To achieve this, responsibilities for quality should be assigned to all key personnel in the rearing facility. Certain workers should be assigned to evaluate insect quality using scheduled bioassays; their only responsibility should be product quality, and they must be answerable only to the programme manager. These people serve as the "eyes and ears" of the programme manager; they must never be answerable to the rearing manager. "Control" in quality control is achieved when there is constructive feedback, from the quality control workers through the programme manager, on activities that may be responsible for the lack of quality.

Quality control procedures both incur costs and provide benefits. The costs are in the appraisal costs of evaluating inputs, processes and product quality, while benefits accrue from savings associated with avoiding: (1) defects during the rearing process, (2) internal failure costs caused by defective equipment, materials, or substandard rearing ingredients, and (3) external failure costs caused by allowing defective products to reach a customer, e. g. insects that are incompatible with the target population or inappropriately irradiated.

3. STRAIN DOMESTICATION, MAINTENANCE, AND REPLACEMENT

In artificial rearing conditions and over time, important behavioural and physiological traits undergo change characterized by Ochieng'-Odero (1994) as acclimatization, selection, and domestication. Examples of such traits are: fecundity, preoviposition period, courtship song, flight, oviposition, rate of development, production of pheromone, response to pheromone, eye morphology, visual sensitivity, metabolic rate, and resistance to stress (Mangan 1992). The importance of changes during this domestication process was discussed by Boller (1972), Rössler (1975a, b), Huettel (1976), Mackauer (1976), Van Keymeulen et al. (1981), and Shimoji and Miyatake (2002).

When insects are brought into the laboratory to initiate mass-rearing, the conditions to which they are subjected are very different from those to which the species is adapted in the field. These conditions exert different selection pressures on the individuals

brought from the field to initiate a colony, selecting a small subset of the population with a reproductive advantage in the new conditions, potentially creating a genetic "bottleneck". The drive to reproduce may overcome some of the inappropriate environmental aspects, but the accumulated changes in courtship and other behaviours resulting from the selection process may be detrimental once the insect is released back into the field, reducing mating competitiveness with wild insects.

The constraints of mass production of insects can lead to selection for rapid larval development, short pupal period, early sexual maturity, reduced pheromone production, abbreviated courtship behaviour, and early fecundity (Miyatake 1993, 1998a, b; Miyatake and Yamagishi 1999; Cayol 2000). Changes in mating behaviour will reduce the competitiveness of the reared insects (Miyatake and Shimizu 1999), but other changes caused by mass-rearing may be linked to detrimental changes. In the melon fly *Bactrocera cucurbitae* (Coquillett), changes in the circadian rhythm and time of mating can be linked to selection for early mating (Miyatake 2002, Miyatake et al. 2002) and rapid development (Miyatake 1996, 1997a), changes in longevity to early fecundity (Miyatake 1997b), and mating success to rearing density (Miyatake and Haraguchi 1996). However, Liedo et al. (2005) showed that quite small changes in rearing conditions may improve quality.

To produce large numbers of insects, an appropriate artificial diet is usually required (Parker, this volume). Often this diet has already been developed, and has proven adequate for the development of immature stages. However, when a large number of eggs is placed on a limited amount of diet, the situation may change. Waste products from larvae can create an intolerable situation for the insects and/or the workers. Metabolic heat could raise the temperature, resulting in too-rapid development. The nutritional value of the diet may change. In any case, selection occurs for individuals that can tolerate these conditions. Pupal handling and storage also create conditions for selection. Each of these parameters can cause a genetic bottleneck that severely limits the number of individuals that survive the first few generations. As each subsequent generation becomes better adapted to the rearing conditions, a larger percentage of insects survives.

Severe "bottlenecking" does not always result in a permanently limited gene pool, but the gene frequency may change. When the population is increased substantially, the number of mutations also increases. Nei et al. (1975) speculated that, when populations expand, genetic diversity increases through mutations. The reduction in average heterozygosity per locus depends on the size of the bottleneck, and on the rate of population growth. If, after going through even an extremely small bottleneck, the population size increases rapidly, there may be significant recovery of heterozygosity. That does not mean that the population returns to the identical diversity of the original gene pool. The western spruce budworm *Choristoneura occidentalis* Freeman, reared for 88 generations in a laboratory, lost 15 alleles, but the quality of the insects (in terms of size, fecundity, vigour, longevity, and disease resistance) was adequate for research purposes (Stock and Robertson 1982); the researchers made no mention of interaction with wild populations. Vale et al. (1976) reported no changes in activity or behaviour of the tsetse flies *Glossina morsitans* Westwood and *Glossina pallidipes* Austen due to laboratory rearing or an artificial *in vitro* feeding regime.

In an AW-IPM programme that applies the SIT, the parameters necessary for the

reared and sterilized males to be effective are: successful emergence, sufficient mobility to find food, shelter, and wild females in a mating arena or other sites, mating competitiveness with the wild male for the wild female, mating compatibility with a wild female, successful transfer of sperm and accessory gland fluids, and good survival.

To ensure that a rearing procedure is producing insects with these necessary traits, it is essential to have a system of quality control tests that measure these parameters, as well as overall compatibility and competitiveness with the wild target population. The tests must be conducted on a regular basis so that the rearing process maintains the product quality in the long term (Parker, this volume). If detrimental changes in insects begin to appear, a feedback loop to the rearing system is necessary so that the shortcoming can be identified, the cause determined, and the problem corrected (Calkins et al. 1988, FAO/IAEA/USDA 2003). A filter rearing system (Parker, this volume) can provide a means to control the selection pressure on the "mother" stock used for the colony while avoiding many of the worst traits from the pressure of mass-rearing.

Strain replacement becomes essential when the properties of the reared strain become too different from the target population, and corrective measures are no longer effective. Replacing a strain can be a major task, takes considerable time, and also involves substantial cost. Newly collected field material usually takes several generations to adapt to colony rearing, with the colony stabilizing after about five generations (Bartlett 1984). If this involves an obligatory diapause, the time investment is clearly large. In the case of tsetse flies, the recent colonization of *Glossina pallidipes* in Ethiopia required more than 60 000 wild females over a period of 14 months to establish a viable colony, representing 4 or 5 generations of adaptation (G. Aboset, personal communication).

4. PARAMETERS OF QUALITY CONTROL

Product quality control covers the biological parameters of the reared insects. While the emphasis in this chapter will be on product control, some aspects of process control are also considered. Measurements for different groups of insects may involve different procedures. Information relating to tephritid fruit flies for most of the following sections can be found in FAO/IAEA/USDA (2003), most of which can be adapted for other insect groups.

4.1. Egg Hatch

Changes in egg hatch may indicate problems with mating in the colony. Samples of eggs should regularly be monitored for hatch rate, and also to ensure correct larval density in the diet.

4.2. Larval Development Time

As observed previously, development time can be related to changes in courtship behaviour. Development period should be monitored, and selection for rapid development avoided.

4.3. Pupal Size

Pupal size is a good indicator of larval diet quality, rearing density and any contamination or infestation problems. Pupal size is measured either by minimum diameter or weight.

4.4. Percentage Adult Emergence

The percentage of insects that emerges successfully determines the number of adults that can be released. Eclosion may be affected by larval nutrition (pupal energy reserve), excessive temperature during the rearing and pupal-holding periods, and inappropriate relative humidity. Mishandling of pupae, such as excessive jarring, tumbling at early pupal stages, and excessive radiation dose, may also influence percentage emergence.

4.5. Sex Ratio and Timing of Emergence

Sex ratio may be affected by poor pupal-holding conditions, or in the case of a temperature sensitive lethal sexing strain, inappropriate temperature at any stage in the rearing. Timing of emergence indicates the uniformity of pupal age (pupal collection and handling control), and may warn of selection for inappropriate accelerated development.

4.6. Flight Ability

The ability of sterile insects to fly, after having been released in the field, is an essential attribute. Those insects that cannot fly to shelter or to food, or cannot reach the mating arena, are lost to the programme.

"Percent flight" is the percentage of insects that can fly, based on the number of pupae put into an emergence/flight tube. This may also be corrected for percentage emergence.

Reductions in flight, dispersal, and survivability due to rearing and/or irradiation have been noted by a large number of researchers, e.g. Dame et al. (1969), Rajagopalan et al. (1973), Sharp (1976), Nelson and Milby (1980), Nakamori and Soemori (1981), and Smith et al. (1981). Little and Cunningham (1978), Sharp et al. (1980), Little et al. (1981), and Sharp and Little (1982) found that sifting of fruit fly pupae at a critical stage in their development was the reason that indirect flight muscles did not insert properly into the cuticle of the exoskeleton during pupal development. This created the "droopy wing syndrome" in which various degrees of droopy wings resulted in non-flying flies (Little and Cunningham 1978).

Dispersal and survival may be measured by field mark-release experiments. Large numbers of sterile males need to be released to obtain adequate recapture numbers, and where possible successive releases should be marked with distinct colours to enable them to be distinguished.

4.7. Pheromone Production and Response

Pheromone emission and response are the earliest interactions that sterile males have with wild females. Continuous laboratory rearing may create subtle changes in components of a pheromone emitted by a sterile male, rendering it unattractive to a wild female. Minks (1971) reported that inbreeding in a colony of the summer fruit tortrix *Adoxophyes orana* (Fischer von Röslerstamm) reduced the amount of pheromone produced by female moths. However, Richmond and Berisford (1980) examined pheromone components from the Nantucket pine tip moth *Rhyacionia frustrana* (Comstock), and found no significant difference between those from wild moths and those reared on an artificial diet. Sower et al. (1973) reported no differences in almond moth *Cadra cautella* (Walker) females emitting, nor males responding to, a pheromone. McGovern et al. (1975) showed that pheromone production in the field by boll weevils *Anthonomus grandis grandis* Boheman was reduced by irradiation. In the case of field-caged males of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), more wild females were attracted to sterile males than wild males. Close examination of the behaviour of the caged males revealed that sterile males "called" more frequently, and for longer periods of time, than wild males (C. O. Calkins and T. R. Ashley, unpublished data). White and Hutt (1975) found that non-irradiated native codling moths *Cydia pomonella* (L.) responded significantly more to synthetic pheromone than did irradiated native or laboratory-reared moths.

Protein feeding by adult male Mediterranean fruit flies enhances pheromone production and hence competitiveness (Kaspi and Yuval 2000, Shelly et al. 2002, Yuval et al. 2002). Protein feeding by males, together with size, also enhances post copulatory sexual selection (Taylor and Yuval 1999).

4.8. Vision

Agee and Park (1975) developed the use of an electroretinogram to measure the quality of the vision of fruit flies; irradiation debilitated both the sensitivity and spectral accommodation. Rössler (1980) discovered that the sexual competitiveness of Mediterranean fruit fly males, possessing an apricot eye-colour mutant, was reduced. Using the apricot-eye strain for mating and acoustical tests, C. O. Calkins (unpublished data) observed that a mutant male could not follow closely the wing waving of a normal female during courtship, resulting in the female rejecting the male's advances.

Visual impairment was also observed in mass-reared New World screwworms (Bush et al. 1976), necessitating strain replacement (Klassen and Curtis, this volume).

4.9. Longevity

In many insects, the nutrient reserves acquired during the larval stage, and carried through the pupal stage, affect the longevity of the adult stage. The longevity/stress test, often related to pupal size, measures the percentage of adults that survives for a set time period, depending on species, without food or water (Orozco et al. 1983, Ashley 1987, Mutika et al. 2002). This test produces stress, and is indicative of the amount of nutritional reserves present when adults emerge.

While protein feeding improves pheromone production in Mediterranean fruit flies, it also reduces survival when they face starvation (Taylor and Yuval 1999). However, Maor et al. (2004) demonstrated that Mediterranean fruit flies fed on protein are as successful at exploiting both protein and carbohydrate resources in the field, and their inability to overcome starvation is not a concern.

4.10. Startle Activity

A common problem in laboratory-reared insects is the loss of irritability. Mass-rearing conditions apparently select inadvertently for insects that, to maximize their fitness, can afford under the protected but very dense colony environment to ignore any movements in their immediate surroundings. As a result, they also disregard any potential dangers after being released in the field, and thus are much more susceptible than wild insects to many of the predators they will encounter (Hendrichs and Hendrichs 1998). The "startle test", developed by Boller et al. (1981), measures levels of irritability, and this or a similar test could be used to select for increased irritability. The level of irritability appears to be inherited genetically (C. O. Calkins, unpublished data), and hence a proper management of the mother colony could probably maintain some degree of irritability.

4.11. Mating Propensity, Compatibility, and Competitiveness

Mating speed or mating virility is one indicator of fitness (Pendlebury and Kidwell 1974; Lance and McInnis, this volume). This test measures the propensity or willingness of mass-reared sterilized unmated males to mate with virgin females. This is appropriate only for male-choice mating systems, and provides no information for female-choice systems (Lance and McInnis, this volume).

Mating propensity presumably measures, by the use of the mating index, how "eager" the flies are to mate. However, as this test is usually conducted in the laboratory with reared males and females, results are often not representative of laboratory male performance when exposed to wild females in the field. This is confirmed by the fact that laboratory-reared flies usually have a higher mating index than wild flies.

Rapid matings tend to be controlled by the male genotype, while the female genotype may assume importance in slower matings (Parsons 1974). Therefore, for female-choice mating systems, this test is often misleading because mating speed largely reflects the fact that males selected under extremely high-density conditions (where most females become receptive at the same time, and thus a 1:1 operational sex ratio to courting males prevails) obtain rapid matings without going through the proper courtship sequence. These males are often rejected by wild females while attempting to court them in leks under natural conditions (Briceño and Eberhard 1998, Hendrichs et al. 2002).

The ability of reared sterilized insects to compete successfully with the target population males to mate is crucial for the SIT. Reduced competitiveness can arise from problems in rearing, irradiation or handling, and from inherent incompatibility between different strains. For example, the Mediterranean fruit fly arrived in southern Europe from West Africa via the Cape Verde Islands in the 1700s, while it reached Hawaii

from East Africa via Australia in the late 1800s, and the populations in South America probably arrived from West Africa. The resulting isolation of these populations might allow the accumulation of changes, which will eventually lead to reproductive isolation and incipient speciation. Some evidence of reproductive isolation in island situations has been found (McInnes et al. 1996, Miyatake 1998a), but an extensive comparison of strains from around the world with the main mass-rearing strains showed no significant mating barriers (Cayol et al. 1999, 2002), indicating that any mass-rearing strain can be used against any wild population.

Reisen et al. (1980) detected assortative mating in releases of *Culex tritaeniorhynchus* Giles, as did Raulston et al. (1976) with the tobacco budworm *Heliothis virescens* (F.). The latter was caused by a change in the temporal mating period of the laboratory-reared population, which initiated courtship 2 hours earlier than the wild population. Zervas and Economopoulos (1982) observed that laboratory-reared olive fruit flies *Bactrocera oleae* (Gmelin) also began to mate 2 hours before wild flies. Wong et al. (1982) noted assortative mating between laboratory-reared and wild oriental fruit flies *Bactrocera dorsalis* Hendel. On the other hand, Spates and Hightower (1967) found that New World screwworm males became more aggressive with each generation reared in the laboratory.

The size of males can also be an indirect indicator of mating competitiveness. It affects mating success in the Caribbean fruit fly *Anastrepha suspensa* (Loew) (Burk and Webb 1983) and Mediterranean fruit fly (Churchill-Stanland et al. 1986; Orozco and Lopez 1990; Bloem, K. et al. 1993a, b; Bloem, S. et al. 1993; Economopoulos et al. 1993). A larger male is more competitive against rival males, and females tend to select larger males over smaller ones, but Hunt et al. (2002) found an effect of size on pheromone calling but not on overall mating success. In field releases, larger males disperse farther and live longer than smaller males (section 4.9., Bloem et al. 1994). Pupal weight is a good indicator of adult size, and Mediterranean fruit fly programmes, to comply with quality standards, require that late-stage pupae should weigh about 7 mg. In New World screwworm programmes, late pupal weight should not fall below 44 mg, but in Lepidoptera large adults (from large pupae) are less competitive due to reduced flight ability.

Laboratory bioassays do not often indicate accurately the field performance of reared insects (Katsoyannos et al. 1999). Quality control tests that are generally more appropriate for field cages containing vegetation are: pheromone attractiveness, mating compatibility, and mating competitiveness (Cayol et al. 1999, 2002; FAO/IAEA/USDA 2003). It has been recommended that AW-IPM programmes releasing sterile insects, at least once a year, obtain insects from the field population being targeted and compare them with mass-reared insects in field cages that permit as large a range of natural behaviours as possible, including female choice (FAO/IAEA/USDA 2003; Lance and McInnis, this volume; Parker, this volume). Field-cage and field tests are discussed in section 7 below.

4.12. Remating

A further important component of mating behaviour for the SIT is remating by wild females (Lance and McInnis, this volume; Whitten and Mahon, this volume). Female

monogamy is not required for the SIT, but if there is a differential rate of remating by females first mated to a sterile or a wild male, or there is sperm selection following multiple matings, there will be an effect on the SIT.

Remating may be controlled by factors transferred by the male during copulation, and if the mass-reared males do not transfer the necessary factors, the incidence of remating may rise differentially (Jang 2002, Vera et al. 2002). This may be measured by an extension to the field-cage compatibility/competitiveness tests listed above. The females from pairs collected in the compatibility/competitiveness test are marked according to the male with which they first mated, and the field-cage test repeated on the second day. Remating pairs are then scored and the results examined for evidence of differential remating (McInnis et al. 2002).

5. EFFECTS OF IRRADIATION ON QUALITY

An essential step in the SIT is the induction of sterility. Initially this was done using chemical treatment, but problems with dose uniformity, toxicity of the chemicals, environmental contamination, and worker safety have led to the almost universal adoption of gamma irradiation. In many insect groups, irradiation results in a reduction in competitiveness (Bakri et al., this volume), and much recent work has aimed at reducing this negative effect. Several factors influence this effect, chief among which are the developmental stage of the insect and the atmosphere used during irradiation.

The timing of irradiation can be adjusted to reduce the effects without creating logistical and handling problems. Normally the least amount of damage to adult males is caused when irradiation is carried out shortly after eclosion, but the problem of irradiating large numbers of adult insects at one time is often insurmountable; therefore usually pupae are irradiated shortly before emergence (Bakri et al., this volume). For Mediterranean fruit flies, pupal development is monitored by eye colour to determine the optimum stage for irradiation (Ruhm and Calkins 1981).

Irradiation in air creates free radicals that are detrimental to the quality of insects. If oxygen were excluded by flooding the containers with nitrogen, this problem did not occur (Robinson 1975). Later it was discovered that, when the containers were sealed and metabolism in pupae quickly exhausted the oxygen and produced carbon dioxide, the resulting hypoxia provided similar protection (Bakri et al., this volume).

In many insects, females become 100% sterile at lower doses than males. Attempts to attain 100% sterility in males usually reduce quality, and it will often be better to reduce the dose so as to obtain a better induction of sterility in the field females by having a more competitive male (Hooper 1972, Toledo et al. 2004, Mehta and Parker 2005). In genetic sexing strains, where minimum numbers of females are released, even the limit imposed by the need to prevent female fertility or egg production no longer limits the minimum usable doses.

In the Lepidoptera and Heteroptera, the holocentric chromosome structure means that very high radiation doses are required to induce dominant lethal mutations. In these groups a lower substerilizing dose can be employed to induce sterility in the subsequent generation. Males treated with lower substerilizing radiation doses normally result in higher inherited sterility (Bloem et al. 1999a, b; Bloem et al., this volume; Carpenter et al., this volume). Also a higher percentage of male moths exposed to substerilizing

doses respond to virgin females than those treated with higher doses. In a large field test in Washington State, USA, codling moth males (partially sterilized with radiation doses of 100 or 250 Gy) and fully sterile females were released in apple orchards. The partially sterile males were more active, dispersed greater distances, and generally were more competitive than males with a higher level of sterility (Bloem et al. 2001; Bloem et al., this volume).

Compared with untreated controls, Opiyo (2001) found no significant difference in the survival of male *Glossina pallidipes* irradiated at doses of 40–140 Gy. Mutika et al. (2002) compared the mating competitiveness of male tsetse flies (*Glossina pallidipes*) irradiated at 120 Gy with untreated controls. Irradiated males formed more mating pairs than the controls, but the spermathecal values (a measure of the quantity of accessory fluid transferred) for females mated with irradiated males were significantly lower than if mated with control males.

6. EFFECTS OF CHILLING ON QUALITY

Before irradiation and prior to shipping, pupae are often chilled to lower their metabolic rate. Long-distance shipments of irradiated Mediterranean fruit fly pupae (either chilled or not chilled) were compared by Brazzel et al. (1986). Pupae shipped for 18 hours in hypoxia averaged 77% emergence and 70% fliers, but those shipped in hypoxia in chilled boxes averaged 82% emergence and 76% fliers. Pupae shipped for 40 hours in bottles in hypoxia averaged 48% emergence and 34% fliers, but those in hypoxia and chilled boxes averaged 73% emergence and 62% fliers.

Prolonged chilling (if the time exceeds 26 hours) can be detrimental to fly emergence at the release point, but not as detrimental as hypoxia with no chilling. Serghiou (1977) discovered that the competitiveness of irradiated sterile Mediterranean fruit flies decreased as their exposure to chilling increased, but chilling did not have an adverse effect on survival.

In view of the need to synchronize releases of sterile male tsetse flies (*Glossina pallidipes*), Mutika et al. (2002) stored pupae at various low temperatures, and found that no significant differences in emergence, survival without a blood meal, or insemination capacity occurred. In fact, male adults, eclosed from pupae that had been stored at 15ºC, started mating more quickly, and formed more mating pairs, than the controls. However, if adult males were chilled (and then removed from the low-temperature container for testing), insemination capacity was reduced, and mortality was 10–32%.

7. FIELD-CAGE AND FIELD TESTS

As noted above, laboratory tests of competitiveness or compatibility may not reliably indicate the situation in the field (Katsoyannos et al. 1999; Itô and Yamamura, this volume; Vreysen, this volume). A much better measure can be obtained by using a field cage (White et al. 1977, Calkins and Webb 1983, Chambers et al. 1983). Standard procedures for field-cage operation have been defined (Cayol et al. 1999, 2002; FAO/IAEA/USDA 2003), and these may be readily adapted for other species, e.g. Mutika et al. (2001). A field cage consists of a mesh cage, approximately 2.3-m high

and 3-m wide, erected over a host plant or other suitable vegetation. Equal numbers of wild males and females and sterile mass-reared insects are introduced into the cage, and mating pairs are recorded. When a genetic sexing strain is being tested, females from the rearing colony stock should be used. A test with only sterile males may also be conducted. The four possible outcomes (SS, SW, WS and WW) are then examined for trends in the mating (Box 1). The low-density and semi-natural conditions permit near-normal courtship and mating behaviour, such that any changes in the behaviour of the mass-reared insects should be apparent. The results of field-cage experiments are affected by the conditions under which the mass-reared insects have been reared, thus it is essential that conditions be tightly controlled, and each test replicated about 10 times.

Box 1. Indices of Compatibility and Competitiveness

A standard field-cage experiment results in four figures, the numbers of each of the four possible pairings formed during the test period, SS, SW, WS and WW, where S represents sterile, W wild (fertile), and males are indicated first. The categories WS and SS will be missing in the case of genetic sexing strains where sterile females are not produced. These four figures have been combined in various ways to produce indices of competitiveness and compatibility, each emphasising a different aspect, and each with advantages and disadvantages. For more details, see section 3.1. in FAO/IAEA/USDA (2003).

The relative isolation index (RII) gives an indication of mating compatibility between two stains. It is calculated as:

$$RII = \frac{SS \times WW}{SW \times WS}$$

A value of 1 indicates random mating, with larger values indicating assortative mating. This index is sensitive to changes in a single type of mating (e.g. a drop in the important category SW), is not affected by the overall level of participation of the different insect types, and does not depend on the ratio of sterile to wild insects in the test. The disadvantages are that the index is undefined if either SW or WS is zero, and it changes rapidly for a difference of only one if a category is small. The value of RII can be interpreted as the number of sterile males that have to be employed to be equivalent to one wild male. Values normally vary between 1.5 and 5, and values consistently above 3 are a cause for concern.

The isolation index (ISI) is defined as:

$$ISI = \frac{(SS + WW) - (SW + WS)}{SS + WW + SW + WS}$$

It ranges from -1 (complete negative assortative mating) through 0 (random mating) to +1 (complete positive assortative mating). The main advantages of the ISI over the RII are that it is easier to interpret values from -1 to +1 than from 0 to infinity, it is not as sensitive to a change in a small category, and is defined even when one category is zero. Values normally lie between 0.1 and 0.4, and values over 0.5 are a cause for concern.

The ISI is normally combined with the male relative performance index (MRPI) and female relative performance index (FRPI) defined as:

$$MRPI = \frac{(SS + SW) - (WS + WW)}{SS + SW + WS + WW} \quad FRPI = \frac{(SS + WS) - (SW + WW)}{SS + SW + WS + WW}$$

which show the proportion of sterile males and sterile females taking part in matings, and should be used to interpret values of ISI over 0.5.

These index values can be displayed graphically to make them easier to interpret, as shown below.

Box 1. Continued

$$ISI = \frac{(SS + WW) - (SW + WS)}{SS + WW + SW + WS}$$

ISI

| -1 | -0.75 | -0.5 | -0.25 | 0 | 0.25 | 0.5 | 0.75 | +1 |

| **Negative assortative mating** | **Random mating** *(Sexual compatibility)* | **Positive assortative mating** |

(Sexual isolation)

$$FRPI = \frac{(SS + WS) - (SW + WW)}{SS + SW + WS + WW}$$ $$MRPI = \frac{(SS + SW) - (WS + WW)}{SS + SW + WS + WW}$$

FRPI

MRPI

| -1 | -0.75 | -0.5 | -0.25 | 0 | 0.25 | 0.5 | 0.75 | +1 |

| **All matings by wild individuals** | **Equal mating propensity** | **All matings by sterile individuals** |

Graphic representation of isolation index (ISI) and female and male relative performance indices (FRPI, MRPI).

It is recommended that field-cage tests be performed against wild collected insects about once per year (FAO/IAEA/USDA 2003; Lance and McInnis, this volume; Parker, this volume). Field-collected larvae must be handled properly. For example, if coffee berries, infested with Mediterranean fruit flies, are placed on screens and the larvae collected in pans below, the berries tend to dry out, causing the larvae to exit the fruit prematurely. Then the emerged flies are smaller than normal, and this can invalidate bioassays comparing wild fly and laboratory-reared fly behaviours.

Field-cage experiments are also useful for more detailed assessments of behaviour such as time of mating, mating duration, and the sequence and timing of specific behaviour components and pheromone calling.

The capacity of sterile insects to survive in the field, and to disperse to feeding, mating, and resting sites, is also critical. Survival and dispersal rates of different rearing strains can be compared by various release/recapture methods (Itô and Yamamura, this volume; Lance and McInnis, this volume; Vreysen, this volume). Such rates are often not correlated with a flight-ability test of the same lot of sterile insects in the laboratory,

or even survival in field cages, since they also measure over time the ability of the sterile insects to find food, to respond to attractants, and to evade predators (Hendrichs et al. 1993). C. O. Calkins and T. R. Ashley (unpublished data), using mass-reared Mediterranean fruit flies irradiated at the usual dose, and at twice this dose, monitored dispersal in the field. Flies that had been exposed to twice the usual radiation dose had very low rates of dispersal and survival. Other dispersal studies by Bloem et al. (1994) compared large flies (from pupae 8–8.5 mg in weight) with small flies (from pupae 5–5.5 mg). A higher percentage of large than small flies was captured initially, and over a longer period of time.

Ultimately, the competitiveness of the insects should also be tested in the field, e.g. White and Mantey (1977) and Villavaso et al. (1980). It is usually not possible to detect mating pairs directly in the field, but the competitiveness of the released sterile males can be estimated by comparing the egg hatch obtained from captured wild females (some with sterile, others with fertile matings) (Haisch 1970, Fried 1971, Hooper and Horton 1981, Iwahashi et al. 1983). Such an estimate takes into account all the factors that affect the induction of sterility in the wild population, including survival, dispersal, mating competitiveness, sperm transfer, remating, and sperm competition. The same test can also be conducted in a field cage (FAO/IAEA/USDA 2003).

8. CONTROL CHARTS

Data generated by quality control assays are not very useful until they can be graphed in a sequential manner to show trends. In 1924, W. Shewhart of Bell Telephone Laboratories first developed control charts (Feigenbaum 1961). A chart consists of sequential plots of a specific quality criterion on a graph that has a central line and upper and lower control limits (Box 2). The control limits may either be defined a priori, or more usually are derived from long-term data. For this, many samples are taken over an extended period and the mean or median computed for the central line, while the upper and lower control limits are usually three standard errors either side of the central line (Charbonneau and Webster 1978). Each new quality control value is plotted on the chart, and both its position and the trend over the last seven points are assessed for conformity with a set of standard rules. A single value out of bounds is less a concern than a continuing trend away from the central line. Control charts have been proposed for the Mediterranean fruit fly (Calkins et al. 1982), Lepidoptera (Fisher 1983), and tsetse flies (Timischl 1980).

9. IMPACT OF LOW-QUALITY INSECTS ON ERADICATION PROGRAMMES USING SIT

Attempts have been made to put an actual monetary value on the impact of producing low-quality insects (Calkins and Ashley 1989). Using Knipling's (1979) model, Calkins et al. (1996) inserted varying values of the quality of Mediterranean fruit flies established during a pilot test for quality control. Using the New World screwworm as an example, with a growth rate of five and an overflooding ratio of 9:1, it would take five generations to achieve eradication. Since the Mediterranean fruit fly has a much greater biotic potential, and based on its life-history parameters, a growth rate of 12 was

established. An overflooding ratio of 9:1 actually resulted in an increase in the population each generation. The "rule of thumb" for Mediterranean fruit flies, selected during the numerous fly invasions into California, USA, is a ratio of 100:1. If all flies were 100% effective, theoretically this ratio achieves eradication in three generations. However, when the fly quality equals the minimum acceptable level of quality, the effective release ratio becomes 23:1, and it takes five generations to achieve eradication (Calkins and Ashley 1989). When the actual level of fly quality was inserted into the model, giving an effective ratio of 54:1, only four generations were needed for eradication. However, if the flies were only half as effective, the cost per hectare becomes almost twice as much to produce and utilize sterile flies that are of only moderate quality. Therefore it is economically beneficial to rear high-quality rather than low-quality insects (Calkins and Ashley 1989).

Box 2. Shewhart Control Charts

Control charts are a well-established tool for stabilizing industrial processes. They are formed by plotting sequentially the successive values of a control parameter. These are then compared with a central line (CL), upper control limit (UCL), and lower control limit (LCL), using certain criteria to determine if the process is in control.

The graphed parameter will usually be the mean of a sample taken from the process. In industrial processes, this may be compared with a priori limits, e.g. the design diameter of a shaft and the specified tolerance limits. In insect rearing, we do not usually have such a priori limits, and base the assessment of the graphed value by comparing it with long-term historical values. The long-term mean is taken as the central line, and the upper and lower control limits are defined as three standard errors above and below the mean. By standard statistical theory, if the parameter plotted is normally distributed, by chance causes about 0.1% of the values will lie above the UCL or below the LCL. If more than one value lies outside the control limits, this indicates a systematic influence, and the process is said to be out of statistical control.

Two examples of control charts are given below — monitoring the fecundity of the *Glossina pallidipes* colony at the FAO/IAEA Seibersdorf laboratories. The parameter plotted is weekly fecundity, expressed as pupae per live female per ovarian cycle (10 days) (P/F/10d). The central line is the average value over a 105-week period (2003–2004). Since only a single measurement is taken each week, variability is estimated by using the difference between successive pairs of values, yielding a moving range, from which the standard deviation is derived.

The first chart shows the individual values plotted for 2004, and the UCL, CL, and LCL. Since increased fertility is not a problem, only the LCL is considered further. Another line has been added, the lower warning line (LWL), at two standard errors below the CL; this draws attention to values approaching the limit.

The second chart uses an exponential weighted moving average (includes all historical data points but weights the most recent more highly). This is less sensitive to individual extreme values, but more clearly shows longer-term trends. In this case, the control limits are not straight lines, but are asymptotic to the control limits in the previous example. Both charts show that, in 2004, fecundity was usually better than average, but fell towards the end of the year.

Sytsma and Manley (1999) provided an explanation of the various types of control chart, detailed instructions on how to construct them, and criteria for evaluating the data.

Box 2. Continued

Individuals Chart
Fecundity of *Glossina pallidipes* colony at Seibersdorf, 2004

Exponentially Weighted Moving Average Chart
Fecundity of *Glossina pallidipes* colony at Seibersdorf, 2004
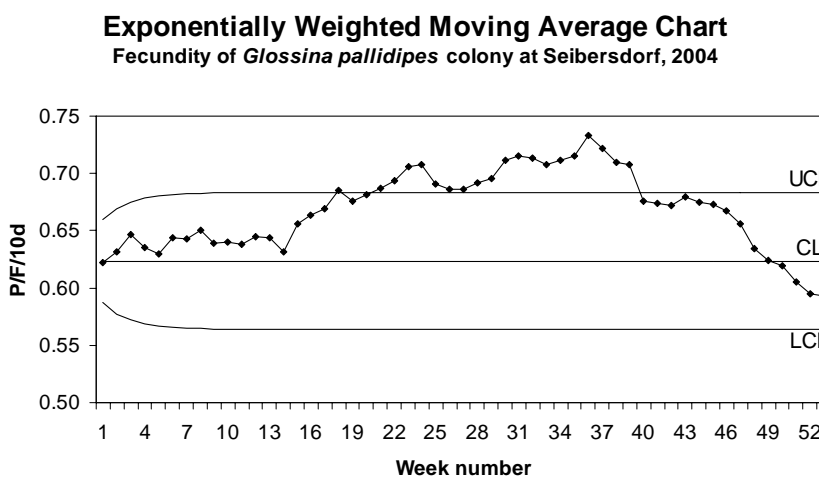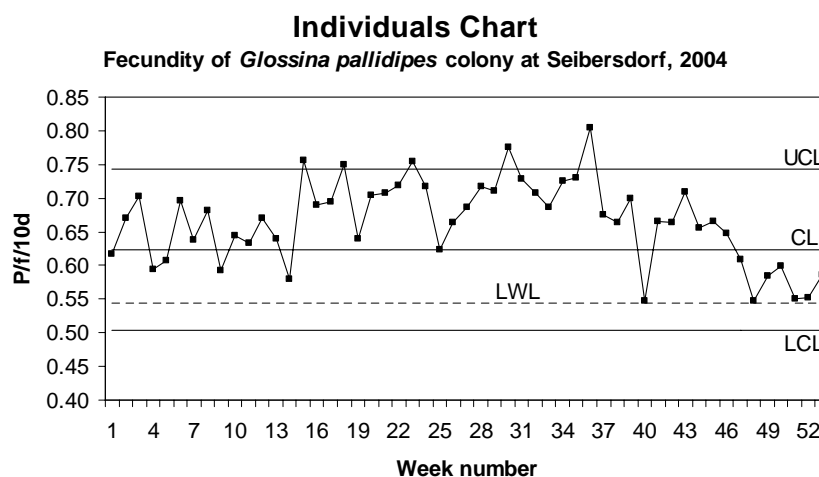
For 2001, Enkerlin (2003) showed a cost figure of USD 216 per million sterile male Mediterranean fruit flies using a temperature-sensitive lethal (*tsl*) genetic sexing strain (El Pino facility, Guatemala). At present, these are probably the cheapest sterile insects in the world. The cost per million *tsl* males is more or less equivalent to the cost per million males of the usual bisexual strain. However, the big difference (in terms of cost savings) is in the transport and release operations, where sterile *tsl* males cost half as much as males of the bisexual strain (Caceres et al. 2004). In addition, male-only releases introduce three or four times more sterility into the target population than do bisexual releases (Robinson et al. 1999; Rendón et al. 2000, 2004).

10.   CHANGES IN INSECT BEHAVIOUR CAUSED BY LABORATORY REARING

Selection during colonization and mass-rearing normally changes the biology and behaviour of an insect species (Iwahashi et al. 1983; Calkins 1989; Miyatake 1993, 1998a, 1998b, 2002; Miyatake and Haraguchi 1996; Miyatake and Yamagishi 1999; Cayol 2000; Shimoji and Miyatake 2002; Maor et al. 2004). The oviposition medium is often completely different from that in natural conditions — in some cases it is fabric, paper, or a membrane, in others it may be a plastic bottle with small holes. Emergence cages may serve as both mating and oviposition cages, and males often harass ovipositing females. In these cages, insects are maintained at abnormally high densities, and there is no space to develop mating aggregations or swarms, or to attract females through producing a pheromone. All insects are of the same age, and thus have an atypical operational sex ratio of 1:1 during reproduction, hence reducing intra-sexual selection among males. Many matings consist of brief courtships or, in some cases, even forced matings. Adult insects are discarded as soon as egg production begins to wane, thereby selecting for early maturing insects. In this situation, it is advantageous for females to mate quickly and maximize the number of progeny. It is disadvantageous for males to retain elaborate courtships, or for females to discriminate in mate selection. If abbreviated courtship becomes fixed in the population, this courtship repertoire may not be acceptable to wild females.

The mating competitiveness of laboratory-reared and wild Mediterranean fruit flies was examined in natural mating arenas (leks) (Zapien et al. 1983, Shelly 1995). Although the laboratory-reared males readily joined leks and displayed calling behaviour similar to that of wild flies, relatively few laboratory-reared males were able to mate with wild females. The sterile males either failed to attract wild females to their positions in the lek, or the courtship repertoire was not acceptable to wild females. In addition, on cloudy days, sterile males refrained from participating in sexual activities, and thus could not compete for wild females during such low-light-intensity conditions (Zapien et al. 1983).

In a mass-rearing environment, unlike the field, light, temperature, and relative humidity are often constant. Exposure to constant and optimal conditions of light, temperature, and relative humidity can select for individuals that are better adapted to these conditions, but lack the ability to adjust to fluctuating environments (Cayol 2000). If insects are reared for generations in constant light, changes in the temporal mating periods of some species may result. In field-cage tests in Florida, laboratory-reared Caribbean fruit flies mated in the afternoon, and completed mating activities long before wild flies began to mate near dusk (C. O. Calkins, unpublished data).

11.   REMEDIAL ACTIONS TO RESTORE EFFECTIVENESS OF REARED INSECTS

When a colony of insects, used for the SIT, begins to deteriorate in effectiveness, there are several ways to restore it. However, if genetic selection is involved, the problem may be more difficult to solve, and a change in colony management procedures may be required, including in some cases the establishment of a new colony from the target population. Changed management procedures may reverse the selection, or at least slow

down colony deterioration (Fisher and Cáceres 2000; Liedo et al. 2005; Parker, this volume).

Specific problems detected by various tests can be addressed to reverse selection, if the causes are identified. For example, the egging cage can be designed to require insects to fly before having the opportunity to eat and drink; this automatically selects against non-flyers. In the cases of the New World screwworm (Wyss 2002) and codling moth (Dyck et al. 1993), adult insects must be able to fly to be collected in a cold room and then taken to the field for release.

Another problem that often develops in a reared colony is flies lacking irritability. As a result, released sterile insects become easy prey to many of the predators that they encounter in the field. If an automatic technique to select against such adults in the egging cage could be developed, this problem would be reduced.

Mating compatibility appears to involve heritable traits that, to a certain extent, can be manipulated. Changes in temporal mating periods may be corrected, at least partially, by implementing egg collection and other regimes for the colony production that maintain the normal distribution of relevant characters of the colonized population (Miyatake 1995), and holding adult colony flies under natural light/dark cycles. Also, fluctuating temperatures in the adult egging-cage room would increase the tolerance for changing field conditions.

The mating performance of sterile fruit flies may decrease under extreme or marginal habitats, such as high altitudes where cooler temperatures prevail. While developing a RAPID quality control system for early warning of poor fly performance, Boller et al. (1981) reared Mediterranean fruit flies in a laboratory at cool temperatures to determine if they would still mate and perform other tasks. They found that there was enough genetic variability in a colony, normally reared at optimal constant temperature, to perform at cool temperatures after being reared at these temperatures for only a few generations (E. Boller, personal communication).

Haynes and Smith (1989) discovered that, when sterile boll weevils were fed on squares (cotton blossom buds) instead of plugs of artificial diet before release, locomotor activity increased, mortality decreased, and mating attractiveness increased by 16% when compared with that of control weevils fed on diet plugs.

There are several possible reasons why a reared insect becomes incompatible with a wild one, such as genetic changes caused by the rearing process, genetic drift, or a change in a wild population that has been exposed to released sterile insects for "too many" generations. Hibino and Iwahashi (1991) reported that wild female melon flies, exposed to sterile males late in an AW-IPM programme that integrated the SIT, became unreceptive to sterile males (Lance and McInnis, this volume; Whitten and Mahon, this volume). Such changes may appear as temporal changes in the mating period, subtle changes in the mating repertoire or in the chemical make-up of a pheromone, changes in acoustical signals, etc. (Sivinski et al. 1989, Briceño and Eberhard 1998). In these cases, there may be no alternative except to replace the mass-reared colony with a new strain from the target population.

However, in situations where the rearing process itself is responsible for the change, changing the strain without correcting the process will not solve the problem. As described above, the changes that occur while domesticating an insect population are major ones, and do not happen in one or two generations. One solution is to continually

develop new strains, just in case the current one deteriorates. In any case, this may be necessary to prevent possible incompatibilities as the SIT activities expand into new geographic areas that have potentially different target populations. However, strain replacement is extremely expensive (Mangan 1992), and a successful strain should not be discarded unless it is found to be inadequate. It is costly to expand a colony, conduct quality control tests, determine rearing characteristics, and evaluate field performance. Nevertheless, regular strain replacement in the New World screwworm colony in Mexico became a standard procedure (Hofmann 1985; IAEA 1992; Parker, this volume).

A less drastic cure is to incorporate wild genes into the colony genome. Wild corn earworm *Helicoverpa zea* (Boddie) moths from St. Croix, US Virgin Islands, were incorporated into a colony in Tifton, GA, USA; as a result, released moths mated much more frequently with wild moths (Young et al. 1975). However, this approach is not always successful. The wild individuals being introduced must compete in the artificial conditions to which moths in the domestic colony have already adapted. In the case of fruit flies, one solution is to introduce only wild males into the colony. For wild females, the most problematic aspect of adaptation is the oviposition device. By excluding wild females from the new introduction, this part of the selection process is avoided.

Sometimes an improvement in the general vigour of a colony can be achieved by reducing the stress of the rearing regime of the mother colony. Boller and Calkins (1984) used a relaxed rearing method for a strain of Mediterranean fruit flies, and within two generations the vigour and size of the males increased. A system for limiting stress on a mother colony, called filter rearing (Fisher and Cáceres 2000), also prevents the accumulation of deleterious traits from the high-density rearing stages back to the mother colony (Parker, this volume). In this system, the mother colony is held under low-density low-stress conditions. Surplus insects from this colony are continuously fed into a sequence of high-density amplification steps to the final release colony, but the mother colony is kept separate and never receives material back from the high-density steps. The use of such a filter system has several advantages. As the mother colony is small, it is possible to keep the insects under low-density conditions, apply sexual competition or directed selection to them to restore or maintain desirable traits, and if necessary, by establishing a new mother colony, the whole production can be easily switched to a new strain.

If a colony is replaced because the rearing process is responsible for developing an inferior strain of insects, and the particular feature of the rearing method responsible for the change in insect behaviour is not adjusted, the new colony will rapidly develop the same inferior behaviour. This domestication takes place in just a few generations. Leppla et al. (1980) reported the complete adaptation of a noctuid moth to a laboratory environment in five to seven generations. The adaptation of the Caribbean fruit fly to a rearing regime, where performances in the laboratory bioassays were the same as those for the reared colony, took three or four generations (C. O. Calkins, unpublished data). Pashley and Proverbs (1981) noted a gradual change over time in the allozymes of reared codling moths, and predicted that this change could affect mating interactions with a wild population.

12.   QUALITY CONTROL MANUALS

The first system of quality control was developed in 1978 by E. Boller. It was referred to as the Mediterranean fruit fly RAPID quality control system (Boller et al. 1981). It consisted of behavioural tests that included a pupal calibration sorting process to determine the array of pupal sizes, a startle test to measure irritability, an olfactometer to determine the age and time of day when males began to produce pheromone and females began to respond, a mating propensity test, a new ratio test, and an isolation index. The system was tested and perfected in 1977–1978 on Mediterranean fruit fly colonies at Wadenswil, Switzerland, and at Seibersdorf, Austria. The field aspects of the system were verified in 1978 in Antigua, Guatemala (Chambers et al. 1983).

A training manual for an International Course on Quality Control in Castellon, Spain, was developed in 1979; it included both laboratory and field aspects of the system (Calkins et al. 1980). An operational quality control manual for Mediterranean fruit flies, used at the Moscamed factories in Mexico and Guatemala and in other fruit fly programmes in Latin America, was developed by Orozco et al. (1983), and subsequently modified or updated by Brazzel et al. (1986), Ashley (1987), and Calkins et al. (1996). Spencer and Fujita (1997) included a section on quality control in a manual for four species of fruit flies reared in Hawaii. To harmonize tests among all these manuals, standardize quality control tests, and allow comparisons for commercial and transboundary shipments of sterile insects, an international quality control manual was produced in 2003 (FAO/IAEA/USDA 2003); it is being used in most fruit fly programmes.

A manual on quality control for production of the New World screwworm was issued in 1995 (Lopez et al. 1995). Quality control procedures to mass-rear tsetse flies are being developed (Feldmann 1994, Gooding et al. 1997). Fisher (1983) developed a quality control manual for mass-reared Lepidoptera (on artificial diet), using the fall armyworm *Spodoptera frugiperda* (J. E. Smith) as a model. He developed process control charts that were sensitive tools for identifying changes in insects during colonization. The parameters used to evaluate the quality of the mass-reared colony were: pupal weight, rotation and mortality, wing-beat frequency, pheromone response, and effects of adult diet and pupal irradiation on mating behaviour and success. A feedback loop back to the production programme was included in the control aspect of quality assurance. The Sterile Insect Release (SIR) Program in British Columbia, Canada, prepared a manual on mass-rearing the codling moth, including quality control (SIR 2003). The IOBC supports the AMRQC to develop and promote quality control in mass-reared insects (Leppla et al. 2002). This group has recommended ways to improve rearing systems (Van Lenteren 2003).

13.   REFERENCES

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CHAPTER 3.5.

STERILE INSECT SUPPLY, EMERGENCE, AND RELEASE

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SUMMARY

Insect mass-rearing for a sterile insect technique (SIT) programme is designed to move beyond the large-scale rearing of insects in a laboratory to the industrial production of consistently high-quality insects for sterilization and release. Each facility reflects the unique biology of the insect reared within it, but there are some generalities for all rearing facilities. Rearing insects in self-contained modules offers flexibility, and increased safety from catastrophic occurrences, compared with using a single building which houses all facets of the rearing process. Although mechanizing certain aspects of the rearing steps helps provide a consistently high-quality insect, successful mass-rearing and delivery depends largely upon the human component. Besides production in centralized facilities, insects can be produced from purchased eggs, or nowadays, adult insects are often obtained from specialized satellite emergence/collection facilities. Interest in commercializing insect production and release is increasing. Shipping sterile insects, sometimes over long distances, is now common practice. Procedures for handling and chilling adult insects, and providing food and water prior to release, are continually being improved. Sterile insects are released via static-release receptacles, ground-release systems, or most commonly from the air. The aerial release of chilled sterile insects is the most efficient method of release, especially when aircraft flight paths are guided by a Global Positioning System (GPS) linked to a computer-controlled release mechanism.

1. INTRODUCTION

Interest in the use of sterile insects for pest management has increased with the realization by researchers, pest management specialists, growers and regulatory officials that the sterile insect technique (SIT) can be utilized in situations beyond the traditional screwworm or fruit fly eradication programmes (Bloem and Bloem 2000, Loosjes 2000, Msangi et al. 2000, Walters et al. 2000, Kohama et al. 2003). Area-wide integrated pest management (AW-IPM) suppression and containment (exclusion) programmes have successfully utilized sterile insects (Hendrichs et al. 1983; Ortiz el al. 1987; CDFA 2002; Hendrichs et al., this volume). All these programmes require a system for emerging, handling and distributing the sterile insects, often from aircraft, over large geographic areas. This chapter describes and evaluates the options available to obtain and disseminate sterile insects.

Sterile insect production and use are still not very widely applied, so this chapter is based largely on systems as they exist today, and not necessarily what they can or should be in the future. As the technology progresses and its application expands, further major advances in mass-rearing, storage, packaging, handling, and release will certainly be possible.

2. STERILE INSECT SUPPLY

2.1. Availability from Established Production Facilities

The easiest way to obtain sterile insects is by purchasing them from a rearing facility. However, there are only a limited number of insect species available for purchase: Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), Mexican fruit fly *Anastrepha ludens* (Loew), melon fly *Bactrocera cucurbitae* (Coquillett), codling moth *Cydia pomonella* (L.), pink bollworm *Pectinophora gossypiella* (Saunders), diamondback moth *Plutella xylostella* (L.), and New World screwworm *Cochliomyia hominivorax* (Coquerel), and there are only few facilities that have the

capacity to rear more of these sterile insects than they need. In general, the full capacity of most rearing facilities is used in the programme for which the facility was built. The Joint FAO/IAEA Programme (Food and Agriculture Organization of the United Nations/International Atomic Energy Agency) maintains a database of rearing facilities, capacities, contact information, etc. (IDIDAS 2004; Bakri et al., this volume).

Often sterile insect release programmes are conducted by governments with limited budgets, and driven by an urgent need to manage a serious pest. The sale of insects by such institutions is done on a full cost-recovery basis, including the depreciation of facilities and equipment. The production cost per million insects (eggs, pupae, adults) can be greatly reduced if economies of scale are taken into account (Parker, this volume). As a rule, due to the cost of construction, most rearing facilities were built undersized rather than basing the production output on a per-insect unit cost. Although the production of a 1000 million insects sounds large, the fixed costs are relatively low compared with a facility that produces only a few ten or hundred million insects.

Much of the "excess" capacity of the facilities, in which output exceeds direct programme needs, may not always be available as it is often dedicated to others through partnerships and used in emergency eradication programmes, e.g. Mediterranean fruit fly, Mexican fruit fly, and the Caribbean fruit fly *Anastrepha suspensa* (Loew). The facility built in Kluang, Malaysia (Mahon and Ahmad 2000), to rear the Old World screwworm *Chrysomya bezziana* (Villeneuve), is a joint venture between the governments of Australia and Malaysia; screwworms would be produced for Australia should this pest be detected there (Tweddle 2002). Private-sector companies have now begun to express an interest in addressing this opportunity.

At present, there is a limit on how far sterile insects can be shipped. Fruit fly and screwworm pupae are sterilized in a late developmental stage (Baumhover et al. 1955, Ohinata et al. 1978, Cunningham et al. 1980, Nakamori et al. 1992), and emergence is delayed by shipping pupae in a low-oxygen atmosphere (hypoxia). Sterile Mediterranean fruit fly pupae have been shipped successfully from Mexico and Guatemala to South Africa, Argentina, Chile, Austria, Israel, and the continental USA, and the FAO/IAEA has successfully shipped sterile pupae from Austria to Israel without serious loss in the quality of the resulting adult flies (Enkerlin and Quinlan (2004) made a systematic summary of worldwide transboundary sterile insect shipments during the last 50 years). In 1990–91, the FAO purchased sterile New World screwworm pupae from the Mexico-United States Commission for the Eradication of Screwworms to eradicate an incipient outbreak in Libya (FAO 1992).

Delays in shipments due to increased security measures at airports are an important logistical consideration. If kept under hypoxia too long, the quality of the resulting adult insects decreases markedly over time, especially regarding emergence and flight ability. Research to identify atmospheres and environments that could lead to extended shipment/holding times of insect pupae should be encouraged. International standards and guidelines on transboundary shipments of sterile insects are currently being developed (Enkerlin and Quinlan 2004).

Mediterranean fruit fly eggs can be shipped with little loss in yield or quality of

the resulting sterile adults (IAEA 2002), and it avoids the problems associated with maintaining pupae under hypoxia for long periods. Also, since the volume and weight of eggs are far less than those of pupae, shipping costs are substantially reduced. For example, 1 litre of eggs is equivalent to almost 5 million pupae. Of course egg shipment requires a facility for rearing the males and sterilizing them. Currently 18 litres of Mediterranean fruit fly eggs of a genetic sexing strain are shipped daily from the El Pino production facility in Guatemala to the Metapa production facility in Mexico. These eggs are heat-treated (to kill female eggs) prior to transfer. Purchasing eggs enables the receiving programme to avoid maintaining a viable colony of fertile adult insects for egg production. Shipments of eggs also minimize the potential quarantine risks associated with accidental insect escape or spillage en route to the final destination.

Codling moths, diamondback moths, and pink bollworms are shipped as chilled adults, since studies have shown that sterilizing adults instead of pupae results in more competitive insects (Anwar et al. 1971, Hooper 1971, Ohinata et al. 1971, Wakid 1973). This may be worth considering for other species as well, especially in locations where the production and sterilization facility is located near release sites. Although not under hypoxia, the quality of adults suffers if they are kept chilled too long (Mutika et al. 2002). Thus new methods would have to be devised for transporting emerged sterile adults from the point of emergence/sterilization to where they are to be released.

The New World screwworm production facility in Tuxtla Gutiérrez, Mexico, provided the sterile flies to eradicate this pest from Central America. However, with increasing shipping distance, both the shipping costs, and the potential for problems including delays in the shipping process, increase (FAO 1992, Wyss 2000). For example, the shipping cost alone to send New World screwworm pupae to Libya was four times the purchase price, and four times the dispersal cost.

The greatest drawback to purchasing sterile insects is that a programme lacks control over the production process. Decisions by the managers of a rearing facility can greatly affect a field programme. These decisions can range from the product being diverted to another programme, unresolved labour problems leading to a work stoppage, scheduling a shutdown of the facility for maintenance at a time when the need for sterile insects is the greatest, changing the strain of insects being reared, to decommissioning an irradiation source (Quinlan et al. 2002). Although these are real possibilities, there are few instances where, in fact, such events have occurred. To the contrary, production levels can be maintained even when major remodelling or construction is underway. Regardless of any potential drawbacks, purchasing sterile insects is an option that needs to be seriously considered during programme feasibility studies.

2.2. Rearing Insects for Release

Three common approaches to insect production are:
- Building a single centralized facility for the mass-rearing and sterilization of the insects, combined with the preparation of the sterilized insects for release (Schwarz et al. 1985, Ortiz et al. 1987, Nakamori et al. 1992, Liedo et al. 1993,

Msangi et al. 1999, Bloem and Bloem 2000, Koyama et al. 2004)
- Building one facility for the rearing and sterilizing of the insects, and one or more satellite facilities where sterile insects are prepared for the releases (Quinlan et al. 2002)
- Receiving eggs from another production facility for local rearing, sterilization, and release as described above (section 2.1.)

2.2.1. Centralized Production Combined With Release

The selection of one combined facility versus separate release facilities is normally linked to the size and distance from the target release area; transporting chilled sterile insects long distances prior to release may reduce insect quality and is costly. In locations such as South Africa or Spain, where the objective is simply effective and more environment-friendly pest suppression, a chain of production and/or release facilities may be more cost-effective from an operational standpoint than a single combined facility.

Production facilities are used to prepare sterile insects for release in many parts of the world. Mediterranean fruit flies, for example, are reared in Argentina, Guatemala, Hawaii, and Mexico, and Mexican fruit flies in Mexico, and the sterilized pupae are shipped to satellite facilities for emergence and release (CDFA 1999a, b, 2002; Barnes and Eyles 2000, Dowell et al. 2000; Rossler et al. 2000).

In the pink bollworm programme in the USA, moths are reared and sterilized in Arizona but released in California (USDA 1995, Walters et al. 2000). For the successful eradication of *Glossina austeni* Newstead in Zanzibar, sterilized tsetse fly adults, produced in Tanga, Tanzania, were directly released in boxes over Unguja Island, resulting in the elimination of the disease trypanosomosis (Msangi et al. 2000). A single centralized facility produces sterile codling moths in British Columbia, Canada (Bloem and Bloem 2000).

Regardless if there is a single centralized production facility, or additional satellite facilities, it is an advantage to have a single management structure for a programme, allowing efficient decision-making and rapid exchange of information among the components of the mass-rearing and release processes (FFEPO 1999; Dyck, Reyes Flores et al., this volume). If problems in post-emergence insect quality are noticed, these can be communicated quickly to the production section. This close link between the production and field components helps to maintain a high-quality sterile insect for field release. Preparing insects for release in the place where they are reared eliminates the extra operations of packing the sterilized insects for shipment and shipping them to a satellite facility.

Aspects of insect quality are addressed through the establishment of international standards for measuring product, process, and production controls, e.g. the FAO/IAEA/USDA (2003) manual for fruit flies.

The rearing facility should be constructed in an area where the escape of fertile females from the brood colony will not cause problems regarding regulatory concerns. This was one consideration in the location of the Mediterranean fruit fly mass-rearing facilities in Hawaii, Mexican fruit fly mass-rearing facility in southern Mexico (Rull Gabayet et al. 1996), New World screwworm in Mexico, and Old World screwworm in Malaysia. Mass-rearing facilities harbouring large numbers of

fertile females of the target organism are generally not recommended to be situated in areas where the species is not known to occur but could establish itself. This situation can of course develop as a programme is successful in eradicating a pest and the facility then finds itself in the quarantined area. Stringent containment measures to prevent the escape of fertile females from the brood colony, as well as the release of sterile insects in the surroundings in case such an escape occurs, are used at the screwworm facility in Tuxtla Gutiérrez, Mexico (Wyss 2000).

2.2.2. Satellite Facilities for Emergence and Release Only

Satellite facilities have several advantages: (1) sterile insect programmes can operate in areas that, for regulatory reasons, cannot have mass-rearing facilities, (2) one mass-rearing facility can provide insects to several satellite facilities, often in different countries, and (3) several different species of insects can be prepared for release.

The shipment of sterile insects to a satellite location allows the distribution centres (insect emergence and release facilities) to be located in areas generally free of the target insect. This is important if the goal of the programme is the eradication of isolated invasions of the target pest, or the exclusion of the pest from an area free of it. Thus sterile pupae of the Mediterranean fruit fly, and sterile pink bollworms, can be shipped from mass-rearing facilities located in areas where these pests occur to areas free of permanent infestations of these pests (USDA 1995, CDFA 2002).

The use of satellite release facilities gives maximal flexibility to the use of sterile insects. One large rearing facility can provide sterile pupae to several satellite facilities scattered throughout an area. The Mediterranean fruit fly mass-rearing facility in El Pino, Guatemala (Fig. 1), has provided sterile pupae for programmes in Guatemala, California, Florida, South Africa, and Israel (Rossler et al. 2000, Villaseñor et al. 2000). Satellite insect emergence facilities are less complex in their design than centralized mass-rearing facilities, and often do not require permanent construction. As programme needs change, satellite emergence facilities can easily be relocated. The satellite facilities used to prepare sterile screwworms for release have steadily moved southwards from Mexico to Panama, as the New World screwworm eradication programme progressed through Central America, but the production facility continued to operate in Mexico (Wyss 2000).

One important consideration in the development of satellite facilities is the cost of shipping sterilized insects (Wyss 2000). Also long transit times can decrease the quality of the released insects. Shipping times of up to 24 hours have not proved detrimental to the quality of the resulting adult insects in sterile fruit fly release programmes in California, Florida, and elsewhere, or in tsetse fly shipments (IAEA 2002).

Figure 1. Mediterranean fruit fly production and sterilization facility, El Pino, Guatemala, in September 2004. (Photo from P. Gomes, reproduced with permission.)

Several species of sterile insects from different rearing facilities can be handled in a single, satellite facility. In California, the satellite fly emergence and release facilities for the Mediterranean and Mexican fruit flies are housed in the same location, and use the same staff.

A satellite facility requires that two facilities be maintained, with two management teams, perhaps with different goals and problems, and located at different sites, often in different countries. One facility is concerned with producing sterilized insects ready to be shipped, while the other is concerned with preparing the sterilized insects for release.

Different goals, and the physical separation of the facilities, can result in more attention and focus on field operations, the real programme target, and in the case of independent customers, provide a more objective sterile insect quality control. On the other hand the separation can increase the potential for poor feedback between producers and users of insects concerning problems in the quality of the sterile insects. Conducting product-quality control tests in accordance with agreed procedures, and exchanging these results, provides a way to monitor quality and address client concerns. By documenting their procedures and other practices, rearing facilities are now beginning to establish ISO 9001:2000 and ISO 14 001:2004 standards (ISO 2005) for insect rearing, and thereby bringing about greater consistency in product quality.

If problems in the rearing process of a centralized facility, that might affect the quality of the resulting insects, are observed, these can easily be transmitted to the rest of the programme. However, experience in California shows that this occurs less frequently than desired. In some instances, the problems observed in the rearing process are only brought to light after the satellite facility notes a decrease in the quality of the resulting insects being prepared for release.

2.3. Commercial Production of Sterilized Insects and their Release

As the number of area-wide suppression programmes has increased, the interest in having private companies, rather than governments, supply sterile insects has also increased. There are advantages and disadvantages to both governmental and private suppliers of sterile insects. Governmental suppliers may be willing to develop facilities to rear insects for which there is a potential use, but not necessarily a continuous demand. They fund programmes against new pests such as the sweetpotato weevil *Cylas formicarius* (F.) (Kohama et al. 2003), or programmes that demonstrate the feasibility of using sterile insects in new ways, such as the codling moth and melon fly AW-IPM programmes. Governments can develop programmes that cross several political entities, such as the Mexican fruit fly preventive release programme along the California-Mexico border, or more recently the release of sterile Mediterranean fruit flies along the Israeli-Jordanian border (Rossler et al. 2000, Cayol et al. 2004). Governments are more likely to invest in research designed to develop potential new technologies for use in mass-rearing programmes..

Government-operated facilities answer directly to themselves, not to growers or ranchers *per se*. Government-funded facilities, however, have several drawbacks, one being the tendency to change policies and/or funding priorities after elections. Thus a programme, that had the strong support of one administration, may find its budget reduced or eliminated by another (Dyck, Reyes Flores et al., this volume). This can make programme managers feel insecure, postpone needed changes in programme procedures or facility upgrades, and make growers or ranchers reticent to support the programme. Another major drawback of government funding is the tendency to ask the programme to do more and more with a static budget. This "do what you can with what you have" attitude leads to decisions that tend to lower the quality of sterile insects produced so as to keep the numbers of insects produced high (Liedo et al. 1993). This can have a negative impact on programme success, which in itself will often lead to funding cuts that continue the cycle of problems.

Privately run facilities are generally created to generate a profit through meeting an existing demand. They are focused on producing a high-quality product for a paying customer. Private suppliers usually use existing technology, and then work to maximize efficiency to reduce costs. Private producers answer directly to their customers, and generally not the government. This direct connection to the end-user makes private producers more accountable, and shortens the feedback loop if problems arise. Private producers can easily cross geo-political boundaries to sell their product, provided that the necessary government permits can be obtained (Quinlan et al. 2002, Quinlan and Enkerlin 2003).

There are also components of producing mass-reared insects that can be privatized, e.g. construction of egging cages, provision of diet ingredients, production of sterile males, emergence and processing of sterile insects, and aerial release operations (Quinlan et al. 2002). Already some of these components are commercially available.

The affected agricultural industries can play a major role in creating public awareness of the importance of sterile insect programmes to themselves, government leaders, and the public (Dyck, Regidor Fernández et al., this volume). They can help develop confidence in the use of sterile insects for eradication or pest suppression.

This will generate a demand for the sterile insects that, in turn, will entice private companies to build the production plants necessary to meet this demand.

As the number of programmes using sterile insects to suppress pest populations increases, so the use of grower or livestock producer funding, and the number of private facilities providing sterile insects, will increase. As this occurs, probably some government-funded programmes will decide to transfer their facilities to commercial producers and redirect funds from facility operation into purchasing sterile insects from private providers.

In the Mediterranean region, bait sprays have traditionally been used to suppress major pests such as the Mediterranean fruit fly and olive fruit fly *Bactrocera oleae* (Gmelin). Encroachment on traditional production areas by "urban sprawl", and the expansion of tourist resorts, makes the future of aerial-bait applications increasingly untenable. Here the integrated use of sterile insects, with the objective of routine area-wide pest suppression, has great potential for commercialization (Hendrichs et al. 1995). The agricultural industry itself is searching for, and promoting the development of, more environmentally acceptable measures to substitute for those technologies developed over 50 years ago. In this respect, the industry is ahead of the technology.

Commercial companies and investors interested in producing sterile insects should be bold in their planning:

- Think big! Economies of scale are important to keeping fixed costs and the cost per insect unit low. The facility and production output must not be undersized.
- Logistical considerations are a key issue. The location of a rearing facility can result in reduced operational costs if diet ingredients do not have to be imported, and the labour costs are low (most rearing skills can be learned by persons with only a limited education).
- "Build it and they will come!" Such a venture can be overstudied and overplanned. The most important thing is to start building; don't wait for everything to be perfect. Be a risk taker!

Programme managers must decide whether to purchase the aircraft used to aerially distribute sterile insects, or contract with a private company to do this. In the USA, all but one programme contract with private firms to provide the aircraft and pilots. Private companies are paid per flight hour, from take-off to landing, or in some instances for actual engine time utilized (based on the Hobbs meter); both are valid practices. An exception is the Mexican fruit fly programme in Texas, where the aircraft is owned by the United States Department of Agriculture (USDA), and the pilot is an employee of the US government. The Animal and Plant Health Inspection Service (APHIS) of the USDA uses this aircraft to design and develop new technologies to deliver sterile insects from the air.

Experience in the USA indicates that it is cheaper to contract a private company, to provide aircraft and pilots, than to purchase aircraft and pay for maintenance, full-time pilots, and mechanics. This may not be true in other countries, and a detailed economic analysis needs to be done for each programme.

3. INSECT PRODUCTION FACILITIES

3.1. *Facility Design*

Rearing large numbers of insects in the laboratory is an "art", but the mass-rearing of insects is an industrial process. These differences must be at the forefront of all planning for insect mass-rearing facilities (Parker, this volume).

Most insects require a colony of adults to produce eggs that are collected and put on a diet to rear larvae. Mature larvae are harvested and allowed to pupate, after which either pupae or adults are sterilized and processed for release. A rearing facility needs rooms allotted to the various insect life stages. This is the situation for most mass-reared insects, including fruit flies, codling moth, pink bollworm, screwworms, sweetpotato weevil, and onion maggot *Delia antiqua* (Meigen) (Nakamori et al. 1992; FFEPO 1999; Bloem and Bloem 2000; Loosjes 2000; Mahon and Ahmad 2000; Fisher 2002; Phillimore 2002; Kohama et al. 2003; Koyama et al. 2004; Shimoji and Yamagishi 2004). However, tsetse flies, which are adenotrophic viviparous, require a different facility design. The reproductive rate of tsetse is low, and thus a large space is needed for holding the adult females that periodically produce mature larvae, which soon pupate. Another area is needed in which the flies are provided with blood meals. After holding pupae for several weeks in a closely controlled environment, adults emerge, and most of the males are separated from females before being sterilized and processed for release (Gooding et al. 1997, Msangi et al. 1999, 2000; Opiyo et al. 2000).

The first consideration in planning a rearing facility is to determine whether a single unit, or whether several modules, each a fully contained rearing unit, will be built. The single-unit approach assumes that the demand will be consistent and rather long-term, and increasing or decreasing production involves facility-wide changes that may be difficult to achieve. During periods of low production, the presence of "empty space", and hence a reduction in the amount of biomass, significantly affects environmental conditions. When operating at full capacity, any perturbation in the process, such as pesticide contamination or an interruption in the electricity supply, may result in the collapse of the rearing process. Equipment maintenance or repair can cause the entire rearing operation to be shut down (Tween 1987). If two or more insect species are reared in the same facility, operational and logistic challenges can arise, especially in trying to avoid contamination.

Using modules allows production to be increased or decreased simply by opening or closing an entire rearing unit (Tween 1987). This does not affect production in other units, and allows the facility to operate just below full capacity and be less susceptible to catastrophic perturbations (IAEA 2002). Contamination, disease, or equipment maintenance or repair in one module does not affect production in other modules. The module system allows two or more insect species to be reared in the same facility, but of course in separate modules. Using individual modules allows production to be increased effectively as demand or the availability of funds dictate. In actual practice, the high demand for sterile insects leads to the maximum use of available space. Facilities with multiple modules may dedicate an entire module to the production of a single phase or stage of rearing rather than

containing all operational phases of production in one module (Stewart 1984, Bloem et al. 2000). For example, several modules at the El Pino facility in Guatemala are dedicated entirely to adult colony/egg production, larval collection, or pupal holding of the Mediterranean fruit fly. A facility located at San Miguel Petapa, 20 km away, is dedicated entirely to egg production.

Both of these systems are used in rearing fruit flies. Mexico has a single unit that rears the Mediterranean fruit fly, and modules for rearing various *Anastrepha* species have been added (Rull Gabayet et al. 1996). The Moscafrut facility in Mexico has a modular system that also rears fruit fly parasitoids for use in integrated management programmes aimed at the Mexican and Mediterranean fruit flies (Villaseñor et al. 2000). However, in Okinawa, Japan, a single unit is used to rear melon flies (FFEPO 1999, Koyama et al. 2004).

Numerous plant designs are available for consideration (Quinlan et al. 2002). The key point is that mass-rearing is an industrial process; the output is a high-quality sterile insect (Vargas 1989). The flow of materials and insects, as well as the movement of workers and equipment, must be considered. The biology of the insects plays a large role in facility design. Usually, except for diapausing insects such as the onion maggot (Loosjes 2000) and codling moth (Bloem et al. 2000), sterile insects cannot be stored for later use. The insects are reared, sterilized, and then immediately sent out for release. This places a premium on fast handling of the insects. For insects like fruit flies, the stock or egging colony is rather small compared with its daily output. The adults lay many eggs per day, and they produce eggs for several days before they are discarded; then the next group of adults is brought into egg production. The larval rearing area is larger than that holding the adult colony (Schwarz et al. 1985, Ortiz et al. 1987, Economopoulos et al. 1993, FFEPO 1999). For tsetse flies, the stock colony is large compared with output, and the breeding colony is maintained for the life of the flies. There is no larval rearing area, and the area holding the adult colony is large compared with the pupal holding area (IAEA 2003).

Fruit fly larval rearing units can be moved from one area of the facility to another (FFEPO 1999). However, at present, rearing tsetse flies involves moving the rearing units to feeding stations on several days a week, and such movement increases fly mortality. Evidently the best approach is to keep the cages with flies stationary, and move the blood to them (Opiyo et al. 2000).

Early facility designs relied heavily on manual labour to move materials and stock — a reflection of the influence of small-scale experiences on facility designers (Liedo et al. 1993). To reduce labour costs and improve facility efficiency, recent work has focused on mechanizing facilities to the greatest degree possible (Roberson and Wright 1984; Stewart 1984; Schwarz et al. 1985; Ortiz et al. 1987; Nakamori et al. 1992; IAEA 2002, 2003; Koyama et al. 2004). Using computers to control the mechanized processes adds additional savings, and improves facility quality-control systems (Bruzzone et al. 1993, FFEPO 1999, Bloem and Bloem 2000; Opiyo et al. 2000; Mahon and Ahmad 2000; Mahon 2002).

3.2. Location, Construction, and Building Materials

It is essential to have a dependable supply of electricity, clean water, and access to a good sewage treatment plant to handle the waste water and solid waste generated in the mass-rearing of insects (Phillimore 2002; IAEA/FAO 2004; Parker, this volume). In some areas the high cost of sewage treatment and/or environmental concerns led programme managers to consider building on-site water-treatment facilities.

Also essential is a consistent supply of high-quality diet ingredients for the insects. For most insects, this means access to locally produced products (Quinlan et al. 2002). It is very important that diet materials are free of insecticide residues (residues caused at least one collapse of the colony at the California Department of Food and Agriculture (CDFA) Mediterranean fruit fly rearing facility in Hawaii).

The facility needs to be close to a good ground-transportation system, and in close proximity to an airport. This reduces the cost of transporting sterile pupae or adults, and also reduces the time that pupae or adults are in transit, thereby increasing the quality of the resulting adult insects.

A mass-rearing facility is usually regarded as a permanent structure. The walls and roof are usually insulated to aid in temperature control. The electrical wiring must be able to operate in high relative humidity, and all mechanical equipment must be resistant to rust and to the metabolic products and reagents present in the rearing process (Quinlan et al. 2002). The whole facility and all equipment must be easily cleaned and disinfected, including all surfaces in all parts of the facility in which the insects are housed. The walls, floors and ceilings must be constructed of materials that can be washed frequently, and the paint must be of high quality to resist peeling. Additional temperature control can be achieved by using air-cushioned transposable material in the walls (Rull Gabayet et al. 1996).

3.3. Environmental Control Equipment

Accurate temperature control is critical in larval-rearing areas (Tanaka et al. 1972, Nakamori et al. 1992, Rull Gabayet et al. 1996), and unless large amounts of heat are dissipated, the quality of the resulting insects will be lowered (Vargas 1989, Nakamori et al. 1992). Extra cooling capacity is always an advantage (Oborny 1998, Mahon and Ahmad 2000); retrofitting to increase cooling capacity is costly. A model Heating, Ventilation, and Air Conditioning (HVAC) system, using chillers to cool water via cooling towers directed to air handlers, was recently installed in the El Pino rearing facility in Guatemala. A computer monitoring system registers data from temperature and humidity sensors. This system also monitors the flow of water and air. Alarms alert maintenance staff when the temperature or humidity changes more than the allowed set points. It also shows if air filters need cleaning or changing. This system relies on polyvinyl chloride (PVC) ducting in the critical rearing areas where metabolic acid fumes are generated, and on stainless steel ducting in areas where adult oviposition/egg collection occurs.

In melon fly mass-rearing, larval-rearing rooms are cooler than those that hold adults for egg production, and the temperature needs to be lowered as the larvae

become larger and produce increasing amounts of metabolic heat, otherwise the high temperatures will be detrimental for the larvae and resulting pupae (Nakamori et al. 1992). Similar procedures are used for the Old World screwworm (Mahon and Ahmad 2000), Mediterranean fruit fly (Vargas 1989, Calderon 1993), and Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Fisher 1996). Recent advances in computer technology make it possible to use personal computers to monitor the temperature and humidity within a rearing facility. Computers can control airflow within the facility or rearing units, moisture content of the rearing medium (which is important for the codling moth) (Quinlan et al. 2002), and temperature and relative humidity in the rearing and other units. Computers provide a greater degree of control than is possible using gauges and facility workers, and also a complete record of the environmental conditions within a facility.

Even though computer monitoring of critical functions is a great asset, it can be a disadvantage if carried too far. In some regions, obtaining local but qualified service personnel to operate and maintain sophisticated equipment is very difficult, and the facility design must take this into account (Mahon and Ahmad 2000).

Air quality is important in the rearing of most insects but especially in the case of moths, as scales from the wings and bodies are potent allergens that can trigger allergic responses in exposed workers (Kfir 1994; Parker, this volume). Therefore, as part of the air distribution system, High Efficiency Particulate Air (HEPA) filters are used in the codling moth mass-rearing facility in Canada (Quinlan et al. 2002), and specially designed collectors attract and deliver newly emerged moths to a cold room without human intervention (Dyck et al. 1993).

Depending on the location of a facility, dehumidification and/or humidification systems are needed. Humidification systems, using stainless steel piping and atomizer nozzles under high pressure, have been used successfully in the Guatemala facility to increase humidity, particularly in the areas of larval initiation. Vapour heat has been used successfully in various facilities. Another option is to control the environment in small rooms or chambers rather than in large rooms, thereby minimizing maintenance costs. Plasticized bags, such as those found in packaged dry cereals, can be used to cover rearing trays during critical phases.

3.4. Facility Operation

3.4.1. Production Management

Mass-rearing sterile insects is an industrial process that maximizes efficiency while maintaining a high-quality product (Nakamori et al. 1992; Bruzzone et al. 1993; Liedo et al. 1993; Calkins et al. 1996; Gooding et al. 1997; Opiyo et al. 2000; Parker, this volume).

Early efforts at judging the quality of sterile insects focused mainly on the adult insect (Calkins 1989; Calkins and Parker, this volume). These insects were subjected to measurement of various parameters: emergence, survival, flight, and mating competitiveness (Ortiz et al. 1987, FFEPO 1999, Koyama et al. 2004). Deviations from accepted norms were noted, and the results given to the managers of the facility who, in turn, would try to determine what went wrong. A better approach is to institute a process-control system that continuously monitors every step of the

rearing process (Bruzzone et al. 1993; Liedo et al. 1993; Calkins et al. 1996; Mahon and Ahmad 2000; Parker, this volume). Such a process-control system provides managers with immediate notice of processes that are not within accepted norms. Process control is greatly enhanced by both mechanization and computer monitoring of the rearing process, including the quality of the ingredients of the larval rearing, or adult feeding, diets (Bruzzone et al. 1993, Liedo et al. 1993, Calkins et al. 1996, Mahon and Ahmad 2000).

The development of genetic-sexing strains has greatly altered the rearing of sterile insects, providing opportunities to increase rearing efficiency and the efficacy of the sterile insects released in the field (Economopoulos et al. 1993, DeLongo et al. 2000, Fisher and Caceres 2000, Pereira et al. 2000). The retrofitting of the CDFA and USDA Mediterranean fruit fly rearing facilities in Hawaii is largely due to the decision to use the temperature-sensitive lethal (*tsl*) strain of the Mediterranean fruit fly instead of standard strains.

3.4.2. *Control of Microbial Organisms Within Facility*

The control of bacterial and fungal contaminants deserves special mention in regards to facility design (Sikorowski and Lawrence 1994). The presence of protein and carbohydrate food sources, in the hot and humid environment of larval rearing areas, creates the perfect habitat for microbial growth. Bacterial contamination has been a frequent problem in the larval diets of the Mediterranean fruit fly, causing population collapse (Ortiz et al. 1987), and fungal contamination is a problem in the rearing of Lepidoptera.

Facility design can greatly reduce the contamination rate of larval rearing areas. In Mexico, it was discovered that minimizing the human foot-traffic among the various parts of the mass-rearing facility reduced bacterial contamination of the larval diets (Rull Gabayet et al. 1996). Other mass-rearing facilities also minimize the movement of staff (FFEPO 1999).

Managing the airflow through a facility can reduce microbial contamination. It is a critical design feature that the HVAC system provides positive atmospheric pressure, and introduces fresh filtered air to "clean" areas, e.g. those used to prepare larval diet, dispense eggs onto the diet, and house the early stages of larval rearing. Conversely, "dirty" areas, e.g. those used to collect larvae, wash rearing trays, and dispose of used diet, should be under negative atmospheric pressure (created by exhausting microbial-laden air from the facility). This will prevent the cycling of airborne microbial contaminants through the facility, and the premature spoilage of the larval diet caused by an ever-increasing load of unwanted micro-organisms. Air-exhaust ports must not be located near or upwind of air-intake ports.

Effective temperature controls, good management of airflow, and regular and thorough cleaning of the area, will reduce bacterial contamination problems to a manageable level (Fay 1989). It must be assumed that bacterial contamination will be a problem, right from the outset, and the design of the facility must be planned accordingly. Failure to do this will result in costly retrofitting of the facility.

4. STERILE INSECT EMERGENCE AND COLLECTION

4.1. Emergence and Collection of Adults

In many AW-IPM programmes that integrate the SIT, sterile pupae are shipped to emergence centres, where they are placed into containers or rooms where adults emerge.

Systems for the emergence and feeding of fruit flies prior to field release have changed over time. An old system was the "bucket method" used for roving ground release. However, the most commonly used system today is the plastic adult rearing container (PARC) system. Figure 2 shows preparations being made for Mediterranean fruit fly emergence using PARC boxes. This system was developed by the USDA (Mabry 1986) to replace the earlier paperboard Tanaka box, developed by the California Department of Food and Agriculture (CDFA). The PARC system is an improvement over the Tanaka box, permitting the containers to be sanitized after each use, and reducing the number of flies lost through escape within the emergence facility. When using either of these two systems, pupae are dispensed into paper bags, the bags are loosely stapled at the top to allow flies to escape, and

Figure 2. Mediterranean fruit fly emergence facility in Retalhuleu, Guatemala, showing a worker (with face mask) placing sterilized pupae into a PARC emergence box. (Photo from J. P. Cayol, reproduced with permission.)

then five or six bags are placed in each plastic container. Screen panels on the sides of the container provide ventilation, and a screen panel in the lid permits the flies to feed on a gelatinous slab consisting of agar, sucrose, and water. This system requires much controlled-environment space to hold a large number of bulky containers during the emergence period. It is also labour-intensive, and the containers do not lend themselves to automation of the loading and cleaning processes. Paper bags are costly, both in direct cost and the cost of waste disposal. Also some flies are lost when the bags are destroyed.

The recently developed "tower system" (Fig. 3) is used for emerging, feeding and immobilizing Mediterranean fruit flies in Florida, and Mexican fruit flies in Texas. This tower system was developed to improve the PARC boxes by the USDA in Mission, TX, USA (Salvato et al. 2004). Each tower consists of interlocking screen-panelled aluminium frames (trays) stacked on a portable base. Pupae are placed into a channel around the inside perimeter of each aluminium tray. One or two slabs of gelatinous food are put on each screen panel. Up to 80 trays can be stacked in one tower. A small, direct-current axial exhaust fan is placed on top of each stack of trays, providing forced upward ventilation in a tower. When flies emerge and move from the perimeter channel to the screen of a tray, the empty puparia are left in the channel. The flies remain on the screen and feed for 4–6 days, the period required for maximum emergence. On the day of field release, the towers are moved into a cold room where high-volume exhaust fans suck cold air into each tower to accelerate fly immobilization. After a short period of exposure to the cold, the puparia are vacuumed from the channels, and the screened trays are manually turned upside down over a hopper (and pan beneath) to collect the chilled flies. After the flies are weighed, they are transferred to the aerial release box and transported to the release aircraft.

Compared with the PARC system, the tower system considerably reduces fly escapes as well as the required controlled-environment space, with a consequent reduction of fixed costs. The only waste materials produced are puparia, food residues, and water from the mechanical tray washer. Overall labour requirements are also reduced, primarily because of the automated pupae loading, puparia separation and disposal, and tray washing process. However, since there are more flies in a PARC box than in a tower tray, the critical time when chilled flies are collected from tower trays requires more manual labour, or is slower, than from PARC boxes. Hence, on balance, even though the tower system requires more hand labour in the final step of the process, i.e. collecting chilled flies, its overall cost is less than the PARC system due to the optimization of space and savings in hand-labour costs in other steps of the process.

For those insect species where adults are positively phototropic and thus show a strong positive phototaxis to an incandescent or fluorescent light source, this behaviour can be used to collect newly emerged adults. Pink bollworm moths, codling moths, and New World screwworm flies are collected using fluorescent or ultraviolet lamps (Stewart 1984, Dyck et al. 1993, Wyss 2002). Cold temperatures are used to temporarily immobilize the adults following collection, while they are being transported to and loaded into an aircraft, and during aerial release operations.

Figure 3. Stages in the emergence and collection of fruit flies using the tower system. Upper left: Screen tray with aluminium frame, showing slab of food, and red-dyed pupae in a channel around the edge; Upper right: Stacks of trays in towers, each with a fan drawing air upwards. Tower system for Mediterranean fruit fly, USDA/APHIS, Sarasota, FL, USA. (Photo from C. Caceres, reproduced with permission.) Lower left: Screen tray in cold room with emerged flies on screen, and puparia removed from channel by suction; Lower right: Chilled flies being collected in a hopper. Tower system for Mexican fruit fly, USDA/APHIS, Mission, TX, USA. (Photo from J. Worley, reproduced with permission.)

It is important to control relative humidity during the emergence and chilling of adult insects. If the humidity is too low, it may be difficult for an adult to emerge, and thus use up critical energy reserves prior to release. However, if the humidity is too high, it can affect insect quality. This is particularly problematic when chilling adults to load them into metal release boxes. Condensation on the inside of the release box can lead to excessive moisture that completely saturates the insects, causing them to become tangled in a large ball at the bottom of the box, resulting in fly damage during release. Commercial dehumidification systems can be used to reduce the humidity during the chilling, transport, and release of sterile insects.

To permit identification after capture in the field, many species are marked prior to release (Parker, this volume; Robinson and Hendrichs, this volume). Calco and tinopal dyes have been used successfully to mark lepidopteran insects. Fluorescent dyes, used on fly pupae, are transferred to the ptilinum of the adult as it emerges (Steiner 1965, Schroder et al. 1972, Vreysen et al. 1999). The transferred dye becomes incorporated into the head capsule as the ptilinum is withdrawn into the insect's head. Upon capture, as adults are viewed under a long-wave ultraviolet (blacklight) lamp, the dye fluoresces (Niyazi et al. 2005).

4.2. Provision of Food and Water Prior to Release

For most adult insects, it is important that they are provided with a source of food and water shortly after emergence. In most cases, the food reserve accumulated during larval development is only sufficient to sustain the adult for 1 or 2 days under ideal environmental conditions. Studies have shown that providing food and water to newly emerged adults results in much higher recapture rates following release.

In addition to improved nutrition that increases field survival and mating competitiveness, a time delay between emergence and release may be needed to permit the insects to become sexually mature, e.g. male tsetse flies, but for most insect species the sterile insects are released soon after emergence to avoid mating between sterile males and females in the rearing facility, and to minimize space requirements in the facility.

5. STERILE INSECT RELEASE

There are three methods of releasing sterile insects: static ground-based receptacles (static release), mobile ground-based vehicles (ground release), and aerial release using aircraft (aerial release). Each release method has advantages and disadvantages.

5.1. Static Release

Static release receptacles typically are containers into which sterile pupae or adult insects are placed (Liu and Yeh 1982, Williamson et al. 1983, Cuisance et al. 1984, Oladunmade et al. 1990, FFEPO 1999, Msangi et al. 2000, Yamagishi and Kakinohana 2000, Koyama et al. 2004, Sutantawong et al. 2004). When pupae are placed into them, adults are able to emerge in synchrony with local diurnal and temperature cycles, leave the receptacle, and disperse from the site during the day as the temperature rises. Static releases require a uniform distribution of release sites throughout the target area, and the pinpointing of areas to receive increased numbers of sterile insects. This is possible only for relatively small-sized areas that are accessible.

Placing pupae in release receptacles eliminates the need for satellite facilities, reducing labour and infrastructure costs. Pupae are easily handled — they can be poured into the release receptacle or included in a pre-prepared insert placed into the

release receptacle. Simple volumetric measurements provide a way of estimating the number of pupae in each receptacle.

Static releases also have major disadvantages: distribution is limited by ground access, sterile insects are clumped, with foci separated by areas of low insect density, provisioning the release receptacles is labour-intensive, and emerged flies are very susceptible to bad weather and predators. Most programmes that have used static releases have now changed to either ground or aerial releases (Liu and Yeh 1982; Koyama et al. 2004; CDFA, unpublished data).

5.2. Ground Release

In a ground-release system, adult sterile insects are dispensed from slow-moving or stopped trucks, or from all-terrain vehicles (ATVs) (Fig. 4). The insects are released by hand or machine, directly into the environment, or in open bags from which they escape after release (Holler and Harris 1993, Fisher 1996, FFEPO 1999, Bloem and Bloem 2000, Covacha et al. 2000, Loosjes 2000, Koyama et al. 2004). To maximize their dispersal away from the release vehicle or bag, the insects may be kept at ambient temperatures during the release procedure. Ground releases can treat a larger area more quickly than static releases. The vehicle routes can easily be changed to meet changing demands of the programme, and additional release trips can be made in areas that need higher numbers of sterile insects. Fewer workers are needed to make ground than static releases. The negative impact of predators on the sterile insects is minimized when the adults are released directly into the environment. Since pupae are kept indoors at relatively constant temperatures, the adults emerge at a predictable rate, making long-range scheduling possible. Adult insects can be held (probably at a low temperature) during periods of inclement weather, and then released when acceptable weather conditions return.

Like static releases, the distribution of sterile insects from ground releases may not be uniform, and greater densities will occur close to areas bordering the path of the release vehicles. There are safety issues associated with having slow-moving vehicles travelling on normal roads, and inaccessible areas of dense vegetation pose problems for ground-release systems. Bird and vespid predators will learn to exploit the insects contained in bags, and the bags may constitute a litter problem.

If adults are released, emergence facilities are needed, and adults will emerge even during periods of inclement weather, when releases are not possible or advisable, and the accumulation of insects may necessitate the destruction of excess insects.

5.3. Aerial Release

Aerial releases directly release chilled adult insects, or eject bags or boxes containing sterile adults, from aircraft (Howell et al. 1975, Nakamori and Kuba 1990, FAO 1992, USDA 1995, Vreysen et al. 1999, Msangi et al. 2000, Pereira et al. 2000, Villaseñor et al. 2000, Vreysen et al. 2000, Walters et al. 2000, Wyss 2000, Yamagishi and Kakinohana 2000, Kohama et al. 2003, Rendón et al. 2004).

Figure 4. All-terrain vehicle (ATV) releasing chilled irradiated codling moths. (Photo from SIR Program (2004), reproduced with permission.)

The direct release of sterile adults requires that they be taken from emergence containers or rooms, chilled (or first chilled in the containers), and put into large refrigerated boxes that are then loaded on to a specially equipped aircraft (FFEPO 1999, Dowell et al. 2000, Villaseñor et al. 2000, Walters et al. 2000, Wyss 2002, Koyama et al. 2004) (Fig. 5). The insects are dispensed from the aircraft using a motor-driven screw auger or belt that delivers the insects to the release tube as they fall out of the release box. The release rate is controlled by varying the speed of the auger and/or the aircraft.

In some programmes, sterile insects are released in paper bags or boxes (Hentze and Mata 1987, FAO 1992, Vreysen et al. 1999, Covacha et al. 2000, DeLongo et al. 2000, Msangi et al. 2000, Opiyo et al. 2000, Villaseñor et al. 2000). Usually pupae are placed in bags/boxes, held in the release containers until adult emergence, and then the release bags or boxes are loaded into larger holding boxes and placed on the aircraft. The release bags or boxes are held at the ambient temperatures and dispensed from the aircraft by hand or conveyor belt. Releasing sterile insects in their emergence bags or boxes reduces preparation time, compared with the chilled-insect procedure.

Aerial releases can cover large areas quickly, regardless of the terrain, lack of roads or density of vegetation. They distribute the sterile insects over the treated area

Figure 5. Loading refrigerated boxes with chilled sterile New World screwworms into an aircraft in preparation for release, Jamaica. (Photo from M. J. B. Vreysen, reproduced with permission.)

as required, especially if the aircraft flight paths are controlled by a Global Positioning System (GPS) guidance programme (Villaseñor et al. 2000, USDA/FDACS 2004). The mechanized release system can be linked to a computer to provide flexibility in the release rate during a flight. This allows more sterile insects to be delivered to those areas needing them, without having to make multiple flights over any given area. If boxes/bags are used, the release rate is increased by ejecting more boxes in a given time period.

Aerial releases are more weather-sensitive than ground releases, and an emergence facility is required. The release of sterile insects in bags or boxes is problematic if litter is a concern. Bags and boxes are expensive, and take up considerable space in an aircraft that could instead be devoted to holding many more chilled insects. Aerial releases of the Mediterranean fruit fly in Argentina, using bagged adults, averaged 1.2–1.3 million flies per flight (DeLongo et al. 2000).

Aerial releases using the direct release of chilled flies in California averaged 3–5 million flies per flight (Dowell et al. 2000). The number of sterile flies that can be released by an aircraft is a function of the release rate and aircraft size. Flight duration is usually determined by the amount of time that chilled insects tolerate without a reduction in quality, e.g. 4 hours. Pereira et al. (2000) found that aerial releases are less expensive than ground releases.

Both fixed-wing and rotary-wing aircraft can be used to deliver sterile insects to the target area. The choice of aircraft type will depend on local environmental conditions, and the availability and cost of aircraft. Rotary-wing aircraft is more expensive, and is usually used for canyons or spot treatment of restricted host areas.

5.4. Global Positioning System (GPS) for Guiding and Monitoring Insect Release

Guidance of aircraft used to aerially release sterile insects has evolved from a system based on compass headings, landmarks and flight times to one that now uses the Global Positioning System (GPS) (Slagell 2000). Using the GPS, programme managers can lay out the exact flight paths for the aircraft. This technology allows the distribution of sterile insects precisely where desired. It has also reduced flight times and pilot error, and eliminated the duplication of flight paths and the unintentional omission of some paths. Today most programmes making aerial releases use the GPS to guide the aircraft.

The GPS aircraft guidance system can be linked to a computer that controls the release of the insects — so as to deliver the sterile insects precisely where they are needed and in the desired numbers, and also have a permanent record of the flight for use in quality-control evaluations (Fig. 6). Such computer-driven release systems will allow two or more different species of sterile insects to be released at the same time. Managers can avoid releasing sterile insects over areas that do not need them. The GPS is especially useful in areas that lack the visual landmarks that pilots may use to determine where they are and where they need to fly.
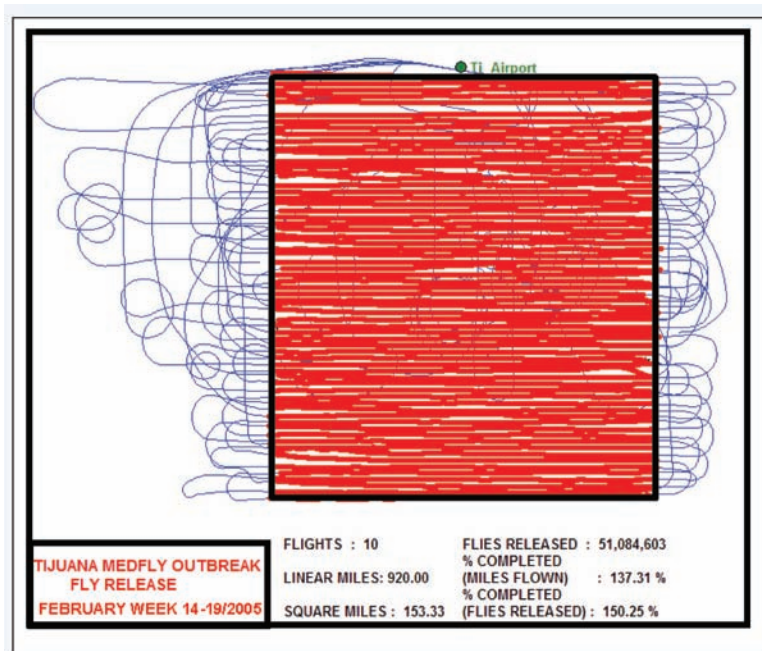
Figure 6. Ten flights (combined) showing flight paths (blue) and sterile Mediterranean fruit fly (medfly) release lanes (red). (Flight record from L. Charles, USDA/APHIS, Tijuana, Mexico, reproduced with permission.)

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CHAPTER 3.6.

MONITORING STERILE AND WILD INSECTS IN AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES

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SUMMARY

Insect pest control programmes, which integrate the release of sterile insects, can be efficient only if the released insects have an optimal biological quality. Frequent monitoring of the quality of reared insects after being released in the field is an important but often neglected component of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT). Parameters of sterile insects, which should be monitored regularly, are sexual competitiveness of the released insects, and related components, e.g. survival, mobility, dispersal characteristics, and spatial occupation of the habitat. A well-balanced monitoring programme will, at any given time, provide essential feedback on the progress being made. This information is prerequisite to efficient implementation of the release and cost-efficient use of sterile insects. The type of monitoring to be done will be determined largely by the particular biology of the target insect species. The most important parameter in relation to the release of sterile insects is the rate of sterility induced in the wild insect pest population; it will provide the best evidence that any observed changes, e.g. in the density of the target insect, are caused by the release of sterile insects.

1. INTRODUCTION

Successful insect area-wide integrated pest management (AW-IPM) programmes using the sterile insect technique (SIT) require reliable data on the biology of the insect, especially its sexual behaviour, population dynamics, and temporal and spatial fluctuations in population density and distribution (Itô and Yamamura, this volume). This information is essential to accurately interpret data accumulated during the monitoring of the programme. Monitoring sterile and wild insects is a critical aspect of any SIT operation, and includes: (1) the performance of sterile insects after release, and (2) the impact of sterile males and other control tactics on the wild target population.

The efficient implementation and success of any control programme using the SIT will depend on factors related directly to the quality of the released insects (Calkins and Parker, this volume). It is imperative that the released sterile insects intermingle rapidly with the wild population after being released, and mate at the same rate as the wild insects. The production of insects in a rearing facility, that have a "biological quality" or "fitness" comparable with that of wild insects, is much more complicated than might be anticipated. This quality can easily be impaired due to: (1) aspects inherent to the colonization and mass-rearing procedures (artificial environment, holding density, stress) (Parker, this volume), (2) the sterilization treatment with ionizing radiation (Bakri et al., this volume), and (3) the physical handling, marking procedures, and transport of the insects to the release site (Calkins and Parker, this volume; Dowell et al., this volume; Parker, this volume). In addition, the behaviour of the reared and released insects could be altered drastically due to changes induced in the genetic traits of the stock kept for numerous generations under artificial conditions. Continuous rearing may select for traits that favour mass production (early maturation, high fecundity, mating at high densities), but which could negatively affect field performance (courtship behaviour, release of

pheromones, territorial behaviour) and even prevent released insects from mating with wild insects (Lance and McInnis, this volume).

Ideally, to assure optimal quality of reared insects, rigorous quality control procedures must be implemented at all times and in all phases of production, not only on an ad hoc basis or after the emergence of a problem (Spradbery 1994). However, regardless of how meticulously quality control procedures are implemented, the laboratory criteria of fitness may have little bearing on the ability of released males to survive and mate with wild females (Krafsur and Hightower 1979). Admittedly, measurement of the "biological quality" of sterile insects in a laboratory or field cage is a convenient way to assess the effect of several factors (FAO/IAEA/USDA 2003) (Calkins and Parker, this volume), but the quality of an insect under such conditions will not necessarily be the same as that of the same insect a day later in the field. Therefore, the frequent monitoring of the "fitness of the released insects" and related parameters, such as survival, dispersal rate, and spatial occupation in the natural habitat, is an indispensable component of AW-IPM programmes that integrate the release of sterile insects.

Efficient programme implementation and technical management of an AW-IPM programme are only possible through the regular and frequent analysis of accurately collected field data. In practice, not all programmes using the SIT grant the same importance to the monitoring aspect, and the emphasis given depends on factors such as: (1) level of experience of the managers, and their confidence in the programme, (2) economics and availability of sterile insects (is it cheaper to release more insects than develop an extensive monitoring programme?), and (3) efficiency and economics of the available monitoring tools. Scientifically sound monitoring activities require significant funding for personnel, equipment, logistics, and recurrent expenses, and as a consequence, insufficient importance is often given to this component. However, when progress does not match expectations, accurate field data are a prerequisite to detecting the causes of the problem and applying corrective measures. Otherwise, programme managers are doomed to "guess work" and making decisions based on assumptions, frequently resulting in technical and financial failure.

History teaches us that any AW-IPM programme will face criticism, especially when the goal is to eradicate the target insect in a circumscribed area (Klassen, this volume). Unfortunately, it is usually the uninformed "outsider", lacking appropriate scientific background or insight into the programme, who is criticizing it. A scientifically sound monitoring programme is an essential element in successfully refuting such criticism.

2. MONITORING INSECT QUALITY IN FIELD

Surprisingly, little attention has been given to monitoring the competitiveness of sterile insects in the field. In part, this is due to the technical difficulties involved in making these types of observations. In recent years, however, significant progress has been made by using large field cages to study a set of parameters (e.g. preferred host location, courtship and mating behaviour, mating compatibility, spatial

distribution related to female location, mating success, etc.) that are relevant to the sexual competitiveness of the reared sterile insects in a semi-controlled natural environment (FAO/IAEA/USDA 2003; Calkins and Parker, this volume). Even with this important development, the successful interaction of mass-reared sterile males with wild females in the field is still the key to the success of the SIT, and every effort should be made to assess the extent of this interaction. A Field Quality Control (FQC) group, that designs a set of tests tailored to the needs of a target species, must be an essential part of all field programmes. The FQC group should be composed of full-time employees of the programme who have the required expertise in conducting field evaluations, and extensive knowledge about the ecology and behaviour of the target insect (FAO/IAEA/USDA 2003).

2.1. Sexual Competitiveness

The competitiveness of an organism is defined as its ability to compete with conspecific organisms for a limited environmental resource (FAO/IAEA/USDA 2003). Fitness in wild insects involves factors that affect the transmission of adapted genotypes from one generation to the next. Sterile insects do not reproduce, and therefore their fitness is largely a matter of survival, dispersion, adequate behavioural responses, habitat finding, and successful mating, i.e. fitness ends with mating or insemination (LaChance 1979). Therefore the general sexual competitiveness of a sterile insect is largely defined and influenced by components such as survival, mating propensity, mating compatibility, post-mating factors, etc. (FAO/IAEA/USDA 2003; Lance and McInnis, this volume). In view of the drastic changes, due to continued mass-rearing, that can be induced in the genetic traits of reared insects, the frequent measurement of sexual competitiveness and its related components under field conditions is indispensable for success (Haisch 1970, Itô and Koyama 1982).

Fried (1971) defined sexual competitiveness c by the following equation:

$$c = \frac{Hn - Hc}{Hc - Hs} \frac{N}{S} \tag{1}$$

where Hn = viability of eggs (i.e. fertility) from an untreated mating (wild fertile male x wild fertile female), Hc = viability of eggs (or expected fertility) from a mating with sterile and fertile males in N/S ratio (from laboratory observations), Hs = viability of eggs from mating between sterile male and wild female (if complete sterility, this value is 0). Using known values of $Hn, Hc,$ and $Hs,$ and the ratio of wild to sterile insects (N/S) in the field, the competitiveness c of the sterile insects can be calculated. The value of c will normally fluctuate between 0 and 1 (Itô and Koyama 1982, Iwahashi et al. 1983).

The importance of regularly monitoring the sexual competitiveness in the field was amply demonstrated by the significant discrepancies observed between the sexual competitiveness of sterile melon flies *Bactrocera cucurbitae* (Coquillett)

measured in a rearing facility and in the field (Kume Island, Japan) (Fig. 1). When the release programme began, mating competitiveness measured in the field was high (0.8), and the c value was comparable with that obtained in laboratory cage tests. However, after only 18 generations of continuous mass-rearing, the field value of c dropped to 0.2, whereas the laboratory value remained high. This difference was attributed to the inferior mating performance of wild males in small laboratory cages, and a real decline in the field competitiveness of sterile males due to rearing-induced genetic changes, i.e. domestication, and the development of SIT resistance in the wild population (Iwahashi et al. 1983; Lance and McInnis, this volume; Whitten and Mahon, this volume). As a result of monitoring the field c value, programme managers were alerted, took corrective action in a timely manner, and the melon fly was eradicated in Kume Island.
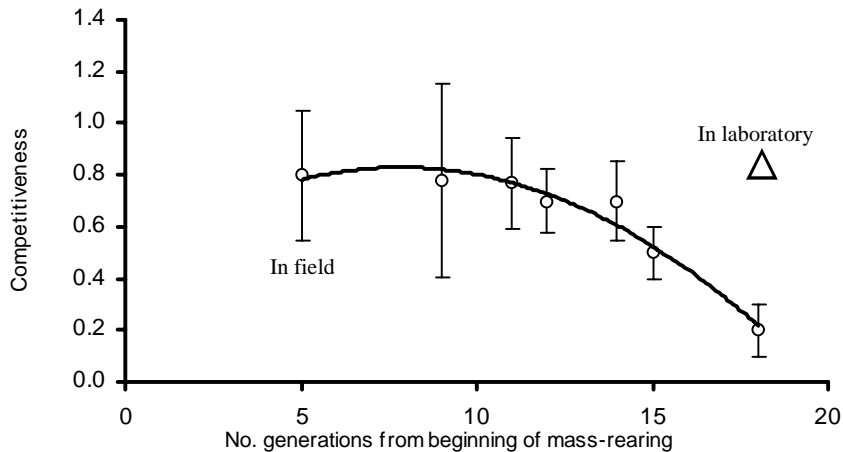
Figure 1. Trend in sexual competitiveness of sterile melon flies measured in the field (circles) and laboratory (triangle). (Figure adapted from Iwahashi et al. 1983.)

An interesting trend in the field competitiveness of released insects, in relation to the operational size of an AW-IPM programme, is provided by the New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programme in the USA and Central America. In the first decade of the programme in the USA (Curaçao, Florida, Texas 1954–1962) (Klassen and Curtis, this volume), less than 50 million sterile insects per week were released at low densities, and competitiveness was 0.29–0.43. However, when the programme progressed to more tropical regions, and production increased to 500 million sterile insects per week, the competitiveness dropped to below 0.1 (Mayer et al. 1998).

Fried's model indicates that the sexual competitiveness of released insects is inherently linked to the sterile to wild insect ratio obtained in the field. Calculations

of critical sterile to wild ratios for any target insect (Barclay, this volume) are usually based on experience or the results of mathematical models, and may vary considerably among insect species, and between theory (models) and practice (operational programmes). Critical sterile to wild ratios varied between 7:1 for the tsetse fly *Glossina palpalis gambiensis* Vanderplank (Politzar and Cuisance 1984), 25:1 for the olive fruit fly *Bactrocera oleae* (Gmelin) (Tzanakakis 1974), 40:1 for the codling moth *Cydia pomonella* (L.) (Dyck et al. 1993), 60:1 for the pink bollworm *Pectinophora gossypiella* (Saunders) (Staten et al. 1993), 80:1 for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Villaseñor et al. 2000), and 25–100:1 for the New World screwworm (J. W. Snow, personal communication). Operational sterile to wild ratios can even be different for species and subspecies within a genus, e.g. a sterile to wild male ratio of 7–10:1, greater than 10:1, and greater than 30:1, was applied against *G. palpalis gambiensis* in Burkina Faso, *Glossina palpalis palpalis* Robineau-Desvoidy in Nigeria, and *Glossina austeni* Newstead in Unguja Island, Zanzibar, respectively (Politzar and Cuisance 1984, Oladunmade et al. 1990, Vreysen et al. 2000). Most likely these differences are related to distinct ecological affinities of the target insect, their mobility, spatial occupation of the habitat, population regulation, sterile male quality, etc.

Even though the critical ratio of sterile to wild males for a target insect can be influenced by factors such as the reproductive potential of the females, climatic conditions, biological quality of the insects before leaving the rearing facility, funds available to disperse sterile insects, and time available to achieve eradication, it is the true density of the wild insect population that is really important. The number of wild insects per unit of habitat surface determines the release rate of sterile insects that is required to achieve the desired sterile to wild ratio in the target area. In turn, the production capacity of the rearing facility must be adequate for the chosen release rate. Underestimating the actual population density can result in a shortage of available sterile insects in the production facility, whereas overestimating it will lead to overproduction and unnecessarily increased costs (Bloem and Bloem 2000). The density of insect populations is usually strongly correlated with habitat and vegetation cover, e.g. melon fly density in Okinawa varied from less than 10 flies per hectare in the mountains to greater than 600 flies per hectare in crop fields and bushy areas (Yamagishi et al. 1993). Therefore, the density in all vegetation types in the target area should be estimated so as to adjust accordingly the number of sterile males released. It is important that the critical sterile to wild ratio be obtained in all trap sites, indicating that sterile insects have been appropriately placed in the target area (Krafsur et al. 1980). Several simple mathematical models are available to assess absolute insect population densities, e.g. Lincoln Index (Southwood 1978), and Jackson's positive and negative method (Jackson 1939), but these methods have rarely been routinely used in operational programmes integrating the SIT (Itô and Yamamura, this volume).

The patchy distribution of most insect species (Itô and Yamamura, this volume; Lance and McInnis, this volume), and the strong correlation between insect density and vegetation type, has implications for interpreting "overflooding ratios" when releasing sterile insects, i.e. one should take into account not only the overall sterile

to wild male ratio obtained in all traps in a given area over a period of time, but also the ratios achieved in each type of habitat and vegetation cover must be adequate. Therefore a suitable network of traps, strategically deployed in all habitats, is needed (Vreysen et al. 2000).

To calculate sterile to wild ratios, sterile insects are usually marked before release (Parker, this volume). However, in programmes releasing sterile New World screwworms, the flies are not marked, and estimations of the ratio of sterile to wild insects are based on catches of female insects. Females are dissected, and the atrophied ovaries of sterile females distinguish them from wild females. However, caution is required in interpreting these data. Sterile to wild ratios are derived from trapped insects, and if the distribution and response to traps is different for sterile and wild male insects, the ratios are prone to error (Meats 1996). One must also be cautious in interpreting female screwworm ratios; they might not reflect actual male screwworm ratios in the field due to sex-related differences in longevity, response to the trapping device, and dispersal characteristics.

2.2. Field Monitoring of Parameters Related to Sexual Competitiveness

2.2.1. Apparent Density and Survival

In routine monitoring, the most immediately available parameter is the proportion of sterile insects recaptured within a certain time frame, i.e. the recapture rate, and a sudden decrease in this rate could reflect a change in the quality of the released insect or in distribution methods (Hutt 1979, Yamagishi et al. 1993).

To increase their chances of encountering a receptive virgin female, sterile males need to have as long as possible an active life in the field (Curtis and Langley 1982). It is important to estimate this parameter, and investigate methods to increase the survival (longevity) of released sterile males (Calkins and Parker, this volume; Lance and McInnis, this volume). After leaving a rearing facility, released sterile insects must find a food source or a host to replenish their limited energy reserves. In the absence of a host, their life expectancy is determined by the available initial energy reserves. Therefore, it is a critical parameter, and can be assessed in the laboratory (FAO 1992) or in field cages under natural conditions (Vreysen 1995).

An exponential decline in the recapture rate of marked released insects gives an indication of the daily survival (or mortality) rate in the area (Fig. 2). The survival rates will determine the frequency of sterile insect releases, ensuring appropriate sterile male densities over the entire target area at all times (Dowell et al., this volume). Insect survival is influenced by factors such as host availability, climatic conditions, environmental stress, and vegetation cover, and therefore the survival of released sterile insects should be assessed in all seasons and in all representative areas of the target zone.

2.2.2. Mobility and Dispersal

Released sterile insects need to be sufficiently mobile, and have adequate dispersal capabilities, to reach (in a timely fashion) all the ecological niches occupied by the

wild insect population (Itô and Yamamura, this volume; Lance and McInnis, this volume). Mobility and the potential of released insects to disperse are often underestimated. The recorded maximum dispersal distance for released New World screwworms is 290 km, 107 km for melon flies, 48 km for oriental fruit flies *Bactrocera dorsalis* Hendel, and 18.5 km for the tsetse fly *G. p. gambiensis* (Hightower et al. 1965, Proverbs 1974, Van der Vloedt et al. 1980). Lance and McInnis (this volume) discuss the implications of this high mobility for programmes using the SIT.
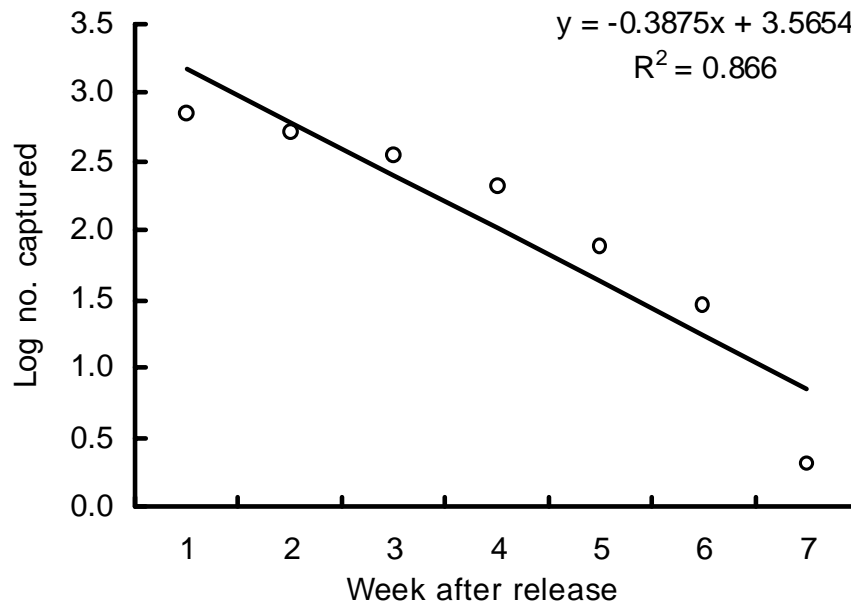
Figure 2. Survival of sterile male Glossina austeni *aerially released in Unguja Island, Zanzibar, Tanzania.*

Knowledge about the mobility and dispersal characteristics of released insects is essential for developing and designing appropriate release strategies. Admittedly this is complicated; there are many variables that influence the dispersal of insects, e.g. wind direction (Peterson et al. 1980), wind velocity (Parman 1945), vegetation density (Krafsur and Hightower 1979), relative humidity (Brenner 1984), host availability (Spradbery 1994), radiation dose (Cuisance and Itard 1973, Wong et al. 1982), and the release of only males or both sexes (Proverbs et al. 1973, Hendrichs et al. 1995). A release strategy should aim at deploying sterile insects in close proximity to wild virgin females. Release lanes for aerial distribution, or release points for ground release, should be separated by a distance not exceeding the

average maximum dispersal distance of the released insect (Dowell et al., this volume). Evidence from New World screwworm programmes suggests that the efficiency of released males, as measured by their mating frequency, is strongly influenced by operational considerations of sterile fly distribution. Distance between parallel flight lanes, and fly density in release containers, are especially critical variables (Krafsur et al. 1980). The sterility of wild populations increased significantly if flies were distributed in small boxes of 400 flies in release swaths of 2 km instead of in larger boxes of 2000 flies in swaths of 8 km (Krafsur and Garcia 1978). Obviously a denser network of aerial release lanes will require more aircraft time and be more expensive, but the programme will be more efficient, and releases of sterile insects would possibly be needed for a shorter time period.

The mobility and dispersal capacity of released sterile insects should be monitored frequently, and compared with those of wild insects. This will require the capture, marking and release-recapture of large numbers of wild insects, and unfortunately these are not always available. Although in the past several complex mathematical models have been developed that describe the movement and mobility of insect species (Williams et al. 1992), a simple but useful index is the "mean distance of dispersal" from a release site to a trap (Itô and Koyama 1982, FAO/IAEA/USDA 2003):

$$x = \frac{\sum_{j=1}^{n} x_j N_j}{\sum_{j=1}^{n} N_j} \qquad (2)$$

where n = number of traps, x_j is the distance between a release point and the j-th trap, and N_j is the number of flies recaptured with the j-th trap.

The mean distance of dispersal can easily be measured by deploying traps along a regular grid of at least 6 x 6 traps, the dimensions of the grid depending on the insect species. Grids are usually easier to set up than a series of concentric circles, but circles are better for measuring dispersal. Caution is required in selecting the sampling device, and especially in using traps in combination with powerful attractants (e.g. pheromone traps for Lepidoptera), that could mask the natural dispersal characteristics of the insect. For lepidopteran species, passive, non-attractant interception traps are an alternative to male pheromone traps; they have been used to determine natural flight paths and flight patterns of insects in search of mates or hosts. The disadvantage of this technique is that catches are usually very small, making statistical analysis difficult or impossible (Knight 2000).

2.2.3. Dispersion (Spatial Distribution within Habitat)

Released sterile insects must also disperse into the ecological niches occupied by wild insects, and detailed data, on temporal changes in spatial distribution of sterile and wild insects are needed (Lance and McInnis, this volume). This is very challenging; most

insects are not uniformly or randomly distributed, but have patchy distributions in both space and time. Spatial heterogeneity is related mostly to host availability and vegetation, making it difficult but not impossible to determine the optimal release rate (number of flies released per km^2) (Krafsur et al. 1979). An adequate trapping network, covering all types of vegetation, is needed to provide frequent (weekly) detailed information on the density and spatial distribution of wild and released insects. Geographic information systems (GIS) and remote sensing (RS) tools can greatly facilitate the selection of these trapping sites, ensuring adequate coverage but also avoiding the deployment of too many sampling devices (Cox and Vreysen, this volume). Data on temporal and spatial changes in occupation of the habitat, by both sterile and wild insects, can be used to regularly adapt the scheme for distributing sterile insects.

On a finer spatial scale, the dispersion of insects such as tsetse flies, e.g. *G. palpalis palpalis*, is influenced by sex, age (young virgin females occupy a different sector than older flies), and the state of gravidity in females (Gouteux 1987). These microspatial differences in an apparently similar habitat should be monitored carefully in important target areas, such as "hot spots" (areas with unusually high insect densities). An example is the spatial occupation of wild and sterile *G. austeni* in an apparently uniform primary forest ecosystem (Fig. 3). Data from the 12 trapping sites indicate that sterile male tsetse flies, in spite of uniform aerial release rates over the forest, redistributed themselves and occupied microhabitat niches similar to those occupied by wild insects (M. J. B. Vreysen, unpublished data).
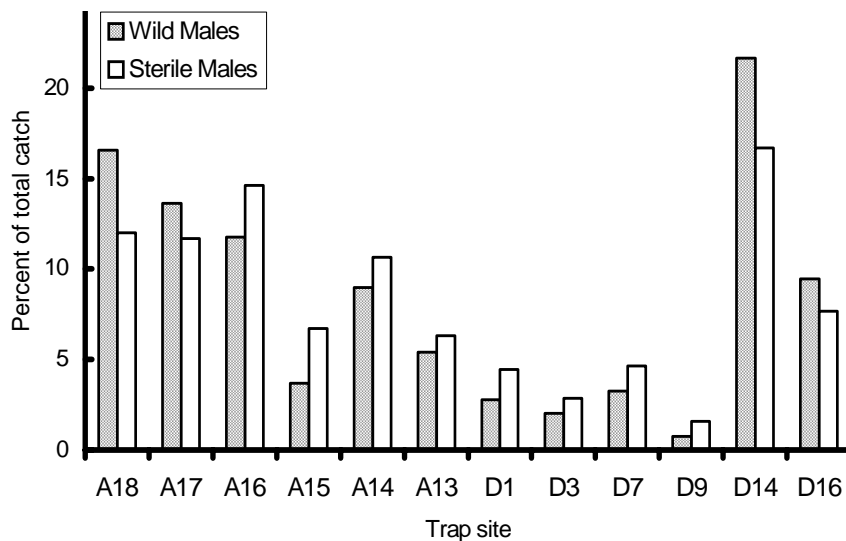
Figure 3. Frequency distribution of sterile and wild Glossina austeni *male fly catches in 12 sampling sites in a primary forest (Unguja Island, Zanzibar) from week 40, 1994, to week 25, 1995 (wild males n = 646, sterile males n = 2549).*

3. MONITORING PROGRAMME PROGRESS

3.1. Monitoring and Insect Biology

3.1.1. Monitoring and Insect Behaviour

The feasibility of developing and deploying efficient insect monitoring tools will be determined and influenced by the behaviour and the biology of the insect. Species with adults that respond to a trapping device may be sampled directly in sufficient numbers to accurately assess fluctuations in population density and structure. The biological mechanisms involved in attracting insects from a distance, and luring them into a trapping device, are usually related to their host-, food-, or mate-seeking behaviour, which is often regulated by volatile components (semiochemicals) emitted by the host/food/mate, and at close range influenced by visual characteristics (Colvin and Gibson 1993, Tan 1993, Green 1994, Hall and Wall 1995). Sometimes direct trapping of insects is difficult (e.g. in inaccessible areas), inefficient (e.g. a good trap is not available), or uneconomic, and therefore alternative indirect methods of monitoring have been developed, usually involving assessments of damage inflicted by the pest on its hosts (Iwahashi 1977, FAO 1992, Bloem and Bloem 2000, Dyck et al. 2000).

3.1.2. Direct Sampling of Adult Insects

In AW-IPM programmes, it is important to be able to monitor both high and low population densities. The densities of pests such as screwworms tend to be low but highly aggregated (Krafsur et al. 1979, Spradbery 1994), and the most efficient and appropriate sampling device should be used (Katsoyannos 1994) for the relevant geographical area (Baylis and Nambiro 1993). However, most trapping systems are biased, and samples are rarely representative of the insect population (Vale and Phelps 1978, Vreysen and Saleh 2001). To correctly interpret trapping data, it is imperative to understand these "trap biases" and the factors that affect the size and structure of trap samples over time and space. Some of the most significant factors that affect insect population samples are: (1) activity of the insects, which depends on the insect's physiological state and on climate (Rogers 1978, Turner 1987, Williams et al. 1990b), (2) efficiency of the trap, which is influenced by the elements of trap construction, habitat, and climate (Hargrove and Vale 1980, Dransfield et al. 1982), and (3) intrinsic trap biases (Williams et al. 1990a).

In addition, when selecting a trapping device for a monitoring programme, the following aspects have to be taken into account: performance of the sampling device in relation to economics (number of traps needed is inversely correlated with trap efficiency), servicing, time required to deploy a trap, unit cost and its components, durability, "user friendliness" (i.e. time required to remove trapped insects), efficiency of the bait (very good baits may overestimate the local population density), and species specificity. Trapping large numbers of non-target organisms is inefficient; e.g. biting flies (Stomoxyinae) caught in tsetse fly traps (more than 1000 individuals per trap per day) (Saleh et al. 1999), species of *Chrysomya*, morphologically similar to

New World screwworms, caught in traps at a ratio of 2600:1 (Spradbery 1994), and a wide range of tephritids and non-tephritids in fruit fly traps baited with food lures (Katsoyannos 1994, Miranda et al. 2001).

Trapping devices deployed in the target area will, of course, contribute to suppression of the target pest population. This becomes important in those programmes that have an extensive trapping network or very efficient traps.

3.1.3. Indirect Sampling

Monitoring a population through direct sampling of adult insects can, in many instances, be supplemented by indirect sampling procedures, both to obtain additional information on the progress of the programme and to verify the data obtained by direct sampling procedures. Indirect sampling is used routinely in programmes against veterinary pests (screwworm and tsetse) and crop pests (Lepidoptera and fruit flies).

Monitoring Host Organisms. In many holometabolous insects, immature stages represent a large percentage of the population (up to 97% for the Mediterranean fruit fly (Carey 1982, Liedo and Carey 1996)), and in view of this demography these stages should also be sampled.

Since it is easy to detect maggots in animal wounds, indirect sampling through the surveillance of myiasis cases in livestock, game animals, and humans has become the standard method of monitoring progress in New World screwworm eradication programmes. Depending on resources, surveillance can be done either passively (livestock owners check their animals and report positive myiasis cases (Robinson et al. 2000)) or actively (programme staff physically inspect at regular intervals all host animals in the target zone (FAO 1992)). Even though passive surveillance is obviously less expensive, the absence of standardization and a "reference-sampling unit" make temporal comparison of such field data very difficult or even impossible. Also, passive surveillance is influenced by: (1) the accessibility that farmers have to their livestock, (2) the willingness of farmers to inspect their animals on a regular basis (and remove screwworm larvae and send them to the responsible authority in the country), (3) the vastness of the grazing area, and (4) the efficiency of veterinary services. Therefore a decrease in "reported cases" does not necessarily reflect a lower population density of screwworms (i.e. progress in the programme), but is probably correlated more with the reporting efficiency and level of farmer cooperation. The highly successful New World screwworm eradication programme in Libya (1990–1992) provides a good example of an efficiently executed active surveillance programme; 94 field teams inspected 16.2 and 30.5 million animals in 1990 and 1991, respectively, in an area of 40 000 km^2 (FAO 1992). Accurate reporting of the number of animals inspected, wounds detected, and wounds infested, provided excellent feedback to programme managers to evaluate the progress (Lindquist et al. 1992).

Monitoring Disease Transmission. Tsetse flies (Glossinidae) are vectors of *Trypanosoma* spp., and insect-trap data can be supplemented with data on the transmission, prevalence, and incidence of the disease in livestock (Barclay et al., this volume). These data become especially valuable if and when the density of the tsetse fly population drops below the detectable limit of the trapping device used. The careful screening of sentinel animals, i.e. not infected with trypanosomes, introduced into the target area can significantly increase confidence that the tsetse fly has been eradicated (Dyck et al. 2000). However interpreting these veterinary surveillance data is complex, and preferably should always be correlated with entomological monitoring data because:

- The density of a tsetse population and the incidence of the disease are not necessary positively correlated, e.g. an insecticide spraying campaign in Kenya reduced the tsetse population by 98%, whereas 6 months after completion of the campaign the disease prevalence was reduced only from 5 to 2% (Otieno et al. 1990).

- The time required for a fly to develop a mature trypanosome infection is between 5 and 25 days, depending on the species and the temperature (Molyneux et al. 1982). Consequently, the potential for a tsetse population to transmit the disease is increased proportionally to its average age (Harley 1965), and the removal of the younger section of the fly population as the result of control actions does not significantly reduce its transmission capability.

- A parasitological survey will not show transmission if a tsetse population is thriving on livestock free of trypanosomes.

Monitoring Crop Damage. The damage that insects inflict to crops can be assessed, providing indirect information on the density and distribution of the pest insect population. Crop damage can be measured at regular intervals, e.g. 80–100 cotton bolls per field are collected each week in the pink bollworm programme in California, USA (Staten et al. 1993). Also crop damage can be assessed at harvest, e.g. in the codling moth programme in Canada, a random examination of fruit is made in about one-third of the treated orchards (n = 600) at harvest (Bloem and Bloem 2000). Caution is required in interpreting the data, because damage to a specific crop is not always caused by the target insect, e.g. all insect damage in fruit orchards in Washington State, USA, was attributed to the codling moth, but careful study revealed that fruit injury from codling moth larvae was only 0.3% while that from leaf rollers averaged 1.1% (Calkins et al. 2000). In some instances, the results of direct monitoring (insect trapping) do not correlate with data from indirect sampling (crop damage), e.g. there is no spatial correlation between damage, i.e. defoliation, from gypsy moths *Lymantria dispar* (L.) and the counts of adult male moths in traps (Liebhold et al. 1995).

3.2. *Monitoring Impact of Sterile Insect Releases on Wild Population*

Three important indicators provide essential information on the impact of released insects on the target population: (1) proportion of the female target population that mated with sterile males (level of induced sterility), (2) changes in the age structure of the target population caused by variations in the recruitment rate of young insects, and (3) decline in the density of the target population. The characteristics of the reproductive biology of the species, and the trap-orientation behaviour of females, will determine which of these parameters can be used. For example, all three parameters can be monitored in tsetse fly programmes integrating the SIT, but in Lepidoptera, pheromone traps attract only male moths, and thus the information obtained is restricted to the apparent densities of wild and sterile males.

Analysing and interpreting temporal and spatial monitoring data can be accurate only if standardized sampling procedures are used. Especially in the case of direct sampling, uniformity of procedures is required for the entire duration of the monitoring programme, particularly with respect to: (1) trapping device (type, colour, material used for trap construction, shape, etc.), (2) sampling sites (number and location), (3) trap mounting and deployment, (4) lures or odour attractants (chemical composition, pH, volatile release rates, etc.), and (5) sampling interval. In addition, data (taken before control actions are initiated) on spatial and temporal fluctuations in the density and structure of the target population (Itô and Yamamura, this volume), collected over a period of at least 1 year, are essential for making correct interpretations of the field data collected during the suppression and sterile insect release phases (Vreysen and Khamis 1999, Vreysen et al. 1999a, De Longo et al. 2000) (Box 1). An alternative is to collect comparable data from an untreated area during the phase when control actions are applied, but it is often difficult to select ecologically comparable areas (Waterhouse et al. 1976).

Quantifying a reduction in the reproductive potential of a target population constitutes the most powerful and straightforward tool to assess progress in a programme using the SIT (Waterhouse et al. 1976, Vreysen 2001). An accurate knowledge of the level and spatial distribution of sterility induced in wild females will permit a more strategic use of the sterile insects, resulting in increased programme efficacy and reduced programme costs. Assessing the rate of induced sterility in a wild target population is only possible when the female portion of the population can be sampled, and when morphological indicators or "visual markers" are present in the female reproductive system to differentiate between matings with wild or sterile males. Alternatively, female insects can be trapped live and then maintained in controlled conditions for a certain time period to monitor the production of viable offspring (result of a fertile mating) or non-viable eggs (result of a sterile mating). In some insects, egg masses can be collected in the field and the level of sterility determined (section 3.2.2.).

Box 1. Pre-Control Entomological Baseline Data: Prerequisite for Correct Interpretation of Trap Catch Data During Control Activities

The application of a control measure against an insect population will change the size of that population (section 3.2.4.) and its age structure (section 3.2.3.). Even when not subjected to a control measure, insect populations (in terms of both size and structure) are not stable in time and space. Consequently pre-control data on the structure of the target population must be collected to correctly interpret sampling data obtained during the control activities.

Physiological age grading is feasible for tsetse flies due to the uniqueness of the female reproductive system (section 3.2.1.). The example below demonstrates the spatial differences in the age structure of the tsetse fly *Glossina pallidipes* Austen sampled in riparian forest vegetation along the Kulfo River and in the Chamo bush thickets of the Nechisar National Park in Ethiopia. The flies were sampled in both habitats during the same period of same day. The data show that the female fly population along the Kulfo River was significantly younger (18.8% young females, tenerals and nulliparous) than the female fly population in the Chamo thickets (6.4% young females) ($\chi^2 = 10.80$, df = 4, $P < 0.03$ — females in the older categories 4 and >4 were pooled in this analysis). A change in fly population structure due to applied control measures must be analysed in relation to these natural spatial (and also temporal) differences.
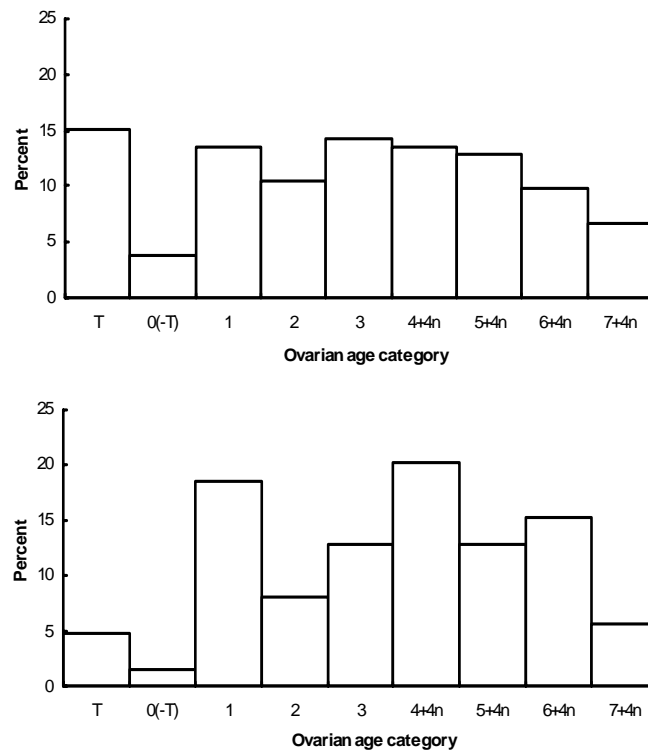
Population structure of G. pallidipes *sampled in (upper graph) riparian forest along the Kulfo River (n = 133) and (lower graph) bush thickets (n = 124) of the Nechisar Park in Ethiopia (T = teneral, 0 = nulliparous, 1–7 = number of ovulations; note that categories i = 4–7 contain flies that have ovulated i + 4n times; more details in Challier 1965).*

The rate of induced sterility is not only the most essential, but also the most reliable, parameter to assess progress in programmes that release sterile insects. Although a reduction in the number of insects caught in traps can be an important indicator, the number trapped is strongly affected by numerous (often unknown) factors (section 3.1.2.), making the interpretation of trap catches complex (section 3.2.4.). A progressive increase in the sterility of the target population, combined with declining numbers of insects trapped, will provide unequivocal evidence that the eventual collapse of a target insect population is due solely to the loss of fertility, without interference of other factors (Vreysen et al. 2000).

3.2.1. Monitoring Reproductive Capacity of Wild Population — Tsetse Flies

Tsetse flies have a unique reproductive system, making them very suited to the assessment of sterility levels in a population subjected to sterile insect releases (Van der Vloedt et al. 1978, Vreysen et al. 1996). Tsetse flies reproduce by adenotrophic viviparity (Hagan 1951). The four polytrophic ovarioles in the reproductive system of females develop sequentially (Saunders 1960) (Fig. 4), with only one oocyte maturing per pregnancy cycle lasting 9 or 10 days (Tobe and Langley 1978). Consequently, the maturation stage of the developing oocyte (the next to ovulate) in fertile females is always in sequence with a particular development stage in utero, i.e. embryogenesis or one of the three larval stages (Challier 1965). Mating a virgin female tsetse with a sterile male will result in fertilization of the egg in utero by the sperm, carrying dominant lethal mutations that will result in the death of the embryo (embryonic arrest) (LaChance et al. 1967), which is later aborted (Van der Vloedt et al. 1978). Consequently, aberrations between the size of the maturing follicle and the development stage in utero (dead embryo or uterus empty due to expulsion of the embryo) will become apparent (Vreysen et al. 1996) (Fig. 4B).

Dissection of a reasonably sized sample of wild female flies from a population subjected to sterile male releases will show the proportion of females in the sample that have aberrations in their reproductive system — a direct indication of the rate of induced sterility in the target population (Vreysen et al. 2000). The only weakness in the methodology is the 1- or 2-day time lag between fertilization of the egg with the irradiated sperm and embryonic arrest becoming visible with a microscope (Van der Vloedt and Barnor 1984). This could result in underestimating the level of induced sterility.

Pre-control data from female tsetse flies on Unguja Island, Zanzibar, showed that 50 and 46.1% of females had a viable egg or larva in utero, respectively, and only 3.5% of females had an empty uterus showing the loss of an egg or larva (natural abortion rate) (Vreysen and Khamis 1999) (Fig. 5). During the initial 8 months of the SIT activities (mid 1994–early 1995), an insufficient number of sterile males was released, and consequently the ratio of sterile to wild males remained below 10:1. In spite of this low ratio, during this period 19.9% of sampled females had mated with sterile males (Vreysen et al. 2000). In 1995, the number of sterile males released constantly increased, and more than 50 sterile males for each wild male were trapped after week 34. Simultaneously, the frequency distribution of the uterus content of the sampled females became progressively more distorted compared with the pre-control
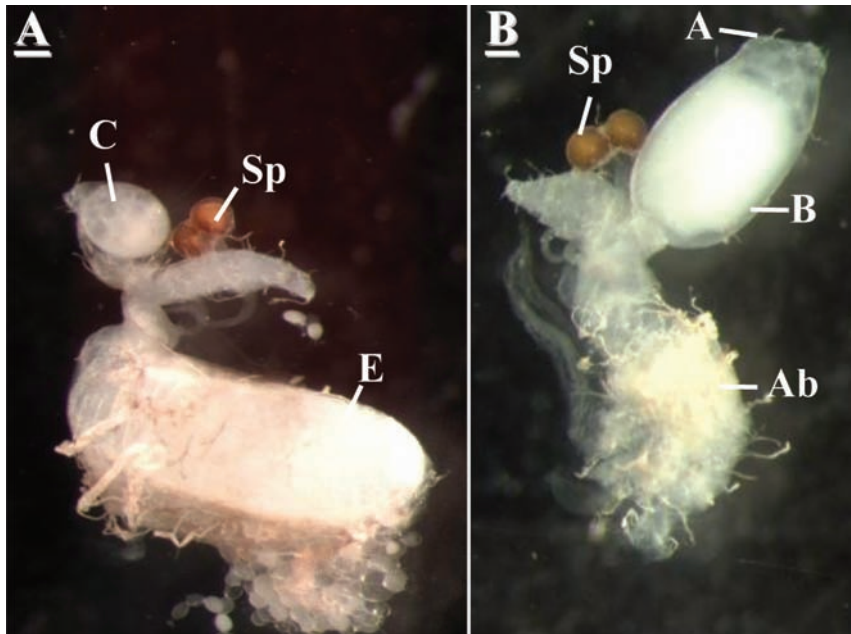
Figure 4. A. Reproductive system of female tsetse fly mated with fertile male, showing one ovulation (C = follicle next in ovulation sequence (FNOS)) and viable egg (E) in utero. B. Reproductive system of female mated with sterile male, evidenced by imbalance between size of FNOS (B) and uterus content (Ab = abortion, Sp = spermathecae).

distribution, i.e. $\chi^2 = 70.3$ in early 1995 and $\chi^2 = 196.6$ in late 1995 (df = 4; $P < 0.0001$), due to a gradual increase in the proportion of females that had aborted dead embryos or displayed eggs *in utero* in embryonic arrest (Vreysen et al. 2000, Vreysen 2001).

A similar trend can be observed in Fig. 6, which shows data on the reproductive status of young parous females (1 or 2 ovulations) that mated with a sterilized male 2 or 3 weeks previously. It is very evident that the sterility level in the young female population gradually increased as the sterile to wild male ratio increased, i.e. from 26% in the last quarter of 1994 to 32, 48, and 72% in the 2nd, 3rd, and 4th quarters of 1995, respectively. Concurrently, as sterility in the young female fly population increased, the proportion of young female flies with a viable larva *in utero* (females mated with a wild fertile male) decreased.

3.2.2. Monitoring Reproductive Capacity of Wild Population — Screwworms, Lepidoptera, and Fruit Flies

Assessing the rate of induced sterility in screwworms, Lepidoptera, and fruit flies is more challenging. No differentiation between sterile and fertile matings can be made
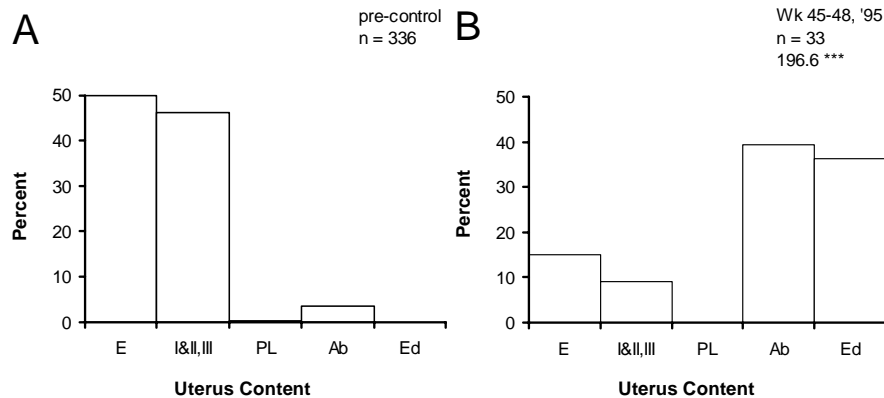
Figure 5. Frequency distribution of uterus content of Glossina austeni *females sampled during (A) pre-control phase and (B) late in sterile male release phase (E = egg, I, II, III = first-, second-, and third-instar larva, PL = post larviposition, Ab = abortion, Ed = degenerating egg). Number is chi-square value of comparison of frequency distribution of uterus content with that of pre-control sample (*** P < 0.001). (Figure adapted from Vreysen 2001.)*

by direct examination of the reproductive system of sampled wild females. Any quantification of sterility levels in wild females requires the collection and maintenance of eggs, and an assessment of the ratio of hatched (fertile) to non-viable (sterile) eggs (Thomas and Mangan 1989). For screwworm and lepidopteran species, special egg collecting methods have been developed, but most are labour-intensive and expensive to implement in large operational programmes. The usefulness and applicability of these methods vary for each insect group.

Induced Sterility and Egg Collection. In female screwworm flies, with 100–150 ovarioles per ovary, egg development is synchronous, and an average of 200 eggs are deposited (Thomas 1993), regardless if eggs are fertilized by fertile or sterile sperm (LaChance and Bruns 1963). Egg masses can be collected in several ways: (1) from artificially inflicted wounds on sentinel animals, maintained in fixed capture stations (Davis et al. 1968, Parker and Welch 1991, FAO 1992), (2) from wounds found by field inspectors during routine surveillance activities (Robinson et al. 2000), and (3) from gravid females that were attracted to, and caught in, liver-bait stations and "egged" in glass tubes (Parker and Welch 1991). Maintaining sentinel pens is cumbersome, and rather problematic in tropical remote environments, because of the paucity of suitable animals, water, shade, and animal food (Krafsur and Highthower 1979, Parker and Welch 1991). (It would be convenient if some kind of artificial wound were available to collect eggs from wild screwworm females; such a methodology needs to be developed.)
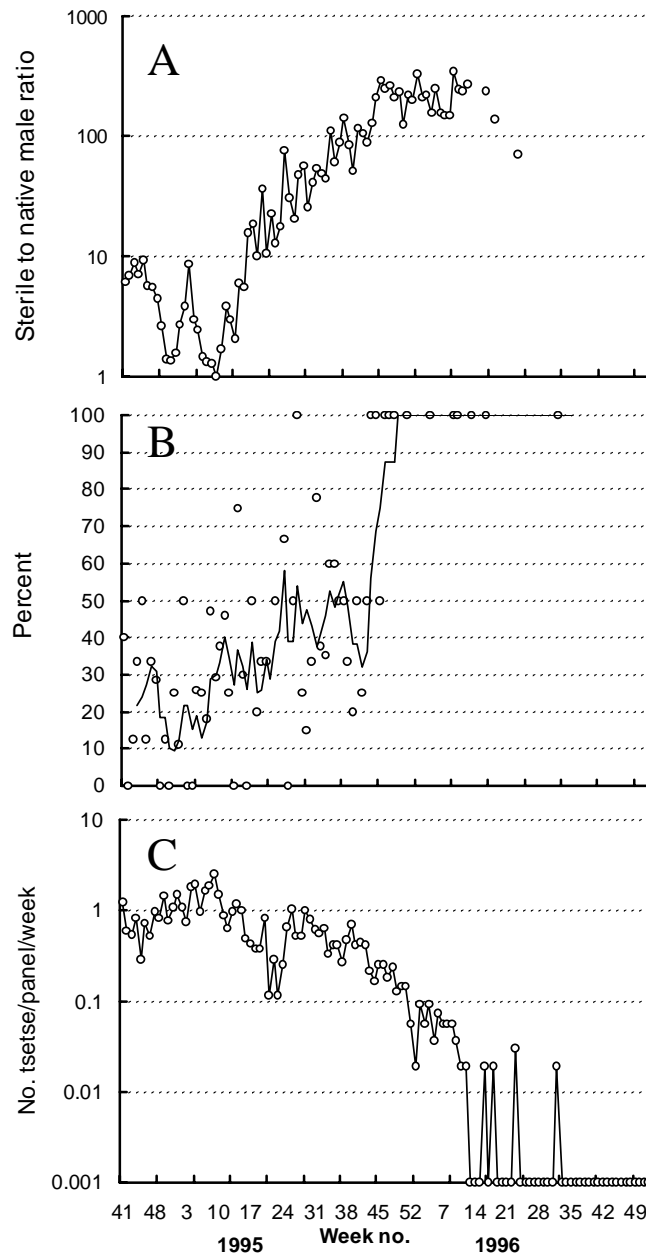
Figure 6. A: Sterile to wild male ratio. B: Rate of induced sterility as proportion of young parous females (1 or 2 ovulations). C: Apparent density (+ 0.001) of wild males and females. Glossina austeni, *Unguja Island, Zanzibar. (Figure adapted from Vreysen et al. 2000.)*

Training and expertise are needed to detect and identify screwworm fly eggs. Also they are short-lived, and inappropriate handling often causes increased mortality, resulting in overestimates of the rate of induced sterility. In addition, one egg mass is not necessarily the output of one female, as was originally assumed (Krafsur et al. 1979, Brenner 1984), but possibly the product of more than one female (eggs laid beside eggs already deposited by another fly) (Thomas and Mangan 1989), making it difficult to interpret sterility data. Nevertheless, the most manageable option at present is the systematic collection of egg masses from animal wounds during routine surveillance along a predetermined grid network, and adequate geo-referencing of the sampling sites for incorporation into GIS (Cox and Vreysen, this volume), providing accurate information on the spatial and temporal distribution of induced sterility in the wild population.

The development of female lures has recently brought about significant progress in monitoring the Mediterranean fruit fly (IAEA 1999) (Box 2). However, as with screwworms and Lepidoptera, dissection of female fruit flies does not reveal any difference between fertile and sterile matings. Therefore, live-trapped female Mediterranean fruit flies can be transferred to a cage containing a natural or artificial oviposition substrate, egg masses collected, and egg hatchability assessed (IAEA 1999, Katsoyannos et al. 1999). Except for experimental trials, methods such as maintaining fruit fly eggs dissected from collected fruits to assess hatchability (Wong et al. 1986, McInnis et al. 1994), or measuring the size of the head of spermatozoids

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Box 2. Monitoring Mediterranean Fruit Fly AW-IPM Programmes that Integrate SIT

Sampling the male portion of the population provides important feedback on sterile to wild male ratios, and on dispersal characteristics of released sterile males. Sampling females is equally vital; they constitute the "reproductive component" of the wild population, are the target of the SIT activities, and provide crucial information on the rate of sterility induced in the population (section 3.2.1.).

In earlier Mediterranean fruit fly programmes applying the SIT, both sexes were released, and population monitoring was done mainly with trimedlure-baited Jackson traps, which attract mostly male flies. This resulted in a reduction in efficiency of the programmes due to the trapping-out of a significant portion of the sterile males. For example, in the Mediterranean fruit fly programme in the Los Angeles Basin, California, USA, about 0.5 million males are captured each week, resulting in a very costly and inexact process attempting to find very few wild males among all the recaptured sterile males (J. Hendrichs, personal communication). The female portion of the wild population is sampled with traps containing food lures, which unfortunately also catch many non-target species, making it time-consuming and laborious to sort out the female Mediterranean fruit flies.

The development of genetic sexing strains (Franz, this volume), the subsequent use of male-only releases in operational programmes (Rendón et al. 2004), and the development of a lure for female Mediterranean fruit flies (IAEA 1999), have given a new dimension to monitoring SIT activities. Female attractant-baited traps focus mainly on the detection of wild females and their offspring (no sterile females are released), thereby significantly reducing the laborious trapping and the sorting of hundreds of thousands of males (Hendrichs et al. 1995). In addition, these traps collect enough males to monitor the ratio of sterile to wild males and the distribution of sterile male releases (Midgarden et al. 2004). The monitoring component of Mediterranean fruit fly programmes can account for up to one-third the total cost during the fly-free stage of AW-IPM programmes (Enkerlin 2001; Hendrichs et al., this volume). The release of male-only strains, combined with the availability of female attractant traps, increase the efficiency of sterile males, and significantly reduce monitoring costs.

collected from spermathecae of female flies to differentiate between sterile and fertile sperm (McInnis 1993), have never been applied because they are cumbersome. Nevertheless, eggs from wild Mediterranean fruit flies were collected from coffee berries in Guatemala and induced sterility calculated (Rendón et al. 2004). Also, an artificial oviposition device, to collect eggs from female melon flies netted in the field, was developed and used successfully to assess egg hatchability during the programme on Kume Island, Japan (Iwahashi et al. 1976, Iwahashi 1977).

Problems Associated with Trapping Female Lepidoptera. Monitoring progress in lepidopteran programmes relies mainly on crop damage assessments and on traps using very potent female sex pheromones as lures (Riedl et al. 1986, Bloem and Bloem 2000, Walters et al. 2000). The pheromone traps attract only male moths, and sterile moths can be distinguished from wild moths if appropriate marking techniques are used (Dyck et al. 1993; Parker, this volume) (section 2.1.). Therefore trap samples will indicate the apparent densities and ratio of wild and sterile moths, but the absence of females in the samples precludes any data on sterility levels induced in the wild population. In addition, the deployment of pheromone traps has to be well balanced to prevent the trapping of too many sterile male moths, which reduces the efficiency of the SIT component of the programme.

Several methods have been developed to assess sterility in moth populations using tethered (Alford and Silk 1983) or clipped-wing sentinel/decoy virgin females placed on mating tables (Snow et al. 1976, Shaver and Brown 1993), in virgin-female traps (Snow et al. 1969), or in mating houses (Mastro and Schwalbe 1980). These female moths will attract male moths. It is assumed that the virgin females mate with sterile and wild male moths at the same periodicity and frequency as occurs in nature. Mated females are then transferred to controlled conditions, where deposited eggs are screened for sterility. The "tethering" or "clipped-wing" method is, however, hampered by the small size of many lepidopteran species, and by numerous escapes, although Teflon®-walled mating tables may prevent escapes (McBrien and Judd 1996). Also, if the design of mating houses interferes with the entrance response of males, such cages would not be suitable.

The recent discovery of a pear-derived volatile (ethyl (2E,4Z)-2,4-decadienoate), which acts as a kairomone (chemical emitted by one species and attracting another) for codling moth males and virgin and mated females, is a very promising development and major breakthrough (Light et al. 2001). The feasibility of trapping live female codling moths not only greatly facilitates the collection of egg masses for screening sterility, but also allows the sampling of females in an economic and systematic way over large geographical areas in operational programmes that use the SIT. Although the efficiency of these female codling moth lures has yet to be assessed under different environmental and geographical conditions, this discovery could promote and accelerate the search for kairomones that are efficient for other lepidopteran pest species.

In programmes for lepidopteran pests that use inherited sterility (IS), an analysis (using light microscopy) of the incidence of chromosomal aberrations in F_1 male larvae can be used to reveal the proportion of moths that mated with released

substerile males. Fragmentation, and non-reciprocal, reciprocal, and multiple translocations, are the most common types of aberrations encountered (Carpenter et al., this volume).

3.2.3. Monitoring Variations in Age Structure of Wild Population

A change imposed on an insect population through variations in mortality, emigration, and invasion, will be reflected in the age structure of that population (Van der Vloedt et al. 1980, Rogers and Randolph 1986, Vreysen et al. 1999a). A method to determine the age of individual insects (or a group of insects) would enable variations in population structure (due to imposed control measures) to be assessed, and would be another powerful tool to monitor progress in programmes. The effects of season, habitat, and other factors on the age structure of a population must be separated out from those of applied control measures.

There are several methods that have routinely been used in operational programmes to estimate the age of tsetse fly populations (Van der Vloedt et al. 1980, Vreysen et al. 2000) — the development stage of ovarioles in females (Saunders 1960, Challier 1965), and the wing-fray analysis (rate of wear of the wings) (Jackson 1946). Wing-fray measurements are a convenient way to give a reasonable, albeit crude, indication of the mean age of a population, but since fraying is influenced by the activity pattern of flies, the rate of wing fraying varies between species and the sexes (Ryan et al. 1980). Determining the physiological age structure in tsetse, using ovarian development, is labour-intensive but very accurate. However, it is not suitable for determining the chronological age of tsetse populations, in view of the influences of temperature and nutritional state on the development rate of each gonadotrophic cycle (Saunders 1972), and inter- and intra-species differences (Wall 1990).

The measurement of fluorescent pigments (pteridines), which accumulate linearly with age in the heads of tsetse flies (Lehane and Mail 1985, Langley et al. 1988) and New World screwworms (Thomas and Chen 1989), and curvilinearly in the Old World screwworm *Chrysomya bezziana* (Villeneuve) (Wall et al. 1990), may provide a cheap, convenient, and rapid indicator of the mean age of these insect populations. However, the level of pteridine accumulation is highly dependant on temperature and fly size. Also, the precise age of individual insects cannot be determined because the levels of residual variation in pteridine fluorescence remain unexplained in all cases studied, and appreciable confidence limits must be placed around pteridine-derived age estimates (Wall et al. 1990). According to field studies, this method was not suitable for accurate age determination in Mexican fruit flies *Anastrepha ludens* (Loew) (Tomic-Carruthers et al. 2002).

Near-infrared spectroscopy (NIRS) is used to estimate the chronological age of stable flies *Stomoxys calcitrans* (L.), house flies *Musca domestica* L., and face flies *Musca autumnalis* De Geer. NIRS has several advantages over the pteridine fluorescence technique for age-grading field-collected insects (e.g. speed, and portability of instruments), and the technique is independent of the sex and size of the insects being studied, and of the temperature to which adult insects were exposed (Mendoza et al. 2002).

Releasing competitive sterile males will gradually increase the proportion of wild females that do not produce viable offspring. Consequently, fewer young insects will be recruited into the population, and the age structure will gradually become skewed towards older age groups, e.g. data from the tsetse programme in Zanzibar (Fig. 7). During the early stages of the release programme, the monthly averages of the proportion of teneral and nulliparous (i.e. young) females in the samples (determined by ovarian ageing) fluctuated between 16 and 19%, whereas the monthly averages of the proportion of old females (with 4 or more ovulations) fluctuated between 18 and 28%. Thereafter, the proportion of young flies decreased progressively, whereas the proportion of old females ($\geq$ 4 ovulations) gradually increased (Vreysen et al. 2000).
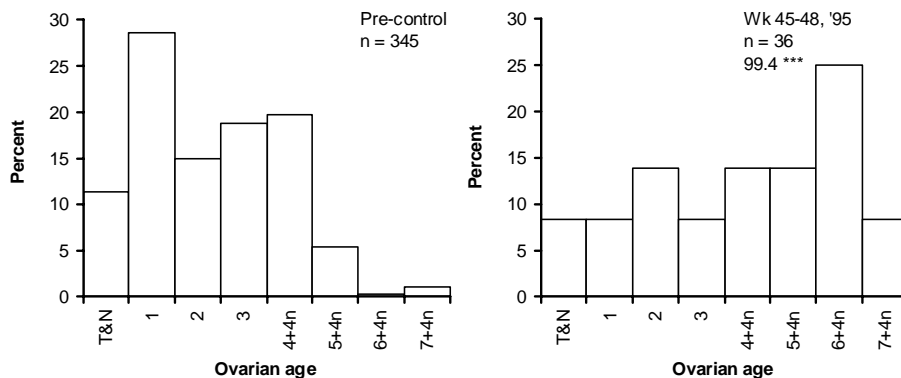
Figure 7. Frequency distribution of ovarian age categories of G. austeni *females sampled during (left) pre-control phase and (right) late release phase (T = teneral, N = nulliparous, 1–7 = number of ovulations; more details in Challier 1965). Number indicates chi-square value of comparison of frequency distribution of ovarian age with that of pre-control sample (*** p < 0.001). (Figure adapted from Vreysen 2001.)*

3.2.4. Monitoring Relative Abundance of Wild Population

Decline in the apparent density of a wild population, as shown by the number of insects trapped in a sampling device, is a commonly used parameter to assess the progress of AW-IPM programmes for tsetse flies (Vreysen et al. 2000) (Fig. 6), fruit flies (Iwahashi 1977), and Lepidoptera (Bloem and Bloem 2000, Walters et al. 2000). In the case of insects with a very long lifespan, such as tsetse flies (Vreysen et al. 1996), monitoring this parameter has an inherent weakness; no insects are actually killed by the SIT technology, and thus there is an inevitable delay in the decline in the number of wild insects available to be trapped.

When sampling insects to obtain an indicator of programme progress, the main difficulty is related to interpreting catch data. For example, is a temporal decline in the number of insects trapped, even when using a standardized monitoring programme, always an indication of progress in a control programme? Numerous factors influence

the size of trap samples (section 3.1.2.), and the importance of these factors for the interpretation of monitoring data is illustrated below.

The density of a natural insect population rarely remains stable, but fluctuates in both space and time. Knowledge of these fluctuations is prerequisite to correctly interpreting monitoring data. In the absence of any control measure, monthly trapping data for the tsetse fly *Glossina swynnertoni* Austen, over a period of 23 years in Tanzania (Fig. 8), show high seasonal variations in the apparent density of the population, with the average highest apparent density being 18 times that of the lowest average density (Glasgow and Welch 1962).

Differences in population density from one place to another can be great. Locations with unusually high densities are called "hot spots", and require special attention in AW-IPM programmes (sections 2.1. and 2.2.3.) (Box 3).
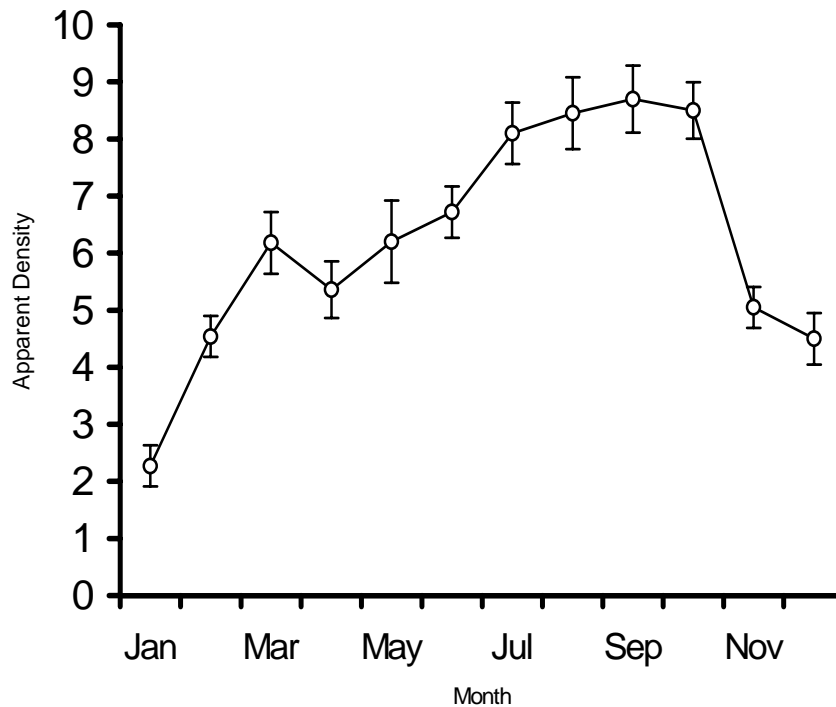
Figure 8. Fluctuations in average monthly apparent density (as percentage of total catches) of Glossina swynnertoni *population sampled over period of 23 years in Tanzania. (Figure adapted from Glasgow and Welch 1962.)*

Box 3. Monitoring "Hot Spots"

Localized infestations or "hot spots" are of particular interest in AW-IPM programmes. Strategies that allow quick action to manage these situations are required. To detect hot spots quickly, programme managers must always be alert throughout the duration of the programme. The early detection of relic populations, or reinfestations in localized areas, is a determining factor in reducing programme cost, in increasing programme effectiveness, and in the ultimate successful completion of the programme (Itô and Kakinohana 1995). Although baseline surveys might reveal that the pest infestation is widespread, hot-spot areas are often concealed, and are revealed only after the programme has advanced.

The reasons for hot spots are numerous — localized ideal climatic conditions, abundant hosts, and presence of natural obstacles that hamper migration and dispersal of the insects.

When hot spots are discovered or persist, surveillance in the vicinity should immediately be increased. The size of the area under surveillance will depend on the mobility of the insect, and should be increased systematically until no more wild insects are trapped. The traps deployed must be serviced/emptied on a very frequent (daily) basis, or the frequency and intensity of indirect sampling procedures (fruit inspection, disease monitoring, etc.) (section 3.1.3.) increased, to amplify confidence in the data. For some insect species such as the Mediterranean fruit fly, DNA analysis can indicate whether the infestation is new, or originates from a relic population or from insects that accidentally escaped from a rearing facility. If the infestation is new, it is essential that live wild insects are collected in the new infestation zone, and their mating compatibility with factory-reared and sterilized insects assessed. In addition, the overall monitoring efficiency could be improved by introducing temporary monitoring schemes, and improving communication and/or feedback mechanisms with crop farmers or livestock keepers to reduce the time between an infestation being discovered and it being reported to programme management or relevant authorities.

After assessing and compiling the necessary information, and possibly using GIS (which can greatly facilitate the detection and management of localized infestations (Cox and Vreysen, this volume)), corrective measures must be taken immediately, e.g. apply an insecticide, increase the number of sterile insects released, create a buffer zone or quarantine programme, and limit the movement of livestock, crops, etc.

In addition to the need for baseline data on the temporal and spatial fluctuations in the density of a wild insect population, the factors that influence changes in the behavioural responses of insects towards trapping devices, in both space and time, need to be understood to correctly interpret monitoring data (Vreysen and Saleh 2001). An analysis of weekly trap catches of sterile *Glossina austeni* released on Unguja Island, Zanzibar, over a period of more than 2 years, showed that, in each 12-month period, the size of catches fluctuated by a factor of more than 10, independent of the actual sterile fly population density (which was estimated from the number of sterile males released) (M. J. B. Vreysen, unpublished data). These data led to some important lessons. Even dramatic increases in trap catches, especially during control operations, could wrongly be attributed to sudden explosions of the pest population, migration from adjacent areas, decrease in mortality, or failure of the applied control method. This study indicates that the probability of trapping insects during a post-control monitoring phase would be increased significantly by deploying traps during strategic periods, e.g. when the behavioural response of insects to the trapping device is at a high point (Saleh et al. 1999).

4. ESTABLISHING ABSENCE OF INSECTS

After the "last" wild insect has been caught, a difficult decision must be made. When should the release of sterile insects be terminated? Stopping the release too soon could jeopardize the success of the programme, but if releases are continued after eradication has been achieved, useful resources are wasted (Proverbs 1974). The time period of continued releases but zero captures will be influenced by the life cycle of the insect, the efficiency of the sampling system used, and the financial resources available.

To increase the confidence of detecting wild insects during the final stages of the programme, the monitoring strategy could be adjusted by: (1) increasing the density and frequency of the direct and indirect monitoring activities (Yamagishi et al. 1993), (2) increasing the proportion of sampling devices biased for the female segment of the population (to obtain more information on fertility or induced sterility) (Vreysen et al. 2000), and (3) releasing sterile females as sentinels (Vreysen and Van der Vloedt 1992). The decision to stop releasing sterile insects is frequently made on an ad hoc basis, and is highly influenced by financial and political circumstances. In the eradication programmes in Central America and in Libya, after the "last" case had been detected, the dispersal of sterile New World screwworms continued for 6–18 months (FAO 1992, Wyss 2000). In Mediterranean fruit fly programmes, it is standard procedure that releases continue for at least three fly generations (using degree-day models) after the "last" fly has been trapped (IAEA/FAO 1997).

A problem related to the issue of when to stop dispersing sterile insects is how long to continue post-release monitoring to obtain sufficient confidence that a pest has been eradicated (Barclay and Hargrove 2005; Hargrove 2005; Barclay et al., this volume). A sample can only confirm that individual insects are present in a given area; sampling can never prove a negative. However, samples can demonstrate that the number of individuals is within a specified range, with a known degree of confidence (Venette et al. 2002). The probability of detecting rare individuals is directly related to the number of sampling units and the density of the population (McArdle 1990, Lance and Gates 1994). Therefore sampling should be implemented so as to maximize the probability of detecting relic insects in the field (McDermotte 2000). If too few samples are taken, an error could be made in concluding that a pest is absent from a habitat (Venette et al. 2002).

As the declaration of the absence of an insect in a target area cannot be guaranteed, it must always be qualified by probability or confidence levels. Unfortunately, standardized probability-based entomological criteria to confirm the status of eradication have rarely been applied in insect eradication programmes. In Queensland, Australia, an established Code of Practice specifies that an area can be declared free of the Queensland fruit fly *Bactrocera tryoni* (Froggatt) after three generations plus 28 days after the "last" fly has been trapped (Clift and Meats 2004). The validity of this code was confirmed by a computer model which indicated that at a mean trapping density of 0.001 flies per trap per week, 16 weeks of zero counts

(with a grid of 100 traps) is the minimum length of time required to conclude, with 95% confidence, that there are no flies in the area (Clift and Meats 2004).

Another option is to follow the approaches used by ecologists to assess species extinction (McDermotte 2000). In one approach, after extinction is assumed to have occurred, the number of negative sightings required to establish extinction at a given probability level is assessed; this requires an accurate knowledge of the sensitivity of the sampling method (Reed 1996). A second approach takes into account data from pre-eradication sampling, assuming a declining population. In this method the probability of extinction is estimated as a function of the frequency of pre-eradication sightings, and the proportion of the total time (pre- and post-eradication) during which no sightings have been made (Solow 1993).

5. DATA MANAGEMENT

It is essential that information from the monitoring activities is reliable, comprehensive, and clear, and is delivered in a synthesized and timely manner to decision-makers (Reyes et al. 1988). The amount and diversity of data that have to be handled and analysed can be staggering, especially in large-scale operational programmes. Thus each programme requires a properly developed data-flow structure, e.g. from field teams via field sub-offices to programme headquarters, and an efficient data-analysis unit. A programme website is very useful; it permits all concerned to have access anytime to the raw and analysed data.

Comprehensive field data recording sheets are indispensable, and must be adapted to the biological characteristics of each target species and to the needs of each programme. Data sheets should include all information relevant to a proper data analysis, e.g. details on animals screened, details on the composition of trap samples, geo-referenced trap deployment sites, baits, types of traps, etc. Using electronic data collectors, and transmitting data to computers at programme headquarters via e-mail, facsimile, or HF radio, even if rather sophisticated compared with traditional paper methods, permit rapid and efficient data collection and compilation (Cox and Vreysen, this volume). In addition, field-monitoring data should be complemented with climatic data from remote automated weather stations in the target area.

Using identical sampling periods, standardized sampling procedures, and uniform compilation methods ensure homogeneous data sets; they greatly facilitate the analysis and interpretation of the data (Box 4). The Gregorian year with 365 days can be divided into equal periods for data collection and compilation, e.g. in the tsetse programme in Zanzibar, traps were checked 1–5 times per week, depending on the importance of the area, but all of the data were compiled on a weekly basis (Vreysen et al. 2000). Data can be compiled using EXCEL spreadsheets or an ACCESS-based database; the latter is more appropriate for large amounts of data, and allows easy incorporation into most GIS. Particularly useful are databases which have been developed to manage the field data of specific pest control programmes, and which can promote the standardization of data reporting and analysis on a regional scale, e.g. the Disease and Vector Integrated Database (DAVID) (Robinson 2001).

Box 4. Concept of Reference Sites for Monitoring or Surveys

Notwithstanding the importance of sound and robust monitoring activities in an AW-IPM programme, funds are needed to deploy each sampling device. Monitoring activities should be planned as a compromise between cost-efficiency and providing adequate and sufficient data. AW-IPM programmes tend to be implemented over large geographical areas, and monitoring (or surveying) in detail the entire area is not feasible, practical or cost-effective. Selecting reference (or fixed) monitoring sites, representative of a certain area, is a useful approach to efficiently monitor or survey large geographical areas.

An area is divided into Universal Transverse Mercator (UTM) squares, e.g. 10 x 10 km, and each UTM grid square is characterized by parameters that are important to the distribution of the pest, i.e. vegetation, land use, land cover, hydrology, soil type, altitude, etc. Pending the availability of these specific data layers, GIS facilitate characterization of the grid squares (Cox and Vreysen, this volume). Therefore each grid square has a certain number of classes, which are of relevance to the abundance and distribution of the pest. After considering accessibility, logistics, personnel, etc., a reference monitoring site can be selected for each class of each grid, and a certain number of sampling devices deployed in each reference monitoring site, which will then be representative for that class in that specific grid square.

This approach was applied to develop and implement an efficient sampling strategy for the collection of entomological baseline data in the tsetse project in the Southern Rift Valley of Ethiopia (Vreysen 2000). In each UTM square (total of 105 squares, each 10 x 10 km) about 15–18 trapping sites were selected to sample the wild fly populations during four surveys within 1 year (with only five field teams). Accurate data on the spatial and temporal differences of the tsetse populations were collected with limited resources over a large geographical area (more than 10 000 km^2) using carefully selected trapping sites in representative areas (Vreysen et al. 1999b, Vreysen 2000).
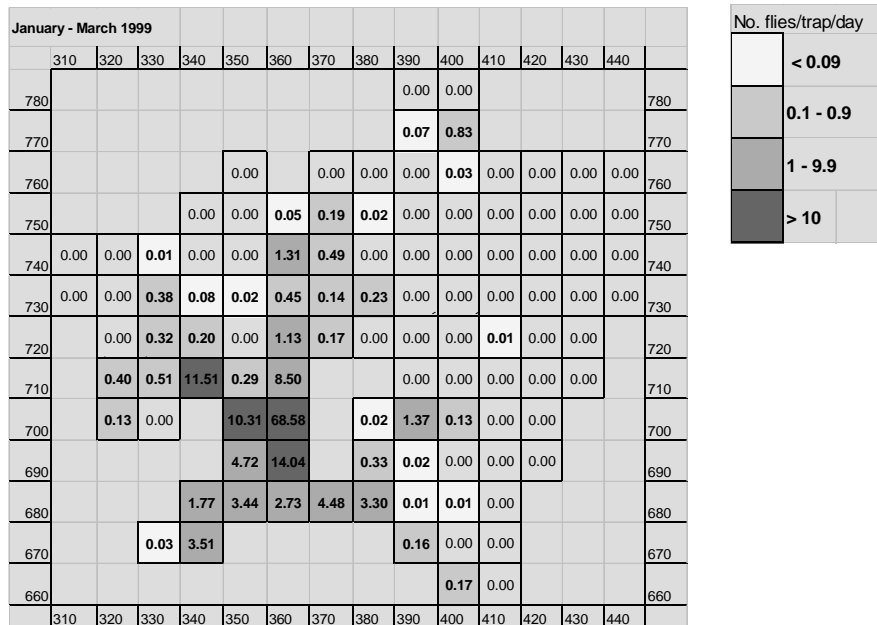
Apparent density of Glossina pallidipes *(number of female flies/trap/day) (expressed per grid square) during January–March 1999 survey in 105 UTM grid squares, Southern Rift Valley, Ethiopia. (Figure from Vreysen 2000.)*

The frequency of analysing the data is related to the regularity of sampling and compilation of the data, but is usually done every 1–3 months; however, if the insect has a high reproductive rate, it will be done much more frequently. In the analysis of trap catches, indices, such as daily or weekly catch per trap, or proportion of positive traps, can be used. However, Clift and Meats (1998) showed that the proportion of positive traps is not a good indicator in the early stages of a programme, since the proportion of positive traps is only slightly reduced when the catch per trap declines from 10 to 1. Standard statistical methods such as analysis of variance in a randomized block design (with fixed time units as blocks) can, after proper transformation of the data, be used for the temporal and spatial comparison of data (Sokal and Rolf 1995).

6. CONCLUSIONS

The importance of reliable monitoring data before, during, and after the release of sterile insects cannot be overstated. In spite of the availability of efficient "direct" and "indirect" surveillance methods suitable for a variety of target species, the monitoring component in operational programmes is too often neglected. Consequently, programme management and decision-making are based more on established protocols, availability of financial resources, and political inspiration rather than on sound scientific principles.

Programmes that release sterile insects are inherently complex, with many critical components in the production process (aimed at delivering high-quality insects), methods of handling and transport, and dispersal procedures. The multifaceted nature of these programmes also implies that the probability of problems occurring is higher than in conventional pest control programmes. Only the availability of reliable field data can: (1) provide clear evidence that observed programme progress is due to released sterile insects and other measures applied to suppress the pest population, (2) identify the causes of problems and suggest possible solutions, and (3) increase the efficiency of the programmes by more strategic deployment of sterile insects. It is acknowledged that monitoring methods are often time-consuming, labour-intensive, and even laborious, but in many instances the financial losses, resulting from inefficient management decisions made without the benefit of reliable field data, outweigh the costs of monitoring activities.

Several shortcomings in implementing the monitoring activities of operational programmes have been pointed out. The need for more standardization, research, and development, to improve several aspects of monitoring the field component of these programmes, has been highlighted. Examples of these aspects are: (1) development of guidelines to standardize sampling procedures (trap types for specific species, lures and attractants, trap deployment, trap densities, etc.) (IAEA 2003), (2) refinement and development of more efficient lure and trapping systems, (3) better understanding of insect ecology, in relation to insect densities, aggregation patterns, and dispersal characteristics, (4) research on visual markers in the sperm of sterile males, (5) better methods of measuring induced sterility, and (6) simple statistical probability methods, which can easily be applied by field entomologists, to assess the absence of a target species.

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CHAPTER 3.7.

PROCEDURES FOR DECLARING PEST FREE STATUS

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SUMMARY

Procedures are presented for declaring an area to be "pest free" following an area-wide eradication programme against a population of an insect pest. These involve two probability models to deal with null trapping results, and a growth model to help verify that pests were no longer present when control actions were stopped. The two probability models are presented for a situation in which trapping for an insect pest is ongoing, and for which the trapping results are all negative. The models calculate the probability of such negative results if in fact insects were present. If this probability is sufficiently low, then the hypothesis that insects are present is rejected. The models depend on knowledge of the efficiency of the traps, and also the area of attractiveness of the traps. The possibility of a rebound of an incipient but non-detectable population, that remains after control measures are discontinued, is considered. Using a growth model, the rate of increase, of an insect population that starts from one or two insects, is examined. An example is given for tsetse flies — both means and confidence limits are calculated for a period of 24 reproductive periods after control has been terminated. If insects are disease vectors, it is also suggested that the progress of the disease be monitored to detect continuing transmission. This should be done in conjunction with a disease transmission model.

1. INTRODUCTION

Following an area-wide programme that has attempted to eradicate an insect population from a specific area, it is important to confirm the pest free status. The criterion of programme success used is that the pest population has been eradicated from the target area (FAO 1996), a very different concept from that of the World Health Organization (WHO) regarding species eradication at a global level (WHO 2001).

There have been several successful insect pest eradication programmes, e.g. the New World screwworm *Cochliomyia hominivorax* (Coquerel) has been eradicated from North and Central America (Galvin and Wyss 1996; Wyss 2000a, b), and from Libya, using the sterile insect technique (SIT) together with wound treatments and quarantine measures (FAO 1992, Lindquist et al. 1992, Krafsur 1998, Wyss 2000b). Many tephritid fruit fly infestations around the world have been similarly eradicated by integrating the SIT with other methods (Sproule et al. 1992, MAG/SAG 1995, Kuba et al. 1996, Reyes F. et al. 2000, Villaseñor et al. 2000, Koyama et al. 2004) or the male annihilation technique (MAT) for *Bactrocera* species that respond to the strong attractant methyl eugenol (Steiner et al. 1970, Koyama et al. 1984, Hancock et al. 2000, Seewooruthun et al. 2000).

If continued attempts to trap insects after the termination of control actions are unsuccessful, this could be taken as evidence that the population has been eliminated from the target area, but with the realization that null results do not necessarily imply non-existence (Mitchell 1980, Richards and Tarry 1992, Clift and Meats 1997, Barclay and Hargrove 2005, Hargrove 2005). Low-density populations are often very difficult to detect. For example, even when trapping the mountain pine

beetle *Dendroctonus ponderosae* Hopkins using pheromones that are known to be effective, if the species is endemic and at a very low density (L. Safranyik, personal communication) (e.g. one infested tree per 10 hectares), it may be almost impossible to trap beetles (Bartos and Schmitz 1998).

Perkins (1989) listed seven scientific questions pertinent to eradication, including:

> What accommodations need to be made for the limitations in the detection methods of ultra-low populations?

In interpreting null trapping (or other sampling) results, some knowledge of the detectability of the species (when using specified traps), as well as a measure of the area of attraction surrounding traps, must be available. Once these measures are known, probability estimates of non-existence can be formulated from null results. An added complication arises if detectability itself is density-dependent, or if the area of attraction depends on weather or other factors.

The Food and Agriculture Organization of the United Nations (FAO) recognizes three categories of pest status: presence, absence, and transience (FAO 1999). Absence is determined by one or more of the following factors: (1) the lack of any records of its presence, (2) the action of eradication, (3) a shift in a pest's range, or (4) the interpretation of positive records as being only temporary. The three stages in the establishment of a pest free area are: (1) systems to establish freedom from pests, (2) measures to maintain freedom, and (3) checks to verify that freedom has been maintained (FAO 1996). However, since these guidelines are very general (applying to all pests — plant, animal, and microbial), they do not include methods specifically applicable to insects.

At present, insect population eradication is usually declared after the pest has not been detected for a reasonable period of time, which includes a considerable safety margin. The usual procedure is to continue control measures for several generations after the last insect was captured, then monitor the area intensively for additional generations (section 2.2.), and finally, if no more insects are detected, declare success. The New World screwworm in Costa Rica was declared eradicated about 1 year after the last fly was found (Galvin and Wyss 1996; Wyss 2000b, 2001). In Libya, sterile releases were discontinued 6 months after the last wild fly was trapped, and eradication was formally declared 8 months later (FAO 1992, Lindquist et al. 1992, Wyss 2000b). The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) was considered eradicated in Chile after trapping (with a parapheromone and food attractants) for three generations without catching any wild flies (C. Flores and J. Gonzalez, personal communication). On the island of Kume, Japan, the melon fly *Bactrocera cucurbitae* (Coquillett) was considered eradicated 6 months after the last capture, and following examination of 70 000 fruits (Iwahashi 1977). When the population of *Glossina austeni* Newstead was eradicated using an integrated approach on the island of Unguja, Zanzibar, sterile releases were maintained for six generations (after the last wild fly was captured) before eradication was deemed complete (Vreysen et al. 2000). The oriental fruit fly *Bactrocera dorsalis* Hendel was introduced into Mauritius in 1996, and, immediately after detection, eradication activities were begun. The last fly was trapped in May 1997, and trapping continued for 2 years, after which time

eradication was declared (Seewooruthun et al. 2000). Following detection of the last wild fly, and a 4-month effort using the MAT and SIT, the oriental fruit fly was declared eradicated from the Mariana Islands (Steiner et al. 1970). One year (about six generations) after the detection of the last wild fly, the oriental fruit fly was considered eradicated from the Okinawa Islands using the MAT (Koyama et al. 1984). Subsequent to seeing the last individual, and trapping for 190 days and examining over 300 000 fruits, this same pest was judged eradicated on Amami Island (Ushio et al. 1982). In Perth, Australia, a large infestation of the Queensland fruit fly *Bactrocera tryoni* (Froggatt) was regarded as an exotic incursion (it is not endemic in the state of Western Australia), and, after integrating the SIT, this population was declared eradicated 12 months after the last wild fly was trapped (Sproule et al. 1992). Using the MAT in north Queensland, Australia, against the Asian papaya fruit fly *Bactrocera papayae* Drew and Hancock, area-freedom was claimed 12 months after the capture of the last fly, and after another 12 months eradication was claimed (Hancock et al. 2000).

Clift and Meats (1997), using risk management software and trapping data up to May 1997, established criteria for 99% probability of localized eradication. Except for this last report, no probabilities were presented in any of these examples of eradication. Most authors simply did not provide the criteria on which eradication was judged, e.g. Schwarz et al. (1989). However, Kuno (1978, 1991) gave a quantitative methodology for destructively sampling biotic units, such as fruit and vegetable shipments, to determine that a particular pest is either absent or at a very low density. His method used a sequential sampling scheme, involving a hypergeometric probability distribution, to determine the required sample size for this verification. In addition, Yamamura and Sugimoto (1995) and Yamamura and Katsumata (1999) adopted a similar methodology for use in the Japanese import quarantine system.

Apart from the above examples, in countries such as Australia, Chile, Mexico, and the USA, cases of the eradication of spot infestations of pests (such as the Mediterranean fruit fly) in areas normally claiming area-freedom are much more common. Such eradications are virtually routine, and are not reported in the scientific literature. Codes of practice for such procedures are also unpublished, and details differ according to both the country and pest involved. Meats et al. (2003) reviewed the codes of practice current in Australia for the Mediterranean and Queensland fruit flies, examined 25 years of data from spot eradications of each species, and calculated the radii of the areas of infestation where the probability of exceeding such radii was equal to probit 9 (1/300 000). If zones of area-freedom are to be established for other species, similar codes will be required. Obviously it would be desirable if there were methods, with a pre-determined degree of probability, to calculate the radius of an affected area (where area-freedom would be suspended), and the length of the no-detection period needed to reinstate area-freedom within the previously calculated radius.

Under the auspices of the FAO and the International Atomic Energy Agency (IAEA), a meeting was held in Vienna, Austria, in August 2003, to review the procedures for declaring areas free of tsetse flies and the trypanosomosis problem

(Barclay and Hargrove 2005). The first three authors of this chapter attended the meeting, and subsequently, along with a fourth author, wrote the chapter.

2. ASSESSMENT METHODOLOGIES

Three methodologies, suitable for addressing the question of the non-existence of insects in a given area, are presented. These include two probability models to assess the results of trapping, a model of a rebounding population following suppression, and the use of disease transmission information in the case of pest species that are disease vectors. These methodologies are illustrated for tsetse flies *Glossina* spp. (Barclay and Hargrove 2005, Hargrove 2005).

2.1. Probability Models

The following probability models are based on trapping (or other method of sampling) with zero results, while assuming that insects are present. The models then give the probability of a zero catch for this assumption; if the probability is sufficiently low, one can reject the hypothesis that insects are present. For simplicity, all sampling systems are referred to below as "traps".

Two models are presented: (1) local sampling involving one trap (suitable for spot infestations), and (2) area-wide sampling (suitable for an area-wide eradication programme involving either an established pest or a large outbreak). Both models involve sampling a population that is close to elimination. Either approach can be used, and the results should be fairly similar; when residual population sizes are very low, the models converge (Barclay and Hargrove 2005).

For an insect to be caught in a trap on a given day, the following conditions must be met:

- A trap must be operative in the vicinity of the insect.
- The insect must be active.
- Given the above, the insect must succeed in finding the trap and be captured by it.

2.1.1. Local Sampling with One Trap

Regarding the probability of a zero catch in each of a number of traps, consider a single trap and the "circle" (area) of attraction around it, within which the probability of catching a given insect with a given trap during one activity period is σ, called the detectability; the probability of not catching a given insect is $1 - \sigma$. In calculating detectability, 1 day would reasonably constitute one sampling period, since it represents one complete cycle of activity. If there are k insects in the "circle", the mean number caught per activity period is $k\sigma$. If there are k insects present, and if the insects are caught independent of each other, then the conditional probability of catching no insects during an activity period (or sampling period) is:

$$p(0|k) = (1 - \sigma)^k \tag{1}$$

The probability of both zero catch and a given number of insects being in the circle is $p(0 \cap k) = p(0|k) f(k)$, where the symbol $\cap$ refers to "and" or conjunction (Parzen 1960), and $f(k)$ is the probability of k insects being present. The conditional probability of no catch, given that there is an undetermined positive number of insects present, is:

$$p(0|k>0) = \Sigma\, p(0|k)\, f(k) = \Sigma\, (1 - \sigma)^k\, f(k), \text{ where the sum is for } k \geq 1 \qquad (2)$$

This equation could be used to construct probabilities of a zero catch, assuming that there are insects present near the trap. The problem arises in stipulating a distribution for $f(k)$, called the prior distribution on k.

Choice of Prior Distribution on k. The choice of a prior distribution is arbitrary, unless prior information is known about it. However, the probability of a zero catch with one insect present is always greater than, or equal to, the probability of using any prior distribution. Thus, the probability of a zero catch with a positive number of insects is: $p(0|k>0) = \Sigma\, p(0|k)\, p(k) = \Sigma\, (1 - \sigma)^k\, p(k) < \Sigma\, (1 - \sigma)\, p(k) = (1 - \sigma)$, as the sum over all k, $\Sigma\, p(k) = 1$, so that $p(0|k>0) < p(0|k = 1)$. Since the choice of a particular prior distribution is arbitrary unless there is some information about it, and since the probability given the presence of one insect has an easy closed form solution (i.e. $p(0|1) = 1 - \sigma$) and is more conservative, the latter probability is used in the development below.

The probability of a zero catch, given that there is one insect present, is $p(0|1) = 1 - \sigma$, so the probability of a succession of n zero catches on n independent sampling occasions is

$$P(0) = (1 - \sigma)^n \qquad (3)$$

and this is true for each trap. If the traps are of different types, then the detectability, σ, is specific to the trap type. The conservative approach is to calculate one probability for each trap, and require that all of them satisfy the criterion for "eradication to be declared" before such a declaration could be made. (If different types of traps and different sampling methods were used to maximize the chance of detecting the insect, they would complement each other and in principle add to confidence in the results, but this is outside the framework of the above conservative model.) This means that, for each trap, the number of trapping sessions needs to be large enough so that $(1 - \sigma)^n$ is lower than the acceptable limit. For example, if the hypothesis (that there are pests present) is to be rejected at the $\alpha = 0.01$ level, and if σ was 0.1, then the number of trapping days, n, needs to be such that $(1 - 0.1)^n \leq 0.01$. This can be found using the equation: $n = \log(0.01) / \log(0.9) = -2.0 / -0.0458 = 43.7 \approx 44$. More generally, the equation is:

$$n = \log(\alpha) / \log(1 - \sigma) \qquad (4)$$

where α is the chosen rejection level. The base of the logarithms is immaterial, as long as both logs are of the same base. Once the rejection level has been chosen, and the value of detectability, σ, is known, the required value of n can easily be computed (however, the number of trapping days should be linked to the generation time under defined climatic conditions). Using a somewhat different model, a similar criterion was obtained by Kuno (1991).

Sampling Fraction of Population. Each trap has an area of attraction such that, within that small area, the probability of catching a given insect approximates the average detectability. If the number of traps is not sufficient to cover the whole area, and therefore the sum of the areas of attraction is less than the area to be evaluated for "pest free" status (called the "assessment area"), then one of two scenarios may occur. If the pests are sufficiently mobile so that they move in and out of areas of attraction in their normal daily or weekly movements, then the detectability is simply reduced, compared with the situation in which they are in the area of attraction all of the time. Alternatively, the traps could be moved from day to day or week to week, so as to cover the assessment area; then the detectability would similarly be reduced. Assuming that every insect spends roughly the same amount of time in areas of attraction to traps, then the detectability will be reduced by the sampling fraction. If the sum of the areas of attraction to traps is a fraction f of the assessment area, then the average detectability will be σf. In that case, the criterion becomes:

$$P(0) = (1 - \sigma f)^n < \alpha \qquad (5)$$

and solving for n:

$$n = \log(\alpha) / \log(1 - \sigma f) \qquad (6)$$

The size of the area of attraction will be crucial to the calculation of the sampling fraction, and this area may depend on weather, since odour plumes from lures in traps will vary in size with wind speed, and may also vary with topography, season, competing natural stimuli, and surrounding vegetation.

2.1.2. Area-Wide Sampling

Another way of looking at the problem, which leads to the same kind of result, is to consider area-wide sampling covering the whole assessment area. Having selected one or more sampling systems, the next problem is to decide on the required intensity of sampling, and for what period of time, before a series of zero catches can be interpreted as indicating eradication at some specified level of probability.

The parameters are defined as follows:

A Area sampled (km^2), assumed to be closed to immigration and emigration (either naturally or through the maintenance of a wide-enough barrier)

k Total number of surviving pests, assumed to be randomly distributed in A

σ Trap efficiency (detectability), i.e. the conditional probability that an insect is caught by a given trap, given that there is only one trap present in a 1-km^2 area containing the insect, and given that the insect is active

s Number of traps present in all of A

n Number of days that each trap is operated

If there are s traps in the assessment area, deployed for n days, and if s is sufficiently small that the traps act independently of each other, then the probability ($C'(k,s,\sigma,n)$) that none of the traps catches any of the k surviving insects is:

$$C'(k,s,\sigma,t) = \exp (- s \, n \, \sigma \, k / A) \qquad (7)$$

assuming that the capture probability is identical for all insects, and is independent of time t (Hargrove 2003). The result of interest is the function relating to the probability, $p(0)$, of observing a sequence of zero results, if in fact insects are in the assessment area.

$$p(0) = \exp (-sn\sigma\rho) \qquad (8)$$

where $\rho = k/A$ is the population density, and other symbols are as defined above. The objective is to know when a series of zero catches is sufficiently long that the null hypothesis of the existence of pests at the assumed level can be rejected. For example, for the probability of a sequence of zero catches in the presence of insects to be below 0.01, then it is required that:

$$\exp (-sn\sigma\rho) < 0.01 \qquad (9)$$

from which

$$-sn\sigma\rho < \ln (0.01) = - 4.605 \qquad (10)$$

One can solve for one of the variables in terms of the others that are known. For example, if t is determined, σ is known, and ρ can be surmised, then

$$s > 4.605 / n\sigma\rho \qquad (11)$$

When this condition is met, the required probability has been achieved. If this calculation leads to an unreasonably large number of traps being required, then a different criterion may be considered. The value of s obtained from the inequality above assumes a risk level of 0.01 in concluding that no insects are present, and this is shown in Fig. 1 for various values of the number of traps used (s) and number of insects present (k).
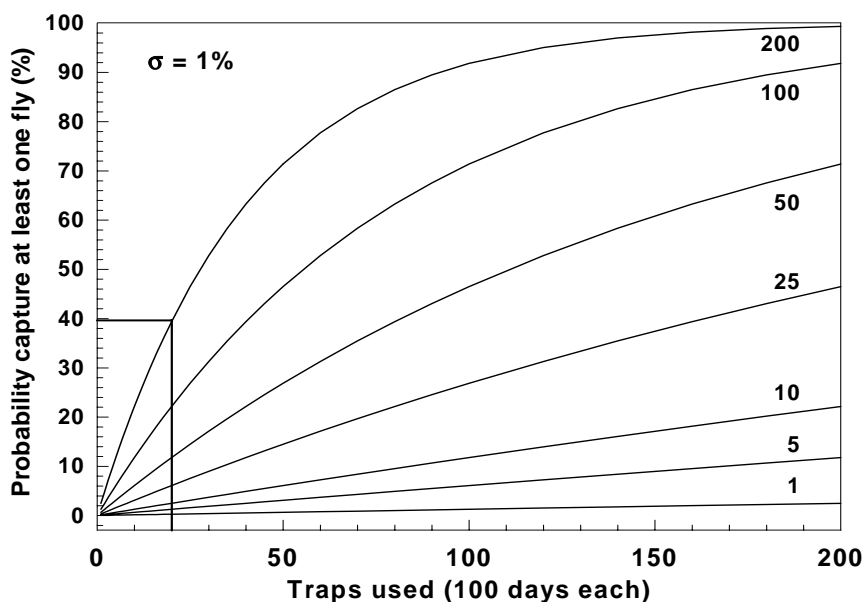
Figure 1. Probability of catching a single tsetse fly in a small remnant population (numbers in body of figure) in an area of 8000 km² as a function of number of traps used and duration of trapping (when using a trap with efficiency of 1%). (Figure from Barclay and Hargrove 2005, reproduced with permission.)

It must be emphasized that the calculated number of traps (s) is independent of the size of the assessment area to be controlled (unless the area is very small). However, with a very large area, there is a greater probability that "pockets" of insects still exist (where the control treatments and/or released sterile males are locally overwhelmed), and these pockets must be identified and treated accordingly (Shiga 1991).

As a simple example of the application of the inequality equation (11) above, consider the insecticide aerial-spraying operation (sequential aerosol technique (SAT)) against tsetse flies in Botswana (Okavango Delta, about 16 000 km²) (Allsopp and Phillemon-Motsu 2002, Tsetse News 2002), where apparently the last fly was caught on 30 August 2002, although very few traps have been installed in the Delta. Possibly the population of *Glossina morsitans centralis* Machado was actually eradicated in the SAT-treated area. However, suppose that 100 insects survived the sprays, what chance would there be of catching one of them? If the insects were distributed at random, 10 traps were deployed for about 3 months (90 days), $\sigma = 0.01$, and using inequality 11, the probability of catching at least one insect is $C(k,s,n) \approx 0.06$. Thus, with this low level of trapping, there is a 94% chance that detection of the presence of insects will fail. To be 90% certain that at least one

insect is detected (given that 100 insects were present), the application of inequality 11 suggests that more than 400 traps must be deployed for 3 months. To be 99% certain, this figure rises to more than 800 traps. Note that these figures assume a rather even distribution of traps. Deploying traps for a longer period, such as 1 year to include all seasons, may permit a reduction in the number of traps. Spreadsheets, that incorporate a variable number of trapping days, are available to calculate the minimum number of traps required. (It is important to be aware of the possible existence of insects in places that were avoided or ignored by field personnel, substantially reducing the chances of detecting a residual population.)

In inequality 11, ρ must be surmised; the size of any residual population is not known. To some extent, this uncertainty is balanced by the fact that, if there is a residual population and it is too small to be easily trapped, naturally over time it will increase since it is no longer under control, and it will become more detectable; this is the subject of the next section.

2.2. *Incipient Non-Detectable Populations*

If control actions have reduced the pest population until very few insects are present, so few that they cannot be detected by trapping, then when control is terminated natural reproduction will cause the population to increase. How long will it take for this small population to increase to a detectable level? (It is assumed that surveillance trapping will continue or be increased (detection trapping/sampling) until there is a decision regarding the "pest free status" of the area.) The population can be modelled by a simple equation:

$$N_{t+1} = \lambda\, N_t \qquad\qquad (12)$$

where λ is the rate of increase each generation, and N_t is the population size at generation t. Starting with an initial (very small) population of size N_0 (following termination of the eradication activity), the size of the population t generations later would be $N_t = N_0\lambda^t$. When t is large enough for the population to have become easily detectable, but continued trapping still yields no insects, then a declaration of "pest free status" can be made. In calculating this critical value of t, allowance must be made for dormant or non-growing periods when equation (12) above does not apply. In addition, the time covered by t generations must be adjusted based on available pest-specific day-degree models to make allowance for slow-growing periods during unfavourable climatic conditions. Furthermore, before such a declaration could be made, the allowance of an adequate time-buffer is needed to permit sufficient time to elapse for the population to increase to perhaps 10 times the minimal detectable level. Since equation (12) is deterministic, and events in nature usually involve random elements, reasonable lower limits of λ should be used, not mean values (often used in calculations of ordinary population growth). This is illustrated below by examples from fruit flies and tsetse flies (which have much lower rates of increase than fruit flies or most other insect pests).

2.2.1. Observed Populations of Bactrocera papayae in Eradication Context

Clift and Meats (1997) described the trap catches obtained during a population resurgence of the Asian papaya fruit fly *Bactrocera papayae* part-way through an eradication programme in tropical northern Queensland, Australia. In discussing localized extinction and reinfestation of various areas within the pest-quarantine area, they noted intervals of up to 12 weeks between catches, and with the data available were unable to define localized extinction. After a declaration of eradication in 1999, subsequent data indicated that at least 16 weeks (favourable for the development of a fruit fly population) were needed to attain confidence that there were no flies in the area. Simulation data for 16 weeks were consistent with this result (Clift and Meats 2004). In practice, after 12 months of no catches, area-freedom was claimed, and after another 12 months with no catches and no control procedures applied, eradication was claimed. Throughout this interval, a 1-km grid of traps, with efficiency of at least 10%, was maintained over an 8000-km^2 area, and traps were checked at least weekly.

2.2.2. Monte-Carlo Simulation of Population Growth of Tsetse Flies

Females give live birth to a larva about every 9 days (Hargrove 1994), which then pupates, and about 30 days later an adult emerges (Phelps and Burrows 1969a, b). Assuming this rate of reproduction, and the least detectable population (one gravid female), a Monte-Carlo simulation was performed to compute the mean population numbers after each of 24 reproductive periods, assuming that gender determination of larvae is a random phenomenon, and that at meiosis males and females are equally likely. At each reproductive period in this simulation, a decision was made (on the basis of a random number) for each adult gravid female (whether her offspring was to be male or female), and then the new numbers were tallied. This was repeated for 10 000 24-generation growth periods.

Mean values for the 10 000 simulation runs were calculated for each of the 24 reproductive periods. A cumulative frequency distribution of population sizes was computed for each reproductive period, and the lowest 1% of the 10 000 runs was noted. Population values bounding these proportions for the 24th reproductive period are shown in Table 1 (the bound for the proportion 0.01 is the upper bound of the first percentile). Four mortality values for adult flies were used (daily mortality of 0, 0.5, 1, and 2 %). If these are accumulated over a 2-week period, they translate into 14-day survivorships of 1.00, 0.93, 0.87, and 0.75, respectively, and these were used in the Monte-Carlo simulation. Since tsetse has a reproductive rate of half a female every 2 weeks (or 10 days, etc.), the fertility rate for 2 weeks is 0.5. Hargrove (1988) suggested that a tsetse population could not sustain 4%-added daily mortality regardless of the amount of density-dependence in its natural mortality.

Table 1 shows that, with 1% daily mortality, the mean number of adult females (after 24 periods) resulting from one gravid female reached almost 711, and one would expect that they would still be within some local common area, if it were suitable. Since these population numbers could vary widely, effective lower limits of the population were established (for the numbers below which it is expected that the total female population would be in 1 out of 100 cases) (Table 1). At this point, the questions become, "What is the minimum detectable density of flies?" and "What is

the maximal tolerable error level?" Once these questions are answered, an appropriate waiting period can be determined.

Table 1. Number of tsetse flies (means and 1% confidence lower values) resulting from 10 000 simulated growing populations after 24 reproductive periods starting with one gravid female or with one male and one female

Initial population		Survivorship per reproductive period			
		1.00	0.93	0.87	0.75
One gravid female	Mean	7480	2176	711	65
	Below 1%	830	1	1	1
One male and one female	Mean	11 211	3397	1157	112
	Below 1%	907	217	51	2

The proportions of the 10 000 simulation runs that by chance ended in population extinction are shown in Table 2 (for the conditions used). Most extinctions occurred during the first five reproductive periods.

Table 2. Proportions of 10 000 simulated growing populations that went extinct during 24 reproductive periods (table from Barclay and Hargrove 2005, reproduced with permission)

Initial population	Survivorship per reproductive period			
	1.00	0.93	0.87	0.75
One gravid female	0.00	0.14	0.26	0.52
One female, one male	0.00	0.08	0.16	0.36
Two gravid females	0.00	0.01	0.04	0.18

2.3. Monitoring Vectored Infections

If pests are difficult or impossible to detect at very low density, but nevertheless act as vectors of a disease, then, in addition to surveillance trapping, one supplementary method is to use antigen tests and assess if the prevalence of vectored infections is decreasing (Delafosse et al. 1996). An epidemiological model using a zero-biting frequency could be applied to test the hypothesis that insects are absent (but bites from non-infected insects would not be monitored by this procedure (Dyck et al. 2000)). The models of Rogers (1988) or Baker (1992) could be used for tsetse,

assuming that the insect density is zero. Infection rates in hosts should decrease with time. Also, if insects are truly absent, new host recruits should not be infected. A similar approach was used in the UK to decide if a warble fly (*Hypoderma* sp.) had been eradicated. In addition to trapping warble flies, blood sera from cattle were examined for antigens to the warble fly (Richards and Tarry 1992), and actually these blood antigens were detectable for several years after the last capture of a warble fly in a trap.

However, as this method has problems associated with it, it should usually be used only to provide additional information. The vector population may be too low for effective transmission, in which case the disease would decline even in the presence of a small vector population. On the other hand, diseases that do not kill the host may linger for a long period in the population after the vector is no longer present. Also, in antigen tests, false positives and false negatives are possible, and disease symptoms may be present without the real presence of the disease (Taze and Gruvel 1978), giving the impression that eradication of the vector is not yet complete. Nevertheless, if the vector species is difficult or impossible to trap, this method may be the only one available.

2.4. Summary of Requirements for Models

- Select sampling methods that will sample all individuals and stages of the population.
- Determine the detectability of each sampling method; to obtain good estimates, this is best done before the eradication programme begins.
- Determine the range of attraction for each trap and other sampling method used.
- Determine the fraction of the assessment area that is actually being sampled (product of the number of traps and the area of attraction of each trap, divided by the total assessment area).

3. ACTIVITIES BEFORE, DURING, AND AFTER ERADICATION PROCEDURES

3.1. Pre-Eradication Activities

Before embarking on an eradication programme for any insect pest, a variety of actions must be taken and information collected. These include the acquisition of scientific background information, and also the creation of a plan for these activities. This information will be of value for monitoring and surveillance activities during, and subsequent to, the eradication programme. At least one, and preferably several, sampling techniques suitable for each of the pest species must be available (Itô and Yamamura, this volume; Vreysen, this volume). Costs and logistic difficulties associated with each trap and other sampling method should be known.

3.1.1. Data Requirements and Complicating Factors

Choice of Risk Level. It is impossible to conclude with complete certainty that a population has been eradicated. The best that can be achieved is to state that the probability, that observed zero or null trapping/sampling results are consistent with the presence of insects, is sufficiently low to reject the hypothesis of insects being present. For this hypothesis-testing approach, a level of probability (the α or type I error level) must be specified; thus, when the probability of null results from trapping falls below this level, the hypothesis that insects are present will be rejected. The choice of α is arbitrary, and in ordinary science is usually 0.05. However, if human and animal lives are at stake, this level is probably too liberal. On the other hand, a lower level of α means that more trapping is needed to satisfy the probability requirement. Thus it is recommended that α be 0.01, and this value is used below; Kuno (1991) also used this level. Kuno (1978) pointed out that null hypotheses are usually framed in terms of absence, rather than presence. Thus, what we refer to as type I error with level α is usually referred to as type II error with level β, the convention used by Kuno (1991) and Yamamura and Sugimoto (1995).

Sampling Methods. Different sampling methods, that sample different portions of a population, are often applied in area-wide integrated pest management (AW-IPM) programmes (Vreysen, this volume). Insects with free-living immature stages will usually require sampling methods different from those applicable to mature individuals, and hungry insects may be attracted to lures that are different from those that attract sexually active or ovipositing individuals (Jang et al. 1999). Traps that minimize the capture of sterile males, and instead capture mainly females, are a special advantage in the case of male-only sterile releases (Katsoyannos et al. 1999). In attempting to sample a sparse (or perhaps non-existent) population, careful planning is needed to ensure that all portions of the population receive attention. If there are portions of a population that cannot be trapped or sampled otherwise, then, before applying the probability models to them, sufficient time must be given for them to become responsive to a trap. Given the propensity of insects to congregate in certain areas, detection trapping needs to be done throughout the assessment area that will eventually be designated "pest free". Any information about the location of aggregated insects, times of greatest activity, and relative efficiency of various trap types should be utilized to improve the chances of detecting insects that are at very low densities. In addition, to cover all life stages and physiological states, all effective trap types and other sampling methods should be utilized.

Detectability. Insect detectability (σ) can be estimated using a variety of methods (Thompson 2002); one method uses mark-recapture techniques (Itô and Yamamura, this volume). Before an eradication programme commences, this should be done for each trap type used. Detectability can be estimated by the ratio of the number of marked individuals in the second sample (r) divided by the total number of marked individuals (m) released in the first sample:

$$\sigma = r \,/\, m \qquad\qquad (13)$$

and σ has an approximate variance given by

$$\mathrm{Var}(\sigma) = (n \,/\, mP)\,(1 - m/P)\,(P - n)\,/\,(P - 1) \qquad\qquad (14)$$

(Thompson 2002) in which P is either the known or estimated total population size. The standard error can be calculated from the variance. A further complication is that detectability may not be constant, but density-dependent. In the probabilistic approach outlined above, accurate measurement of detectability is of crucial importance; the way that detectability varies with density must be determined. If detectability declines at low density, then even estimating it presents a sampling problem since sparse captures will give a poor estimate of detectability. As an example of detectabilities that may be encountered in some pests, Table 3 shows the approximate estimated mean efficiencies for two tsetse species. The results in Table 3 underline the importance of selecting, for each pest species, the appropriate sampling systems, and of providing at least approximate estimates of the efficiency of each system that is used. If trap efficiency is unknown, it is necessary to conduct studies that estimate this parameter; only then can one use the technique to make defensible statements about the probability of an area being free of a pest species.

Range of Trap Attraction. To apply the concept of detectability, it is important to define the area in which a trap will attract insects. This area will differ for each trap type, and therefore each type must be estimated independently, and perhaps also in different seasons and weather conditions. If there are areas not covered by a trap, then the traps should be moved around so that the total area is susceptible to trapping.

Problem of Immigration. Unless formidable natural barriers to movement exist, most insects will eventually move into a favourable habitat. Therefore eradication is possible only if such barriers exist or eradication programmes have long-term commitments to address large eradication areas sequentially (Wyss 2000a; Hendrichs et al., this volume). Even with barriers, such as large lakes, oceans, mountain ranges, forests, large tracts of grassland, etc., invasions may still occur occasionally as a result of phoresy, strong winds, persistent flying, etc. If trapping is not carried out systematically and at sufficient intensity, it may be impossible to distinguish between a reinvasion and an incipient population that went undetected. Indeed, to verify the eradication of the oriental fruit fly and melon fly in the Mariana Islands, trapping continued for several years; several reintroductions were detected (Mitchell 1980). (In this case, another fly species that was also attracted to the traps verified the traps' attractiveness.)

Unequal Propensity to be Trapped. There is genetic variability in virtually all biological characteristics. Many insects exhibit variability in their attraction to

pheromones and other natural attractants (Yaninek and Geraud 1989, Clearwater et al. 1991, Whittle et al. 1991). If eradication is attempted using only traps with odour attractants, there is a possibility that insects with a lower propensity to be trapped will eventually predominate (Barclay 1990, 1996; Shelly 1997). As the population declines, ultimately it could form a residual population that would not be detected by further trapping, and from which a rebound could occur. To avoid this problem, eradication should follow an integrated approach combining various control tactics, and assessment of the status of the population should deploy simultaneously several methods of sampling.

Table 3. Estimates of probabilities (converted to percentages) of catching Glossina morsitans morsitans *Westwood and* G. pallidipes *Austen using a variety of stationary and mobile baits (capture probabilities in this table for stationary baits apply to the probability for 1 day for flies in a 1-km^2 neighbourhood of the sampling device; for mobile baits the system may sample more than 1 km^2, but the time frame is the same (Vale (1974a, b) and Flint (1985) described the various sampling systems); table from Barclay and Hargrove 2005, reproduced with permission)*

Baits	*Glossina m. morsitans*		*Glossina pallidipes*	
	Males	Females	Males	Females
Mobile baits				
Standard fly-round	4.0	0.3	0.2	c. 0.1
Ox fly-round	4.0	0.5	0.8	0.2
Land Rover (electric net)	12.3	2.4	1.5	0.7
Decoy (electric net)	5.6	c. 0.1	< 0.1	< 0.1
Trolley (electric net)	9.5	2.1	3.9	1.4
Stationary baits				
Stationary ox	c. 0.1	< 0.1	0.2	0.2
Odour-baited F3 trap	c. 0.1	c. 0.1	1.0	1.0
Odour-baited electric net	c. 0.1	c. 0.1	1.9	2.0

3.1.2. Characteristics of Targeted Pest Species

- Population density. Population density is important in assessing both the control method and the progress towards eradication, and in determining the detectability of the trap types and possibly the effects of density on detectability.
- Reproductive capacity. In population growth and ease of control, the following parameters are important: fertility rate, generation length, number of

reproductive periods per individual, number of generations per year, probability of finding a mate at low density, and density-dependence of fertility.

- Mortality. Age-specific and stage-specific mortality, and density-dependence of mortality, are important in assessing both the method and difficulty of eradication, and also the speed of rebound from a small population after an attempted eradication or a pest reintroduction. Mortality is also important in assessing the probability of a small population becoming extinct by chance.

- Population movement. Dispersal rate and distance, probability of exotic introductions, home range or territoriality, and density-dependence of movement are important in determining the size of eradication areas and the area to be covered with monitoring traps. Individuals may move from a suboptimal habitat into areas of known concentration that have been eradicated.

- Periods of inactivity. Certain portions of the population may be inactive and therefore cannot be trapped or otherwise sampled. Periods of inactivity are important in determining how long each trap type has effectively been trapping flies, and thus how many sampling periods may be counted in determining the probability that no insects are present.

- Spatial distribution patterns. Knowledge of spatial distribution is important in placing the control effort and simultaneous monitoring in the most effective positions. The distribution of the pest population within the targeted area, with particular regard to terrain, vegetation, and other features likely to affect distribution and density, should be ascertained. This information is important in case it is unnecessary or undesirable to uniformly apply control actions over the whole target area.

3.1.3. Characteristics of Control Area

- Nature of terrain. This will determine the ease and cost of control and monitoring, and the logistics of trap location and visitation.

- Boundaries, size of eradication areas, and barriers to reinvasion. To prevent reinvasion, these must be known before eradication activities proceed.

- Vegetation patterns. These will affect the monitoring of insect density and distribution in suitable habitats.

- Temperature and precipitation regimes. Temperature will affect the speed of pupal development, influencing both the reproductive rate and the time required to ensure that no relict population exists after an eradication attempt, and precipitation regimes will affect the seasonal changes in vegetation patterns.

3.2. Pest Control Phase: Suppression

If the target insect is detected after eradication measures have stopped, more traps must be deployed immediately in the area where the insect was caught, and further control measures initiated. If, on continued monitoring, more insects are caught, control treatments should be continued.

By collecting field data before and during control operations, the success of the control treatment can be assessed (Itô and Yamamura, this volume; Vreysen, this

volume). As the density of the target population declines, the probability of a positive catch decreases. Simulation studies for tephritid fruit flies indicated that at very low population levels (capturing 0.001 flies per trap per week), consecutive zero catches for 16 weeks occurred in at least 5% of simulation runs (Clift et al. 1999).

However, given that the trap grid includes all the areas where the insects could be present, any relict population will increase and hence be detected eventually. In the case of tephritid fruit flies, this occurs after three generations (Lance and Gates 1994). Trapping records from an Asian papaya fruit fly programme showed that 12 weeks of successive zero catches occurred, even though wild insects were still present (Clift and Meats 1997, Clift et al. 1999). Often this represents a trade-off; it is not practical to have an optimal trapping grid of, for example, 400 m throughout the assessment area, but if the existing 1-km grid is maintained, some insects may go undetected until numbers build up to a detectable level.

3.3. Post-Eradication: Detection Trapping

The intensity of surveillance or detection trapping should be highest after control ceases (Hendrichs et al., this volume). Immediately following an eradication attempt, surveillance traps must be increased throughout the target area. These surveillance traps should be chosen for their efficiency in detecting the target species at low populations (Lance and Gates 1994, Katsoyannos et al. 1999, Papadopoulos et al. 2001). The trapping time needs to be sufficient to conform to the probability model, and allow the eventual probability of occurrence of any insect in the trapping area to be lower than the chosen risk level, e.g. a probability of 0.01 of being wrong.

Vreysen and Van der Vloedt (1992) described a sensitive biological method to determine whether or not eradication has been achieved. This consists of the release of virgin sterile female flies (female sentinels), and their recapture and dissection, to determine if, while in the field, they became inseminated by wild males.

It should be recognized that remnant insects might still exist in areas where control actions were less effective, e.g. sterile insects that did not reach narrow canyons or insecticidal sprays that did not penetrate dense vegetation. If there is a way to identify such potential problem areas before eradication activities start, then particular attention must be given to these areas immediately after eradication.

3.4. Evaluation Phases

The first evaluation phase would normally be the calculation of the probability of insects being present given a sequence of null trapping results while control actions continue. The calculation can be done using either of the probability models. The first one is on a trap-by-trap basis (most useful for spot infestations), and all traps must satisfy the risk criterion. The second is on an area-wide basis (more useful for most programmes dealing with established pests), and gives one probability. When this probability becomes small enough, then the conclusion is reached that no insects are present at that trap site.

If, after terminating control actions, the number of insects remaining is extremely low, such as one insect in 10 or 100 km^2, the small number of traps indicated by the second probability model might not catch any of the remaining insects, simply because the insects never come into contact with the traps (they are too widely separated). In this case, either more traps must be deployed, or they should be moved around to ensure that for some time they are located in the vicinity of the insects. Alternatively, the SIT may be continued for some time as insurance, as in the eradication of *G. austeni* in Zanzibar (Vreysen et al. 2000), or it is necessary to wait for the numbers to increase until they are detectable.

The second phase involves stopping control actions but continuing detection trapping and waiting for an appropriate period (its length dictated by the population growth model (equation (12)) to assess the possibility of resurgence of a small remnant population. Generally, before declaring "pest free" status, it would be advisable to wait long enough to allow the potential small population to grow to at least 10 times the minimum detectable level. If continued trapping proceeds without catching any insects, confidence in the eradication procedure will increase, either because elimination has succeeded, or an increase in the small remnant population would make detection easier.

In addition, there may be species for which, at present, no efficient trap exists. In this case, the trapping time would be unrealistically long or may even be incalculable, and there is little alternative except to wait (while still trapping) and see if a population rebound occurs during the minimal period. In such cases, a supportive technique proposed by Vreysen and Van der Vloedt (1992) is to release only virgin sterile females and, after recapture, assess if any of them had mated with wild males.

In both phases, it is advisable to monitor the progress of any diseases vectored by the insect pest, possibly using sentinel animals, trap trees, or other highly attractive features in highly suitable pest habitats. This is especially important in cases where detection trapping is very inefficient.

3.5. Criteria for Declaration of "Freedom from Pests"

The criteria for making a declaration of eradication involve the above two phases of monitoring, and both should be satisfied before making a declaration. In the first phase, a series of zero results will have been obtained from surveillance traps (while still continuing control actions). From these results, the probability of such a sequence is calculated in accordance with one of the two probability models. If this probability is sufficiently low (below 0.01), then control activity can be stopped but surveillance trapping must continue. These probabilities may be calculated from the inequality (6) above, i.e. $n > - 4.605 / \ln(1- \sigma)$ for the first probability model, or from inequality (11) above, i.e. $n > 4.605 / s\sigma p$ for the second probability model. When condition (6) or (11) has been met, the required probability has been achieved. If this calculation results in an unreasonably large number of traps, then only the second phase of the criterion might be required.

The second phase involves calculating the minimal expected size of a population resulting from the rebound of a remnant population after a period of time.

Calculating the population growth rate for the species is done using an appropriate population growth equation, such as the one in equation (12), and waiting until the expected population is at least 10 times the size of the minimally detectable population, assuming that a rebound is occurring. When both phases pass the numerical test, then eradication may be declared.

Throughout both phases, any diseases vectored by the pest should be monitored. By observing the presence or absence of new infections, especially in young hosts, the existence of continuing disease transmission can be ascertained (section 2.3.).

As an illustration, the practical implementation of both phases has occurred in Australia, where experience in eradicating incursions of both endemic and exotic tephritid fruit flies has accumulated for over 50 years (Madge et al. 1997, Hancock et al. 2000). If a fruit fly species is introduced (usually in infested fruit) into an area that was free of that species, the young adults will disperse from the point of introduction, and could take from 1 or 2 days to several weeks to mature, mate, and infest more fruit. Dispersal can happen during and after the pre-maturation period. In a fly-free area, an invading propagule of flies will disperse into a mate-free void, so that only the few that stay around the origin will be at a sufficiently high density to encounter each other and breed (Meats 1998a, b). However, once mated, a female can disperse any distance that her lifetime permits, and spread the infestation as a new generation. Thus, we can expect that the occurrence of adults in a usually fly-free zone would be clustered around the origin, and that the occurrence of larvae would be even more clustered.

In the case of the Mediterranean fruit fly, catching one or more male insects in a male-targeted trap indicates that supplementary traps (preferably baited with food lures attractive to females (Katsoyannos el al. 1999)) should be set up around it (CDFA 1999). In addition, a search for larvae is made within the array of supplementary traps. A catch of three male insects in the same or adjacent traps within 14 days, a catch of one female, or the detection of larvae in fruit, indicate the beginning of an outbreak and the need for the localized release of sterile insects within a 1-km radius. Also, the localized movement of soil or fruit is prohibited, and a wider "suspension zone" imposed. A formula is then applied to establish criteria for the reinstatement of "area-freedom" status, involving a period free of both control measures and fly detection. This period is equivalent effectively to 12 weeks, or the length of one generation plus 28 days, whichever is longer (Madge et al. 1997, Hancock et al. 2000).

3.6. Quarantine Issues

For agricultural pests that are transported in produce, it is feasible to impose quarantine on fruits, vegetables, etc. entering an area where a pest has been eradicated. Such quarantine would not cause financial hardship to local growers who benefit from the absence of the pest, although the quarantine process itself might be expensive. For pests of veterinary importance, it is advisable to establish a programme of quarantine and testing of hosts that are imported from infested areas. For pests of medical importance, humans are often the host and quarantine is not feasible. Therefore a medical history should be required of people travelling to areas

in which a disease has been eradicated; luggage should also be inspected to prevent the unintentional introduction of exotic pests.

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PART IV

SUPPORTIVE TECHNOLOGIES TO IMPROVE THE STERILE INSECT TECHNIQUE

CHAPTER 4.1.

ROLE OF POPULATION GENETICS IN THE STERILE INSECT TECHNIQUE

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SUMMARY

The detection and analysis of genetic variation in natural and laboratory populations are reviewed. The application of population genetic methods and theory can help to plan and evaluate the implementation of area-wide integrated pest management (AW-IPM) programmes that use the sterile insect technique (SIT). Population genetic studies can play an important role in estimating dispersal rates and thus gene flow among target populations, determining if sibling species exist, establishing the origin of outbreaks or reintroductions, and supporting the quality control of mass-reared colonies. The target's population history may be examined, in terms of "bottlenecks", range fragmentations, and expansions. Genetic methods can be helpful in distinguishing wild insects from released sterile or substerile ones, and in

ascertaining, together with mating cross-compatibility studies, the compatibility of mass-reared colonies with target wild insects.

1. INTRODUCTION

Population genetics, in the broad sense, is the study of gene frequencies in and among subdivided populations. It encompasses estimations of variation in terms of allelic and genotypic frequencies. Given appropriate data, the application of population genetic theory allows estimates of gene flow within and among populations, estimates of effective (i.e. reproductive) population sizes, tests for past "bottlenecks" and rapid expansions of population size, and pairwise genetic distances. Since all evolutionary change is accompanied by change in gene frequencies, population and evolutionary genetics overlap.

Methods of population genetics can be used to support the application of the sterile insect technique (SIT) for the control of target pest populations in nature. Briefly, the degree of genetic isolation of target populations from each other, and from untargeted populations, can be estimated. The existence of sibling species may be investigated by careful sampling and genetic analysis of pest populations. Immigration rates from unchallenged populations may be estimated, in terms of reproducing females per generation. The origin of pest outbreaks in treated areas may thereby be specified. The relatedness of mass-reared strains and target populations may be estimated in terms of allelic composition and gene frequencies. The question of laboratory "degradation" of strains destined for release can be examined in terms of inbreeding and genetic distance from founding and target populations.

Given what may be done by using modern techniques and adequate sampling of natural populations, it should nevertheless be noted that the New World screwworm *Cochliomyia hominivorax* (Coquerel) and Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) area-wide integrated pest management (AW-IPM) programmes were executed successfully without any substantial knowledge of the population genetics of these species (section 8) (Enkerlin, this volume; Vargas-Terán et al., this volume).

This chapter outlines the kinds of genetic variation that may be examined, and briefly reviews tests of hypothesis and analytical methods that may be applied to genetic data. In addition, the causes of genetic differentiation are discussed.

Population genetics is built on well-developed and sophisticated mathematical and statistical theory (but not reviewed here). Crow and Kimura (1970) and Wright (1969a, b; 1978a, b) covered the theoretical basis of population genetics. The analysis of population genetic data was examined by Nei (1987) and Weir (1996), and more recently by Rousset and Raymond (1997). Luikart and England (1999) reviewed the most recent methods of data analysis, and tabulated the necessary software. Wright (1969a, b; 1978a, b), Avise (2004), Hartl and Clark (1997), and Li (1997) provided general treatments of population and evolutionary genetics. Roderick (1996) and Black et al. (2001) have written particularly useful reviews of insect population genetics.

2. WHY POPULATION GENETICS?

- Sibling or cryptic species are reproductively isolated forms that are morphologically undifferentiated. If pre-mating isolating mechanisms exist, the application of sterile males of one member of a species complex may be totally ineffective against other members. Sibling species are common, e.g. the *Drosophila willistoni* Sturtevant species complex, the *Anopheles maculipennis* Meigen complex (Bates 1949), the *Anopheles gambiae* Giles complex (Coluzzi 1992, Coluzzi et al. 2002), the *Aedes scutellaris* (Walker) complex (Khambampati et al. 1992), the *Simulium damnosum* Theobald complex (Vajime and Gregory 1990), the sand fly *Lutzomyia longipalpis* (Lutz and Neiva) (Lanzaro et al. 1993), and the coccinellid *Coleomegilla maculata* (De Geer) complex (Krafsur and Obrycki 2000). Most complexes uncovered to date are medically important Diptera or genetically interesting *Drosophila* species, but this is probably sampling bias. Complexes important to agriculture include the tephritid flies *Anastrepha fraterculus* (Wiedemann) and *Bactrocera dorsalis* Hendel. Colonies of medically important Diptera are routinely established to study insecticide resistance, vector-pathogen transmission, etc., and intercrosses among strains may produce sterile progeny. Examination of polytene chromosomes, well developed in many Diptera, may reveal reproductively isolated forms, as demonstrated in some *Drosophila* species (Dobzhansky 1970). Many insect pest species are not easily reared or studied genetically, particularly herbivorous beetles and moths, hence the question of cryptic speciation may not arise and cannot be investigated easily.
- Natural populations tend to be more or less discontinuously distributed. Gene flow varies among them according to many factors, including the historical, geographical, and environmental. There may be local selection regimes that cause one or more populations to differ biologically in ways that could make the SIT less effective due to mating barriers. Such barriers might be ecological, temporal, or behavioural, and their strengths, in principle, could vary greatly. It should be noted, however, that no such mating barriers were detected in the New World screwworm and Mediterranean fruit fly programmes.
- Estimating rates of gene flow among populations can provide an estimate of the rate of invasion or reinvasion of eradicated areas from unchallenged populations. The rates would be described in terms of the average numbers of reproducing flies per generation. Moreover, the likely source(s) of pests in an outbreak can be examined and, in many instances, specified. For easily transported pests such as the Mediterranean fruit fly, the geographic origins of new infestations can, in principle, be specified. Determining the source of new infestations is an important undertaking because embargos may be levied on produce originating from areas where it is suspected that Mediterranean fruit fly populations have become established.
- Construction of laboratory strains for sterilization and release can be improved. If adequate numbers of insects are to be sterilized and released, it is necessary to establish thriving colonies. How much of the existing natural genetic diversity should be incorporated into a release strain? The geographic range of many pest

species is very large, and locally adapted populations may exist. How then should the genetic make-up of release strains be formulated?

- An analysis of genetic variation may also reveal genetic markers in laboratory colonies that can be used to differentiate released insects or the offspring of substerile insects from their wild counterparts during the monitoring of programmes that release sterile insects.

3.   CHIEF KINDS OF GENETIC VARIATION

Chromosome morphologies and structural rearrangements may vary within species and provide markers that distinguish populations (Robinson, this volume). Examples include paracentric inversions, easily detected in stained squashes of polytene chromosomes found in *Drosophila*, *Anopheles* mosquitoes, and the supernumerary chromosomes of some *Glossina morsitans* group tsetse flies (Gooding and Krafsur 2005). These kinds of variation rarely provide the high degree of resolution afforded by molecular variation in DNA or its transcription and translation products such as allozymes. Moreover, chromosome rearrangements are not selectively neutral as has been demonstrated in *Drosophila* species and *Anopheles gambiae* (Coluzzi 1992, Coluzzi et al. 2002). They may nevertheless provide important information about reproductive isolation and the operation of natural selection.

Allozymes are allelic forms of an enzyme. Isozymes are enzymes that act on a common substrate. Alleles are codominant. They are readily demonstrated by electrophoresis of tissue homogenates on starch, cellulose acetate, or polyacrylamide followed by histochemical staining that involves a dye or UV-fluorescence (Richardson et al. 1986, Murphey et al. 1996). Allozyme banding patterns correspond to genotypes, i.e. one band for a homozygote, two bands for a heterozygote at a monomeric locus, and three bands for a heterozygote at a dimeric locus. Most allozyme loci occur on the nuclear genome, but some loci, e.g. nicotinamide adenine dinucleotide phosphate (NADP)-dependent malate dehydrogenase, are mitochondrial. Allozyme analysis requires that tissue samples be fresh or frozen so as to preserve enzyme activities. This restriction does not apply to analysis of DNA.

Mitochondrial DNA (mtDNA) is single-copy, hence haploid, self-replicating, and is maternally transmitted as a circular molecule of about 16 kilobases (kbp). It encodes 22 transfer RNA genes and 13 proteins, including the cytochrome oxidases, NADH, RNAase, and ribosomal RNAs. There are many highly conserved regions from which oligonucleotide primers have been designed (Simon et al. 1994) that are commercially available in kits. These primers allow the amplification of regions of the mitochondrial chromosome by the polymerase chain reaction (PCR). Mitochondrial variation can be assessed by a number of methods. Nucleotide sequencing of amplified regions can be followed by the comparison of sequences among individuals. A less expensive method (appropriate for large sample sizes) is the single-strand conformational polymorphism (SSCP) method (Black and DuTeau 1996, Hiss et al. 1994). The SSCP technique involves PCR amplification of mtDNA, electrophoresis of the products on acrylamide gels, and visualization of the DNA by silver staining.

Restriction fragment length polymorphisms (RFLPs) are generated following the digestion of DNA with restriction endonucleases (REs) that cut at defined 4- or 6-base sequences. These enzymes cut non-methylated double-stranded DNA at specific locations, thereby generating a reproducible array of fragments that may be separated by size using gel electrophoresis (Dowling et al. 1996). End-labelling is required with nucleotides tagged with ^{32}P or ^{35}S, followed by exposure to X-ray film. There are hundreds of REs available, most with different recognition sequences. RFLPs are expensive to demonstrate, and are not ideal for large inter-population surveys. A further development for demonstrating restriction fragments in genomic DNA is the amplified fragment length polymorphism (AFLP) technique, in which the presence or absence of restriction sites is demonstrated (Vos et al. 1995). These DNA "fingerprinting" methods do not lend themselves to extensive population surveys, and are not discussed further in this chapter.

The use of randomly amplified polymorphic DNA (RAPD) involves PCR amplification using commercially available 10-mer random primers (Operon Technologies Inc., Alameda, CA, USA). Under low-stringency conditions, the primers anneal to complementary regions of template DNA, and products are obtained when the primer 3'-ends face each other on opposite strands. Amplicons may then be electrophoresed on acrylamide gels and silver stained (Black and DuTeau 1996).

Microsatellites are simple sequence repeats (SSRs) of nucleotides $(CA)_n$, $(GA)_n$, $(TA)_n$, $(CAG)_n$, etc. (where C is cytosine, A is adenine, G is guanine, T is thymine) that may occur throughout the nuclear genome. Each locus may vary among individuals in the number of repeats. Alleles are codominant. Conserved flanking sequences allow the design of oligonucleotide primers with which to amplify via PCR the microsatellite region. Comparatively sophisticated procedures are necessary to find microsatellites in the genome, and to develop oligonucleotide primers to amplify them. A substantial number of protocols are available to do so, e.g. Dowling et al. (1996). Molecular biological expertise is required to carry them out. Some protocols involve enrichment of DNA for repeat sequences to maximize the yield of desired repeats, e.g. Kijas et al. (1994) and Hamilton et al. (1999). Alternatively, one may choose to hire a commercial organization to isolate and characterize microsatellite loci, and for some laboratories this may be the fastest and least expensive way to proceed. Microsatellite loci have become the method of choice to evaluate the genetics of populations (Luikart and England 1999).

4. RELATIVE ADVANTAGES AND DISADVANTAGES OF PRINCIPAL METHODS

4.1. Chromosomal Polymorphisms

Chromosomal polymorphisms occur in many species. The most common can be a paracentric inversion, where two breaks occur in a chromosome arm followed by a 180-degree rotation of the interstitial piece and a rejoin of the three pieces. Most, if not all, seem to respond to selective forces, and therefore do not provide unbiased estimators of gene flow. They may nevertheless provide striking indicators of

genetic differentiation of populations. Cytological analysis is time consuming and requires much skill, and therefore adequate and representative sampling can be a problem.

4.2. Allozymes

The chief advantage of using allozymes is that it is probably the most cost-effective and rapid means of estimating gene frequencies. Capital expenditures are small compared with DNA methods, and much of the necessary equipment is readily available in many laboratories or can be made locally. Since alleles are codominant, heterozygotes may be detected unambiguously. The disadvantages are that very small insects may not yield enough homogenate for the examination of more than a few loci. Fresh-frozen insects are required in which enzyme activities are preserved. The availability of dry ice or liquid nitrogen can be a problem, particularly in tropical countries, and shipping frozen material is expensive and uncertain. There may be insufficient variation at allozyme loci. About 25–30% of the underlying amino acid variation is detected by protein electrophoresis. Some banding patterns may not be consistent with Mendelian inheritance patterns. Some allozyme loci may respond to natural selection via balancing selection, i.e. heterozygote advantage. A large chemical and biochemical inventory is required to carry out allozyme surveys.

4.3. Mitochondrial Variation Detected by Single-Strand Conformational Polymorphisms (SSCPs)

The SSCP method is particularly useful for the examination of mitochondrial variation (Zhang and Hewitt 1996). Large sample sizes may be evaluated, and DNA contamination from other species is seldom a problem (Rand 2001). Regions of the mitochondrial genome are highly variable, and some may be useful as population-specific markers. The mitochondrion is inherited as a single locus, and the effective population size (Box 1) is about a quarter of the size estimated by nuclear loci. This makes mitochondrial variation more sensitive to the effects of historical reductions in population size ("bottlenecks") and colonizing episodes. The reason is that the mitochondrial genome is single copy (haploid) and maternally inherited. Consistent and careful interpretation of silver-stained banding patterns must be practiced. Sequencing of SSCP phenotypic variants is necessary to confirm their allelic status (Norris et al. 1996). It will often be found that a gel phenotype consists of more than one haplotype. Thus, SSCPs may not detect all nucleotide variation present, but where it does so, the data may be used also for phylogenetic purposes, e.g. Hiss et al. (1994). SSCPs have been used to evaluate maternal gene flow in tsetse flies (Krafsur and Wohlford 1999, Wohlford et al. 1999, Marquez et al. 2004) and house flies (Marquez and Krafsur 2002).

Box 1. Basic Formulae Used in Population Genetics

Gene heterozygosity (diversity) at a locus is one minus the frequency of homozygotes expected by Hardy-Weinberg criteria, corrected for sampling bias (Nei 1987):

$$h_e = 2n(1 - \Sigma x_i^2)/(2n - 1)$$

where x is the frequency of allele i, and n is the sample size. For measuring diversity at haplotypes, use $n/(n-1)$ as the correction factor. The average heterozygosity over s loci is,

$$H_e = \Sigma h_e/s$$

with variance of

$$\text{Var} = \Sigma(H_e - h_e)^2/[s(s - 1)]$$

Under Hardy-Weinberg assumptions, the relationship between diversity H_e, mutation rate v, and effective population size N_e is:

$$H_e = 4N_e v/(4N_e v + 1)$$

Effective population size is, roughly, the number of successfully reproducing individuals. Note that $4N_e$ is likely to be a very large number and the mutation rate a very small number, of the order 10^{-6} for allozyme loci; both are difficult to measure.

The index F describes departures from random mating by taking the difference between expected heterozygosity h_e and the observed h_O as a fraction of the heterozygosity expected on Hardy-Weinberg criteria:

$$F = (h_e - h_O)/h_e, \text{ or } F = 1 - (h_e/h_O)$$

It gives the equilibrium value of the proportion of homozygous genotypes. F is a sensitive index of breeding structure, and may be expressed for hierarchical levels in subdivided populations. (It is described further in Box 3.)

4.4. Randomly Amplified Polymorphic DNA (RAPD)-PCR

RAPDs enable the quick demonstration of multiple loci. For many arbitrary primer pairs, numerous bands are quickly obtained, and many marker loci can be scored. However, about 90% of RAPD loci are dominant, heterozygotes cannot be demonstrated, and departures from random mating within populations cannot be estimated, thereby limiting studies of gene flow. Interpreting the banding patterns in acrylamide gels requires extreme care. Reproducibility is problematical, and results from one laboratory do not carry over to another laboratory. Slight changes in annealing temperatures, ramp times, buffer concentrations, quality of DNA, etc. can have large effects on the amplification products. Amplification of contaminating DNA can be a problem because the low annealing temperatures encourage binding to imperfectly homologous template. Black (1993) provided helpful information on, and discussed the pros and cons of, using RAPD-PCR.

4.5. Microsatellites

SSRs can be well distributed throughout the genome and highly polymorphic, which has both advantages and disadvantages. Microsatellite alleles can prove useful in pinpointing the source or sources of immigrant insects in a treated area. Automated scanning and scoring of acrylamide gels by using Genescan® and Genotyper® software allow high throughputs. However, it is time consuming and expensive to find and develop microsatellite loci, and molecular biological experience is necessary. Null alleles, usually caused by mutations in primer annealing sites, lead to underestimates of heterozygotes. Some taxa have few SSRs, including some culicine mosquitoes and ticks (Fagerberg et al. 2001). High mutation rates occur at some loci, violating a key Hardy-Weinberg assumption, and leading to homoplasy and biased estimates of gene flow and genetic differentiation of populations. Homoplasy, the convergent development of phenotypically similar repeats, is common in microsatellites. Highly variable loci with many alleles provide downwardly biased estimates of gene flow and genetic differentiation. Since many microsatellites do not conform well to the infinite allele model (the stepwise mutation model would be more appropriate) (Box 2), some analytical procedures (e.g. *F* statistics estimation) may be inappropriate, and it then becomes necessary to use other methods. Nevertheless, microsatellite loci have become the principle tool with which to examine the genetics of populations, leading to an explosion of powerful new analytical methods to examine the torrent of data now available (Luikart and England 1999).

5. BASIC PRINCIPLES OF POPULATION GENETICS

The Hardy-Weinberg theorem, or rule, is the fundamental template on which genetic data are tested. The rule specifies that in diploid, sexually reproducing organisms, genotypic frequencies will remain unchanged from generation to generation if the locus under consideration is selectively neutral, mating is random, generations are discrete, the mutation rate is negligible, and population size is infinite, so that no sampling errors occur in the transmission of gametes from one generation to the next. The foregoing assumptions, however, apply to few, if any, natural populations. Violations of Hardy-Weinberg assumptions may cause departures from random

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Box 2. Stepwise Mutation Model (SMM)

Change in microsatellite repeat number is thought to occur principally in a stepwise fashion by unequal crossing over and replication slippage. Two or more independently arisen alleles can have the same molecular weight, and therefore appear as one on gels (homoplasy). Clearly, there are upper and lower limits to the number of repeats a polymorphic locus can have, so the infinite alleles model of mutation is inappropriate. Thus, in theory, the stepwise mutation model (SMM) is most appropriate when dealing with microsatellite loci. Slatkin (1995) developed a model based on the SMM analogous to Wright's *F* statistics. He termed the index of gene flow R_{ST}. The basic difference is that *F* statistics are based on variance in allele frequencies, and R_{ST} is based on variance in repeat number. However, simulations have shown that, when sample sizes are less than 50 and the number of loci scored are less than about 20, *Fst* provides less biased estimates of gene flow than R_{ST} (Gaggiotti et al. 1999).

mating within and among populations. The principle cause is genetic drift — the random change in small gene pools due to sampling errors that occurs when the gametes of a parental generation unite to form the individuals of the next generation.

For diploid genomes, the probability of fixation (i.e. attains a frequency of 1) of a neutral allele is $1/2N_e$ where N_e is the "effective" population size (Box 1). For uniparentally inherited loci, the probability is $1/N_e$. Thus fixation, i.e. loss of diversity, is inversely proportional to the effective population size N_e. It should be noted that the effective population size may be much less than the census number because not all individuals reproduce, and some generate more progeny than others.

Most species are distributed more or less discontinuously in space and time, so individuals in a population will have a greater opportunity to interbreed with receptive individuals in their own population than with individuals in other populations. Thus, matings will not ordinarily be random among populations separated by distance, altitude, or age structure. The degree of genetic differentiation among populations can be used to measure gene flow.

Selection acting on a locus can also cause departures from Hardy-Weinberg expectations. Most genetic markers used in population genetics research are selectively neutral. However, some allozyme loci are not selectively neutral, but respond to balancing selection. An interesting example of balancing selection is that of the tsetse fly *Glossina pallidipes* Austen. Populations in Zambia and Zimbabwe show about the same level of heterozygosity as diverse populations in Kenya, but with much reduced levels of microsatellite and mitochondrial variation (Krafsur 2002, Marquez et al. 2004, Gooding and Krafsur 2005).

6. PROCESSING AND INTERPRETING POPULATION GENETIC DATA

Having scored haplotype or genotypic frequencies, it is necessary to test hypotheses about their distributions within individuals (if dealing with nuclear variation) and at hierarchical levels of population structure. Much software is available for these tasks. (A website maintained by the University of Washington (Felsenstein 2004) comprehensively lists, with annotations, many free software packages.)

The first step is to examine gene diversities for each population, and record the observed heterozygosity h_o. Next, calculate the expected single locus heterozygosities h_e, together with the expected heterozygosity averaged over loci H_E (Box 1). Allele or haplotype frequencies can be tested for homogeneity over sampling units by using the Chi-square contingency tests (Rice 1989) (these are incorporated into most software packages). If gene frequencies at all or most loci are homogenous among populations, then there is little likelihood of genetic differentiation among populations, assuming one is dealing with selectively neutral variation. It is then likely that the sampled populations behave as a single randomly breeding unit, and the SIT would likely have to contend with high immigration rates. Such a result does not necessarily argue against the successful application of the SIT, as is discussed below. However, it is most likely that allele frequencies among populations will differ, a consequence of population structure and genetic drift. Over time, drift and genetic differentiation can lead to the development of reproductive

isolation and, eventually, to speciation (Dobzhansky 1970, Futuyma 1998, Avise 2004).

The magnitude of genetic drift is inversely proportional to the effective population size (Box 1) (i.e. mean number of individuals that contributes progeny to a subsequent generation). Genetic drift is most expeditiously quantified by estimating F_{ST} or its analogues (Box 3).

The inverse of F_{ST} is proportional to the level of migration that satisfies the equation, $F_{ST} = (4N_e m + 1)^{-1}$, where the term $N_e m$ is the mean number of reproducing migrants per generation (Box 4). Figure 1 shows the relationship between $N_e m$ and F_{ST}. Wright (1978a, b) suggested that a critical level of migrants is about one reproducing organism per generation. A lower sustained migration rate among equilibrium populations would lead to further genetic differentiation by drift.

For discontinuously distributed (i.e. subdivided) populations, it is helpful to make estimates of F_{ST} between all possible pairs of populations. In this way one can test hypotheses that F_{ST} increases with geographic distance or other physical measure of interest. Highly differentiated populations can easily be identified in this way. Such local populations could, in principle, have a measure of pre-mating reproductive isolation that might work against the SIT. If deemed to be of interest, colonization, and laboratory and field-cage testing for pre-mating isolation, could

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Box 3. F Statistics

S. Wright's *F* statistics and analogues are used to describe the breeding structure of subdivided populations. The original statistical theory was formulated in terms of two alleles segregating at a single locus. *F* can be defined in terms of inbreeding, as departures from random mating, and as correlations between uniting gametes.

F_{IS} describes departures from random mating within populations; it is the correlation of random alleles within individuals averaged over populations, relative to the correlation of two random alleles from the population as a whole. Thus, the heterozygote deficiency in individuals is (Hartl and Clark 1997): $F_{IS} = (H_S - H_I)/H_S = 1 - (H_I/H_S)$. Here H_I is estimated as the average observed heterozyogosity among populations, and H_S is the expected heterozygosity.

F_{ST} indicates departures from random mating among populations; it is the correlation of two random alleles in a population relative to the correlation of two random alleles chosen from the population as a whole ($F_{ST} = 1 - [H_S/H_T]$), where H_T is the heterozygosity averaged over all populations). It was termed by Wright as the standardized measure of genetic variance among populations.

F_{IT} describes departures from random mating accruing from all causes ("inbreeding"); it is the correlation of alleles in individuals relative to that in the entire population ($F_{IT} = 1 - [H_I/H_T]$). Important extensions of *F*-statistics include Nei's G_{ST} (Nei 1987) and Weir and Cockerham's (1984) theta (θ).

F_{ST}, G_{ST}, and θ progressively underestimate differentiation of subdivided populations as gene heterozygosity h_e increases because as diversity $h \rightarrow 1$ in Hardy-Weinberg demes, F_{ST}, G_{ST}, and θ will approach zero. Indeed, they cannot take a value equal to or greater than the mean level of homozygosity (Hedrick 1999). Slakin (1985), Barton and Slatkin (1986), and Slatkin and Barton (1989) offered a way round the problem of high diversities. Slatkin observed a nearly linear relationship between the mean frequency of private alleles p_1 and the mean number of reproducing migrants per generation $N_e m$: $\log p_1 = a \log_{10} Nm + b$. Constants a and b vary with mean sample size. The method was found to be robust.

F_{ST}, G_{ST}, and θ are variance statistics. θ, for example, may be used in an analysis of variance for both diploid and haploid loci (Weir 1996). Thus, one can obtain variance statistics and estimates of θ for each level of hierarchy (Rice 1989).

Box 4. Gene Flow

According to Wright's island model of gene flow, F_{ST} can be defined in relation to the effective population size N_e, mutation rate ν, and migration rate m. $F_{ST} = [4N_em + \nu + 1]^{-1}$ for populations at mutation-drift-migration equilibrium. This equation allows the estimation of a migration rate in terms of the mean number of reproducing organisms per generation N_em. Thus, where the mutation rate ν is negligible (i.e. $\rightarrow 0$), $N_em \approx (1 - F_{ST})/4F_{ST}$ for diploid loci. For mitochondrial haplotypes, $N_em \approx (1 - F_{ST})/2F_{ST}$ (where the sex ratio approaches unity).

F_{ST} is non-linear because it is a reciprocal function of N_em (Fig. 1). Therefore, mean values of N_em can be quite meaningless, and caution is required in their interpretation (Whitlock and McCauley 1999). Pairwise estimates of F_{ST} and its linear transformations are very useful, however, and can be obtained by using "Arlequin" software (Schneider et al. 2000).

Some assumptions of the island model of gene flow include populations of equal size in which drift does not occur, all of which exchange alleles with equal probability.

Other models have been formulated, including the stepping-stone model, in which only adjacent populations exchange alleles.

However, the island model has proved sufficiently robust for many purposes, and it remains the most generally used. Theoretical developments have led to new applications, for example, the cladistic nested analysis of phylogeographical data (Templeton 1998).

An additional caution is necessary when using microsatellite loci to estimate F_{ST}. Mutation rates can be substantial at some microsatellite loci, leading to downwardly biased estimates of F_{ST}.

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then be carried out (Cayol et al. 2002).

Do high rates of dispersal argue definitively against the SIT? History indicates that they do not. Epizootiological and experimental studies have shown that the New World screwworm has a great capacity for dispersal, as might be expected in such a colonizing species. Genetic evidence indicated that mating was random among New World screwworm populations (Krafsur and Whitten 1993), but the high dispersal rates did not fatally compromise the effective application of the SIT to this species wherever it was applied.

7. POPULATION GENETICS AND CONSTRUCTION OF RELEASE STRAINS

How may the rules of population genetics be used to construct strains for eventual release? Thriving mass-reared colonies of the insect are required, the progeny of which, when radiosterilized, have to be competitive with wild males in seeking to mate with wild females (Calkins and Parker, this volume; Lance and McInnis, this volume; Parker, this volume).

It is often assumed that the "degradation" of laboratory stocks occurs via adaptation through selection and inbreeding, with a correlating loss of field competitiveness. Work on *Drosophila* showed a great loss of gene diversity (Briscoe et al. 1992) and fitness that was proportional to the duration of laboratory colonization (Jungen and Hartl 1979). On the other hand, no substantial loss of mitochondrial variation was detected in *Glossina pallidipes* (Krafsur and Wohlford 1999) or *Glossina morsitans* Westwood (Wohlford et al. 1999). Each case must be taken on its own merits.
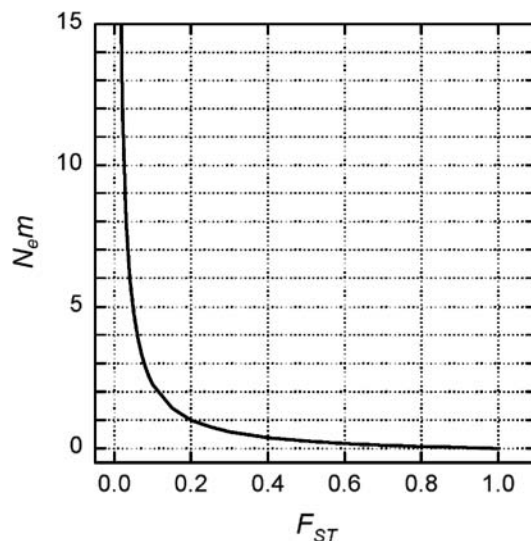
Figure 1. Mean numbers of reproducing migrants per generation exchanged among populations ($N_e m$) as a function of the mean departures from random mating (F_{ST}) according to the island model of gene flow. $N_e m = (1 - F_{ST})/4F_{ST}$.

During the course of laboratory adaptation, sexual selection that operates in nature may be relaxed because of the close proximity of mates in cages. For example, contact cuticular hydrocarbon pheromones in higher Diptera (Carlson et al. 1993, Brown et al. 1998), and swarming behaviour in nematocerous Diptera, could, in principle, inadvertently become modified during laboratory adaptation (Eberhard 2000, Briceño and Eberhard 2002, Briceño et al. 2002). The genetics and evolution of pheromone signalling and response behaviour has been studied in moths (Löfstedt 1993, Phelan 1997). Inbreeding causes a loss of genetic variation that could, in principle, reduce competitiveness. Are these realistic concerns? Is competitiveness in the field inversely proportional to laboratory adaptation? Do laboratory strains/colonies "deteriorate" with time?

The theory of inbreeding is well known; a simple account of elementary inbreeding theory is given in Box 5. A substantial loss of heterozygosity may occur if a release strain was formed from few founding insects, followed by a prolonged "bottleneck" in colony size. However, it is by no means certain that even a great loss in diversity will have pronounced effects on laboratory fitness or mating competitiveness in the field. Many colonizing species undergo periodic genetic bottlenecks in nature, with little adverse effect. The question of laboratory adaptation and competitiveness must be addressed on a case-by-case basis. In the New World screwworm, for example, estimates of sterile mating rates among

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Box 5. Inbreeding

Some consequences of inbreeding and drift include high frequencies of homozygosis and fixation of mildly deleterious alleles. Correlations between uniting gametes in "inbred" populations are greater than the correlations drawn from reference populations higher in the hierarchy. Loss of genetic diversity through drift is to be expected in closed populations. The inbreeding coefficient F (probability of identity by descent) in generation t is related to the effective population size N_e as follows:

$$F_t = 1 - (1 - 1/2N_e)^t \quad \text{As } t \text{ increases, } F \to 1, \text{ hence diversity} \to 0$$

Manipulating this equation shows that about half the diversity at selectively neutral loci is lost in $1.4N_e$ generations. For a breeding colony established from 2, 5, 10, 25, and 100 pairs the initial loss of diversity in a single generation is 22, 10, 5, 2, and 0.5%, respectively. These are trivial decreases. In a constantly expanding insect culture there would be little additional loss of variation. After 10 generations of "inbreeding" at the foregoing populations densities, however, heterozygosity losses become far greater, at 92, 63, 39, 12, and 5 %, respectively. Thus the duration of a "bottleneck" has profound effects on genetic variation. It is well to remember that these formulae apply to selectively neutral loci, but insects in culture are subject to various forms of selection, some of which may be for traits not advantageous in the wild. It should be noted, however, that weak selection intensity in very small populations can be overridden by drift (Black et al. 2001).

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geographically diverse target populations were found to be unrelated to the strain of sterile flies released (Krafsur 1985, 1994). Experience with *Glossina morsitans* suggested that colonized and irradiated flies dispersed and mated with their wild cousins at approximately the expected frequencies (Vale et al. 1976, Dame 1979).

In some cases, genetic phenomena have been invoked to explain pest outbreaks during the course of programmes that released sterile insects (section 8), but few genetic data were available to support the contentions. Nowadays, however, it is fairly easy to obtain genetic data for target populations, and for laboratory colonies and their source populations. Inbreeding coefficients (F) and genetic distances can (and should) be estimated continuously as part of a quality control programme. A progressive loss of diversity in laboratory colonies, coupled with a progressive decay in physiological quality control indices, could provide prima facie evidence of degradation, and possibly predict a decline in field competitiveness. If no data are collected, little of substance can later be said about genetic causes of, or correlations with, the failure of the programmes.

The rationale for constructing strains for propagation and release should include the number of founding organisms and their geographical origins. If there is good reason to believe that a target pest species may constitute a species complex, then it is necessary to ensure that only the pest species is cultured. Isofemale strains can be constructed for insects that reproduce readily in colony (an isofemale strain is based upon the progeny of a single mated pair). Many such lines can be constructed, and each evaluated for laboratory fitness and field competitiveness. If it can be shown that geographically adapted populations exist (Vera et al. 2005), then it would be prudent to conduct mating preference tests (Cayol et al. 2002). Such tests have strong environmental correlations, and thus experimental designs must be adequate to detect unambiguously a true genetic component. Where strong, genetically

determined mating preferences exist, of course it would be advisable to rear, sterilize, and release the most compatible strains.

The question of loss of diversity and fixation of deleterious alleles as a consequence of small effective population size can, in principle, become acute when constructing genetically engineered strains; the progenies are descended from a unique genome that may or may not be subject to recombination with wild-type alleles (Franz, this volume).

Finally, it is most important to put in perspective the issue of strain degradation. It is difficult and expensive to conduct field experiments that estimate the genetic and environmental components of competitiveness. In programmes that release sterile insects, however, sterile mating rates in target pest populations should be monitored routinely (Vreysen, this volume). Experience has shown that rearing, handling, irradiation, and release procedures are much more important for competitiveness than the laboratory degradation of release strains. A good exemplar is the North and Central American New World screwworm eradication programme (discussed below).

8. APPLICATIONS AND EXAMPLES

Experience suggests that studies of gene flow throughout a species' range are not necessary to implement and successfully carry out programmes that release sterile insects. The Mediterranean fruit fly and New World screwworm programmes were both conducted successfully for years without any population genetic data available. However the spectre of reproductively isolated screwworm populations was raised repeatedly, particularly when progress was slow or releases deemed ineffective (Krafsur et al. 1987). Some investigators were so certain of the existence of assortative matings between wild and released flies, and of reproductively isolated forms, that numerous claims were made in anticipation of their certain discovery. Indeed, a few geneticists played a controversial and unhelpful role (in the New World screwworm eradication programme in the USA and Mexico) by claiming evidence of assortative matings (Bush et al. 1976), or later, by claiming evidence that screwworms constituted a hitherto unrecognized complex of reproductively isolated forms (Richardson 1978, Richardson et al. 1982).

This situation, precipitated by screwworm epizootics in 1972–1976, was exacerbated by the practice of presenting and evaluating eradication programme results solely on the basis of screwworm case incidence. Programme officials invoked rainfall to explain the outbreaks, but no meteorological data were ever provided. The possibility of genetic changes in target populations was also acknowledged. In the absence of published meaningful data to support programme claims, interested commentators offered their own explanations, attracting much notice in the scientific community, and leading to attempts to stop the development of the Mexico screwworm eradication programme until critics could solve the very problems they claimed to have identified.

There was very little evidence that the screwworm outbreaks could be explained by powerful genetic phenomena or the existence of cryptic species. Actually there was much evidence to falsify the claims, had it been recognized and invoked by

programme officials (LaChance et al. 1982, Krafsur et al. 1987, Krafsur 1998). One of the lessons to be learned from this episode is that periodic estimates of gene frequencies from wild and mass-reared insects of a targeted species, together with predictive quality control measures, can be used to track changes that could, in principle, point to a decline in strain effectiveness for SIT implementation. The availability of such data, particularly when published in peer-reviewed scientific journals, can do much to defuse potentially damaging and confounding criticism from the scientific community. It would also be necessary, in those species where it is practicable, to maintain estimates of sterile mating frequencies, to show a relationship between sterility and sterile insect dose rates and target population densities (Krafsur and Hightower 1979; Vreysen, this volume).

Pest outbreaks during a sterile insect release programme can reasonably be expected, and it will be necessary to be able to specify the most likely causes. It must be anticipated that natural events, such as weather or the evolution of assortative mating, can, in principle, occur, but experience has shown that it is much more likely that failures in insect production, the distribution of sterile insects, or the implementation of complementary suppression measures will have caused this breakdown in control.

9. ACKNOWLEDGEMENT

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CHAPTER 4.2.

POPULATION SUPPRESSION IN SUPPORT OF THE STERILE INSECT TECHNIQUE

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SUMMARY

Suppression or eradication of insect pest populations by the release of sterile insects is often dependent on supplementary methods of pest reduction to levels where the target pest population can be overflooded with sterile insects. Population suppression activities take place in advance of, or coincide with, the production of sterile insects. Supplementary methods to remove breeding opportunities, or management methods that prevent access of pests to the hosts, may reduce the population or prevent damage. Insecticides have been used widely in direct applications or applied as baits, in traps, or on specific sites where the pest makes contact or reproduces. As sterile insect release does not kill the pest, adult biting pests or fertile mated females of the pests will continue to attack hosts after the release of sterile insects. Thus supplementary pest suppression programmes and quarantine measures are essential to prevent

damage or the spread of disease. Eradication or effective pest management requires that the entire population of the pest be treated, or that the programme apply immigration barriers. When supplementary pest control activities benefit the human population in areas being treated, such as in mosquito or screwworm eradication programmes, these activities are usually acceptable to the public, but when the public receives no direct benefit from supplementary control activities such as in fruit fly programmes, social resistance may develop.

1. INTRODUCTION

The sterile insect technique (SIT) is highly species-specific and non-polluting, and the target is the reproductive system of sexually reproducing pests. Supplemental systems to reduce pest populations are required, prior to the release of sterile insects, to reduce the target pest population to the degree that the sterile insects have an advantageous numerical ratio to induce sterility. Most of the successful programmes releasing sterile insects were applied when field populations were at low densities.

The decision to use suppression before the release of sterile insects may also be for economic reasons. Quarantine decisions, on market access of commodities attacked by pest outbreaks, are frequently based on adult trapping data. Reducing the adult population to below the detection level with adulticide sprays adds the benefit of meeting quarantine protocols and reopening markets sooner than when the sterile insects have eliminated the population.

In other cases, the action of released sterile insects on the pest population is indirectly associated with reduction in pest damage. Mosquitoes and tsetse flies can continue to bite and spread disease after they are mated to sterile flies. Fertile-mated screwworms can, for the rest of their lives, continue to destroy livestock. These activities are not reduced by the release of sterile flies. Decisions to use pesticides or other methods to protect hosts will, in these cases, be largely independent of the success of the sterile insects.

In this chapter the various pest control techniques that are used to suppress pest populations, in conjunction with the application of the SIT, are reviewed. Suppression activities applied as precursors, or in tandem with sterile insect release, will be emphasized. Quarantine treatments will not be considered.

2. OVERVIEW OF PEST CONTROL TECHNIQUES

Major benefits of the SIT are species specificity and the possibility of eradication. Knipling (1979) outlined the techniques for reducing insect pest populations with respect to the species specificity (Table 1), and to their effectiveness with respect to pest density (Table 2). Table 2 is of particular relevance to the SIT as it relates to the density of the pest population. He followed this overview with a discussion on integrating these techniques with the SIT.

Knipling (1979) also discussed a series of exceptions and modifications to this classification, and recent research has greatly extended the number of available suppression methods. The method of application was also recognized as being of crucial importance in selectivity. For example, aerial application of broad-spectrum insecticides will have a greater impact on non-target organisms than application of

the same insecticide to a specific location on a plant or animal that is to be protected from a pest.

Natural biological control is inevitably a part of any area-wide integrated pest management (AW-IPM) programme, but specific releases and manipulation of parasitoids and predators are being investigated as part of AW-IPM systems that include the SIT (Wong et al. 1992; Knipling 1992, 1998, 1999; Bloem et al. 1998; Vargas et al. 2004). Carpenter et al. (this volume) describe the synergism between inherited sterility (IS) and natural enemies. In a recent programme in New Zealand where irradiated male painted apple moths *Teia anartoides* Walker were released, *Bacillus thuringensis* Berliner variety *kurstaki* was also applied to suppress the pest population (O'Callagahan et al. 2003; Suckling 2003; Bloem et al., this volume).

Table 1. Classification of degrees of selectivity of various methods of insect control
(Table from Knipling 1979)

Highly selective	Moderately selective	Non-selective
Insect-resistant plant varieties	Predators (general)	Conventional insecticides
Insect pathogens (specific)	Parasites (general)	Cultural measures
Insect parasites (specific)	Light traps	
Insect predators (specific)	Attractants (baits)	
Insect attractants (specific)		
Genetic techniques		

Table 2. Classification of insect control techniques by efficacy at various pest densities
(Table adapted from Knipling 1979)

Methods equally effective at all densities	Methods most effective at lowest densities	Methods most effective at highest densities
Conventional chemical insecticides	Sterile insect and other genetic techniques	Host-specific pathogenic organisms
Chemical sterilants applied to natural populations	Sex- or aggregating-attractant traps	Host-specific parasites
Cultural and mechanical control methods	Sex-attractant diversion sources	Host-specific predators
Insect-resistant crop varieties	Sex-attractant vapours	
Light traps		
Attractant baits		
Trap crops		
Synthetic non-pheromone attractants		
Sex pheromones that block responses		

Knipling (1979) followed this table with an introduction to the concept of IPM, including descriptions of the purposes of pest management: to slow population growth, suppress and maintain populations below a certain level, or eliminate populations.

3. CULTURAL AND MECHANICAL CONTROL

Activities in agricultural production, property management or lifestyles can all influence insect pest densities, and a major advantage of cultural control is that it is pest density-independent. The disadvantage is that many cultural control activities reduce the population but do not protect the crops or animals being attacked. In cases when very low pest populations can have high economic or health impacts, cultural control through habitat manipulations is not likely to provide the desired level of suppression.

Development of the SIT technique for control of the New World screwworm *Cochliomyia hominivorax* (Coquerel) relied heavily on activities that prevented infestation of livestock. The pest was subjected to considerable cultural control because of the value of livestock and the critical damage from infestations to animal and humans Programmes for treating wounds, and preventing reinfestations of individual animals, were the primary actions taken to control screwworm damage. Dove (1938) proposed a combination of livestock management such as special care with wound protection, protection of females and offspring following pregnancy, curing tick bites, protecting castrated and branded animals, and other management activities. Protecting animals from infestation, and wound treatment, are still the key suppression activities in the screwworm programme.

Cultural control of the pink bollworm *Pectinophora gossypiella* (Saunders) (Nobel 1969) in the USA was developed in the early 1950s as part of an area-wide approach as the pest spread across Texas, New Mexico and Arizona. Activities included evaluation of stalk-shredding machines for killing the potential overwintering insects and incorporating the use of shredders. Devices were developed that killed bollworm larvae in cotton gin trash. In contrast to the screwworm programme, the impact of these activities was to decrease the pink bollworm population rather than directly protecting the crop.

Early reviews of fruit fly pest control focused on environmental modifications to reduce reproduction and the survival of immature stages, and chemical control to kill adults. Back and Pemberton (1918) described covering of immature fruit with a bag or cloth material to prevent infestation. They also described a system used in Australia of bagging the canopy of trees with mosquito netting, but considered the method too expensive for large-scale use. Individual bagging of fruit was successful, but the bag had to be impermeable to oviposition, and problems with scale insects on the protected fruit developed.

Another cultural control approach to fruit fly pests was described as "clean culture" (Back and Pemberton 1918). This method is based on removing all hosts from the infested area. Crawford (1927) reviewed clean-culture methods used in Mexico and determined that the approach was effective for the Mexican fruit fly *Anastrepha ludens* (Loew), but extreme measures such as the destruction of trees

were not practical or effective. He compared ranches that cleaned up fallen oranges once a week, and found the approach ineffective for complete control but more effective when coordinated with the application of poison bait. Crawford also recognized the value of trap crops in controlling fruit fly damage; he suggested that a few grapefruit trees (a preferred citrus species) could be used for this. He also recognized that the trap-crop trees could be sprayed.

The application of "clean culture" to fruit fly management programmes has been widely practiced in AW-IPM programmes integrating the SIT in the USA and the joint programmes in Mexico and Guatemala. Destruction of host material is widely carried out in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) eradication programme (Moscamed) in Mexico and Guatemala. During a series of outbreaks in the Palenque district of northern Chiapas, from 8332 hectares treated (e.g. bait sprays), 33 978 kg of host material were collected and destroyed (Moscamed data sheets for 2002). In Guatemala, from 411 739 hectares treated, 345 633 kg of host fruit were destroyed (USDA/APHIS/IS 2002). As an immediate response in 2003 to a large infestation (106 hectares) of Mexican fruit flies in Valley Center, California, the California Department of Food and Agriculture removed and buried host fruit. A total of 2 941 070 kg of fruit (mostly citrus) was collected from the ground and stripped from trees at sites ranging from 0.26 to 36 hectares. The removal and destruction of host material is a logical tactic because this activity destroys immature stages of the pest that cannot be controlled by other methods. However, there is a possibility that the removal of host material induces adult females to migrate in search of hosts, and actually causes an outbreak to spread.

Where the primary purpose of the SIT is to eliminate diseases through eliminating the vectors, cultural and mechanical controls have been applied widely. In addition to pesticide application as a supplementary treatment incorporated into programmes that also release sterile insects for mosquito control, Musil (2002) outlined improvements in integrated activities that reduce the vector populations, and in health care supplemental activities:

- Habitat management that interferes with the mosquito life cycle, including physical barriers and the use of beneficial organisms
- Community education and participation to improve public understanding of the mode of disease transmission
- Public health strategies that integrate detection, diagnosis, and prompt treatment of the disease to prevent spread

4. TRAPPING FOR CONTROL

The first area-wide programme for screwworm control was attempted by setting out a series of meat-baited traps in Menard County, Texas, USA, and the fly population and rate of infestation in the treated and untreated areas were compared (Laake and Cushing 1930). At that time both the New World screwworm and the secondary screwworm *Cochliomyia macellaria* (F.) were classified as *C. macellaria,* so counts represented both species. A total of 300 traps was maintained during the entire fly season (March-October), but 361 traps were added on August 10 to bring the total to 631 traps in 62 677 hectares. The non-trapped area was 58 623 hectares.

The data in this report show an interesting relationship. The population estimates of *Cochliomyia* spp. in the two zones showed that the adult population in the trapped zone was reduced by more than 83% (550 in the trapped area versus 3409 in the control area). However the myiasis rate was only reduced by about 50%, even after the data were corrected for cases due to movement of animals and off-season births. This discrepancy could be due to the pooling of the two species, and the fact that *C. macellaria* is normally many times more abundant than *C. hominivorax.* Also Laake and Cushing (1930) found that the removal of a big portion of the population did not proportionally reduce the rate of infestation.

Leak (1999) reviewed the use of targets and traps for tsetse fly control. These techniques used artificial odours, colours, or targets attached to workers. Targets on workers were a component of the eradication of tsetse from Principe in 1910, and a tsetse-control technique using odour attractants and traps was proposed by Balfour in 1913. DDT-treated traps were proposed by Vanderplank (1947) and tested successfully.

5.   CHEMICAL CONTROL

5.1. Direct Insecticide Application for Adult Population Suppression

Broadcasting insecticides has often been done in conjunction with AW-IPM programmes that integrate the SIT. However, public acceptance of insecticide application in this context has usually been limited to situations where the treated properties are owned by persons who receive some benefit. Eradication experiments, and programmes with medically important pests such as mosquitoes and tsetse flies, have frequently used broadcast insecticides. Programmes for pests such as the pink bollworm, with a distribution largely restricted to certain hosts, were also implemented with insecticides but with little publicity, though more recent supplementary suppression activities are based on attractants (Walters et al. 2000a). Suppression of the codling moth in Canada required insecticide treatments to reduce the pest population (Bloem and Bloem 2000). If an insecticide is applied at the same time that sterile insects are being released, the chemical may kill some of the sterile insects; however, as long as the ratio of sterile:wild insects remains the same, this would not impair the efficacy of the SIT.

In the 1960s, when the first attempts to use the SIT or other genetic modifications of released mosquitoes were made, broadcasting insecticides to suppress mosquitoes was widespread. Insecticides were also applied for the control of tsetse flies in Africa, so as the programmes for these species developed, applying insecticides was a component. Langley (1999) summarized the control techniques that are presently used for tsetse flies (but also used in similar applications for mosquitoes) as follows:

- Sequential aerosol technique (SAT). Spraying ultra-low-volume formulations of insecticides from the ground (fogging) or air (fixed-wing aircraft or helicopter) with limited environmental impact. The goal is to kill adult tsetse flies in the first spraying cycle by direct contact, and kill emerging flies in subsequent cycles.

- Stationary attractive devices (traps and insecticide-impregnated targets). The goal is to impose a modest daily mortality on tsetse females by attracting them to a device that either kills the flies by contact with an insecticide or retains them in a non-return cage. Pyrethroid compounds were identified as the principal insecticides used, but sterilizing compounds and compounds that inhibit reproduction such as triflumuron may also be effective (Oloo et al. 2000).
- Live-bait technology (pour-on). An efficient technology in tsetse-infested areas with a high density of cattle, but disadvantages are the high frequency of treatment and high cost of insecticides.

According to Douthwaite (1992), the first area-wide attempts to control tsetse flies with insecticide sprays began in 1945 in South Africa using organochlorine insecticides. This programme resulted in the successful elimination of *Glossina pallidipes* Austen from 11 000 km^2 through the aerial application of DDT or lindane, and supported by game destruction, habitat clearing and massive trapping operations (Du Toit 1954). Although the negative effect of these treatments on beneficial insects was recognized, and an impact on bird populations was reported by Graham (1964), persistence and bioaccumulation of residues was not understood at the time.

The first tsetse programme using sterile mass-reared insects was carried out against *Glossina morsitans morsitans* Westwood in Tanzania (Dame et al. 1980, Williamson et al. 1983). The strategy of this test was to suppress the tsetse population with two aerial applications of endosulfan (28-day interval) and then control the population with sequential releases of sterile males. A 195-km^2 area was surveyed for 14 months using various trapping methods, and the reproductive status and density of the population were assessed. A 105-km^2 area was selected for treatments. A 1-km barrier was cleared and treated with manual backpack spray applications of DDT in a 300-m-wide swath to prevent the migration of flies into the treated area. Fly surveys showed that, after the first endosulfan application, there was 100% reduction in *G. m. morsitans* and 91.5% reduction in *G. pallidipes*. Following the first spray, sterile *G. m. morsitans* males were released twice per week at a rate of 135 males per km^2, resulting in an average male sterile:wild ratio of 1.12:1. A comparison of the female reproductive status between control and treated areas showed that, following the second spray, the sterile males were highly effective. Over the 15-month sterile-treatment period, Williamson et al. (1983) reported an 81% reduction in *G. m. morsitans*. The population of *G. pallidipes*, which received only the insecticide treatment, recovered to pre-spray levels within 5 months.

A similar strategy using trapping techniques, insecticide-impregnated targets, and the release of sterile males, successfully eliminated *Glossina palpalis gambiensis* Vanderplank, *Glossina tachinoides* Westwood, and *Glossina morsitans submorsitans* Newstead from 3500 km^2 of agro-pastoral land in Burkina Faso (Politzar and Cuisance 1984). Prior suppression of the native fly populations was achieved by placing insecticide-impregnated screens along 650 km of gallery forest at a density of 10 screens per linear km for 4 months during the dry season. Subsequent releases of sterile males, at the rate of 20–35 per linear km, were sufficient to obtain sterile:wild ratios of 10:1 and to eliminate the target populations.

A trial against *Glossina palpalis palpalis* Robineau-Desvoidy was carried out in central Nigeria. First the population was reduced by deploying insecticide-impregnated screens and by removal-trapping with traps (Oladunmade et al. 1985, Takken et al. 1986) that reduced the native fly population by 90–99% over a 6–12 week period. Extending the period of control, using traps and targets, did not achieve eradication. A major concern was the loss of the screens due to theft, flooding and fire (Takken et al. 1986). Then the target population was eliminated over the entire 1500-km^2 area by weekly releases of sterile males from the ground (Oladunmade et al. 1990).

The AW-IPM programme against *Glossina austeni* Newstead on Unguja Island (Zanzibar) relied on the use of live-bait technology (in areas of high cattle density), and the deployment of insecticide-impregnated screens (in the forested areas), to reduce the native tsetse population (Vreysen et al. 2000). The fly densities in the primary forest habitats were reduced 80–98% by using insecticide-impregnated screens, deployed at densities of 40–70 per km^2 for a period of 18 months (Vreysen et al. 1999).

Patterson et al. (1980) described a field trial to control the stable fly *Stomoxys calcitrans* (L.) with the SIT as an adjunct to insecticidal and physical methods.

The need for supplemental programmes in mosquito management was expressed by Rao (1974) in an early review of the World Health Organization and Indian Council of Medical Research programme:

> Simultaneous progress in accomplishing control of mosquitoes by conventional methods is essential, for it is a well-known axiom in the theory and practice of genetic control that it can, in the present state of knowledge, only supplement the general methods and cannot supplant them.

Pal and LaChance (1974) reviewed early trials of genetic control during the late 1960s and 1970s. They cite the need for supplemental suppression activities to reduce the number of released sterile insects that are required. They suggested that, if control methods are to be applied concurrently with the release programme, the best chemical control would be a larvicidal programme that killed target insects without affecting released insects. If a pre-release suppression programme is used, then an adulticide treatment would be preferable.

Weidhaas et al. (1962), Morlan et al. (1962), and Patterson et al. (1970) performed field trials with sterilized mosquitoes in Florida, USA. In these tests DDT applications were made in perimeter areas to prevent the immigration of pests or as treatments to reduce populations. Patterson et al. (1970) concluded that:

> Obviously, other population suppressants such as insecticides, reduction in breeding sources, and biological control will have to be used to decrease a total population in a large area to a level commensurate with the mass-rearing capabilities.

Trials of genetic control of *Culex pipiens fatigans* Wiedemann in villages near Delhi, India, in a programme developed with the World Health Organization, were among the first to apply integrated supplemental population suppression as an experimental component. After several attempts to introduce sterility into the populations (reported from 1971 and 1972 tests with negative results), tests were designed to distinguish the effects of rearing, chemosterilization and strain genetics

from the effects of strain contamination with females and immigration (Pal and LaChance 1974, Pal 1974). Yasuno et al. (1976) reported the results of a 5-month sterile insect release (February to July 1973) of chemosterilized males into an area with mosquito fish *Gambusia affinis* (Baird and Girard) or larvicide-treated (temephos) breeding sites in buffer zones. A second treatment of an adulticide, pyrethrum, was applied to one set of villages. Although the Delhi programmes, summarized by Pal (1974), Yasuno et al. (1976) and Curtis (1977), were not permitted to continue to the stage of measuring population suppression, the programmes proposed in these tests included population monitoring and integration of adult and larval control. A more recent review of the potential for mosquito control (Curtis and Andreasen 2000) cited the importance of barriers to immigration of females into treated areas. Immigrant females not only serve to increase the target population and impede eradication, but may also reintroduce the disease and set back the ultimate goal of eradication.

Recent proposals (Jayaraman 1997) to begin SIT treatments for *Anopheles* spp. will need to consider the increased resistance to pesticides (Mattingly 1957, Pal and LaChance 1974, Asman et al. 1981, Whalen 2002). Classic alternate methods of population reduction (Ross 1902), through habitat modification and more recent alternative methods both to reduce mosquito populations and protect people from bites and disease, will be required in any new programme as alternatives to the general spray programmes used in previous SIT experiments.

5.2. House, Bednet, and Other Treatments

The use of insecticide-impregnated bednets has proven to be a successful treatment to reduce malaria morbidity. The development of the pyrethroid insecticides that are safe for human contact has supplied a substitute (for DDT, organophosphate and carbamate insecticide treatments) that gives more direct protection than outdoor sprays to control populations. According to Curtis (2002), treated nets can irritate, drive away or kill biting mosquitoes. Numerous tests have shown that this treatment greatly reduces both populations of mosquitoes and rates of disease. The use of insecticide-treated bednets, as well as treatment of curtains, wall hangings and clothing, have also been tested, but bednets, which act as a trap baited with the sleeping person, have proven more effective.

More than 20 tests of bednets have demonstrated a reduction of 20–63% in malaria disease rates. Tests carried out in The Gambia, Kenya and Ghana showed a significant (25–39%) reduction in mortality in children. Mathenge et al. (2001) found that bednets reduced the rate that some mosquitoes (but not others) entered houses, and the action of bednets against mosquitoes was also species-specific. Slight shifts in feeding times were also noted for one species (but not the other). The success of bednets is reduced for mosquito species that bite earlier in the day. Treatment of other things in a household may help control disease transmission by these species.

5.3. Chemical Treatments to Protect Hosts from Biting Adults or Immature Stages

Wound treatments have been an important and consistent part of the New World screwworm programme in North America (Graham 1979). In addition to reducing overall screwworm populations and protecting livestock from larval damage, the research programme to develop wound dressings had direct effects on the programme. The early development of a treatment called "Smear 62" (Knipling 1939) led to research methods that included rearing larvae on artificial diets. Not only are wound treatments an essential supplementary component of this programme that releases sterile insects, but research in developing these treatments also led to the implementation of the programme.

The use of small packets of coumaphos, chlorfenvinphos or similar insecticide, applied either as a spray on cattle or as an individual treatment to infested wounds, was an essential part of the screwworm eradication programme in Florida, southern Texas and Latin America. Unlike attempts to trap out or reduce adult screwworm populations by applying insecticides area-wide, larvicidal applications of insecticide directly saved livestock, and producers could directly observe the application's benefit. During the breakdown of the programme in 1972, larvacide treatments were the main mechanism that saved livestock and slowed the spread of cases. During the late 1970s, when the programme was stalled in northern Mexico (Coppedge et al. 1980a), the lack of progress was attributed to both ineffective sterile flies and the lack of animal protection by livestock producers.

The efforts of the Mexican-American programme to use the coumaphos packets in conjunction with releasing sterile insects were very successful. Through grower education and publicity, producers were informed that these packets were provided by the inspectors of the programme. The ability to provide producers with an effective and free treatment for infested cattle was surely a major factor in gaining access to ranches in Mexico and Central America. Historically and culturally, ranches in these regions were not open to outsiders.

5.4. Pour-ons — Insecticide Applied to Livestock

A programme to control tsetse flies or trypanosomosis by treating livestock with insecticides can be effective, either by killing flies as they attack animals or by a repellent effect so that animals are not attacked. Leak (1999) stated three conditions required to achieve optimum control of tsetse populations by pour-on treatments: (1) a large proportion of the feedings are taken from domestic rather than wild animals, (2) a large proportion of the livestock are treated, and (3) the level of fly reinvasion is relatively low.

Initially pour-on insecticides consisted of DDT mixed with resins (Leak 1999). Other tests were done feeding lindane to cattle. Although applying these pesticides to cattle was terminated because of environmental concerns, the development of synthetic pyrethroids revived this treatment method. These insecticides have the advantages of low human toxicity, high insect toxicity (especially to tsetse flies), and rapid movement through the epidermis. Ivermectin compounds were also

discussed, but the effective dose is very close to the limit for toxic effects to the hosts, and cost is prohibitive.

The eradication programme in Zanzibar was specifically planned to meet environmental concerns (Vreysen et al. 2000) for supplementary population control. A combination of insecticide pour-ons and insecticide-treated screens (targets) was the principal means of population control.

Vreysen et al. (2000) concluded that the pour-on treatment alone was not sufficient to eradicate the tsetse population from the island. Possibly this was due to flies feeding on hosts other than cattle, such as bush pigs, which enabled some flies to be unaffected by the cattle treatment. They also cite tests in Burkina Faso where the application of deltamethrin to cattle failed to eradicate *G. p. gambiensis* because the preferred hosts, monitor lizards, were available for feeding by tsetse. Apparently pour-on insecticides can drastically reduce tsetse fly populations, but untreated wild animals may serve as alternate food sources for the sustenance of the population.

5.5. *Use of Insecticide Baits*

An area-wide bait-insecticide treatment to control screwworm adults was developed in the 1970s in conjunction with activities improving baits for monitoring populations. Mackley and Brown (1985) reviewed the development of swormlure, an attractant, and SWASS (Screwworm Adult Suppression System) pellets. In Texas, screwworms were believed to migrate hundreds of kilometres, so extremely large plots were needed to avoid the confounding effects of flies migrating into the treated areas.

SWASS pellets were tested, through "before and after treatment" observations, in Curaçao, Texas (Coppedge et al. 1980b), Colima (Tannahill et al. 1982), and Veracruz (Spencer and Garcia 1983). The bait swormlure was used to attract and sample adult screwworm flies. Wounded animals were used to collect eggs and thus sample the reproductive capacity of the fly population. The general pattern in the experiments was a reduction in populations trapped and in reproduction. It was concluded that swormlure and SWASS were attractive and toxic, respectively. However, since the experiments were not replicated, no statistical conclusions about the effects of the SWASS treatments can be drawn. One site did not fit the pattern; in Veracruz, reproduction in the fly population did not decrease but increased. The most likely reason for the failure of applications to reduce reproduction in screwworm populations in Mexico may be related to the very patchy distribution of target females. This situation may be similar to the attempts to trap out populations (section 4). That is, although the baits reduced the total population, they may not have reduced the reproductive potential of localized populations. Another consideration is that, in wet areas such as Veracruz, the formulation of the SWASS pellet was such that it dissolved when wet (Mackley and Brown. 1987), and the pellets may not have persisted long enough to reduce the populations.

Moreno and Mangan (1995, 2002) reviewed the development of improved insecticide baits for fruit flies. At the beginning of the 20th century entomologists discovered that fruit flies would feed on toxic chemicals contained in baits composed of various sugars. In search of an insecticide programme for the control of

the recently established Mediterranean fruit fly in Hawaii, Back and Pemberton (1918) reviewed the research status of edible baits for use with arsenic poisons. The principal baits were carbohydrates and fermenting substances such as sugars, molasses, syrups, and fruit juices. McPhail (1937) found that sugar-yeast solutions attracted several species of *Anastrepha*, and, in 1939, found that protein lures were attractive to these species. In 1952 Steiner demonstrated the use of hydrolysed proteins and partially hydrolysed yeast in combination with organophosphate insecticides to control fruit flies, leading to the attracticides currently used. The first protein-hydrolysate baits contained protein hydrolysate, sugar, and parathion (Steiner 1952). The early fruit fly eradication programmes in the USA relied on attracticide baits using DDT or organophospate pesticides. Flies responding to the attracticide needed only fume exposure, or to contact, taste, or ingest the mixture, whereupon in a short time they died.

Bait formulations that meet both the attraction and gustatory requirements of the pest permit the use of a wide range of contact and stomach insecticides (Moreno and Mangan 1995, 2002). The concentration of the active ingredient in bait can be reduced more than 90%. To be active, the formulations require consumption, either because the toxicant cannot penetrate, or the concentration is not sufficient to penetrate, the insect cuticle. Other important components include conditioners such as oils, humectants and adjuvants. These components protect the spray drops from evaporation and running off vegetation, help keep the drops wet for fly ingestion, and enhance the toxicity of the insecticide. Mangan and Moreno (2001) showed that a series of commercial adjuvants varied widely in their interaction with dyes and fruit fly mortality, and under field conditions adjuvants could significantly increase bait effectiveness by about 30%.

A series of insecticides was tested in the laboratory with SolBait formulated for tropical fruit fly control (Moreno and Mangan 2002). In that study, 16 insecticides that had mammalian toxicity values at least 40x lower than malathion were identified. As part of this study, Moreno and Mangan developed, and adopted for commercial use, spinosad for fruit fly control. This spinosad-based toxic bait, currently marketed as GF120, was formulated with proprietary modifications by Dow Agrosciences to optimize attraction, edibility, and stability. In addition, since spinosad is derived from a naturally occurring soil fungus, after being combined with a selected series of bait components, the product was eligible for organic registration.

6. BEHAVIOURAL MODIFICATION WITH CHEMICALS

The use of pheromones for the detection and management of lepidopterous pests has become a standard procedure. Tamaki (1985) summarized the chemistry and application of pheromone technology for pest management. The chemical structures of pheromone compounds are known for 160 lepidopterous species in 20 families. Pheromone technology could be applied in three ways (Tamaki 1985):

- Monitoring and surveying for early detection of introduced exotic insects, forecasting pest outbreaks, and estimating population density
- Mass-trapping for population suppression and detailed monitoring

- Communication disruption to inhibit mate-finding and suppress the population

Carde and Minks (1995) reviewed the use of pheromones for mating disruption as a pest control strategy. Success with this strategy has been restricted to moths that have a mating behaviour that involves males following a pheromone plume as the principal means to locate females. Carde and Minks described a series of modes of action that results in mating disruption, including effects on the sensory mechanisms of the target males, and control of behaviour and orientation. They described programmes for 9 pest moth species that have successfully used mating disruption, and 14 additional species that, at that time, had formulations available. The best example of combining mating disruption with the SIT is the use of gossyplure in the pink bollworm control programme in the south-western United States (Walters et al. 2000a; Bloem et al., this volume).

Gossyplure is a mixture of two isomers of 7,11-hexadecadienyl acetate (Hummel et al. 1973). This mixture was shown to be the effective attractant, with more than 56 times the attraction to males than hexalure (cis-7-hexadecenyl acetate) or than the less effective propylure mixtures reported to be sex pheromones (Jones et al. 1966, Jones and Jacobson 1968, Keller et al. 1969). Flint et al. (1974) used gossyplure for monitoring early season populations. The delta trap, previously used to control the gypsy moth *Lymantria dispar* (L.), was shown by Foster et al. (1977) to be a superior *P. gossypiella* monitoring tool. The first registered use of a pheromone to control the pink bollworm through mating disruption was developed by Gaston et al. (1977) in tests carried out in Arizona and California, USA.

Jenkins (2002) discussed current commercial formulations of gossyplure and modes of action of disrupting pink bollworm mating. Formulations exist as three types: (1) reservoir type such as the PB-ROPE L, which is containerized into a plastic tube or band, has a long field life (60–90 days), and is applied at 250–1000 units per hectare, (2) a low-rate, female equivalent, sprayable product has a field life of 7–21 days, contains an insecticide additive, and is contained in a paste, flake, or hollow fibre at 750–32 000 units per hectare, and (3) a low-rate, microdispersible system that can be applied as a fog or in a capsulated form, and has a field life of 7–28 days.

In addition to the application of insecticides, one of the pest suppression tactics used in the Sterile Insect Release (SIR) Program in British Columbia, Canada, against the codling moth *Cydia pomonella* (L.) was mating disruption (Judd et al. 1992; Dyck et al. 1993; Bloem et al. 2001; Bloem et al., this volume). The integration of mating disruption and the SIT has also been successfully implemented against the codling moth in Washington State, USA (Calkins et al. 2000).

7. RESISTANT PLANTS

The development of cotton cultivars resistant to the pink bollworm has been a long-term component of the pest management programme. Nobel (1969) reviewed the characteristics of the components of resistance used in breeding programmes initiated in the 1950s. The cultivars screened, *Gossypium thurberi* Todaro and *G. thurberi* x *Gossypium hirsutum* L., were recognized as the least attractive for oviposition. Pink bollworm larvae attacking these varieties experienced reduced

larval survival and lengthened development time, apparently due to a protective response by the seeds.

Characters in varieties that induced oviposition away from the bolls exposed larvae to increased contact with pesticides and increased predation or parasitism. Boll, stem and leaf morphologies that reduced oviposition were also developed. Nectariless cultivars were developed to reduce the supply of food for adults. Although these characters appeared to reduce survival or oviposition, field trials did not show an increase in protection from attack. Increases in gossypol in the plant, though reducing pink bollworm survival, rendered the seed unusable for animal feed and the seed oil unusable for human food. Wilson et al. (1992) reported a 36% reduction in seed damage in a breeding line with a combination of nectariless, okra leaf, and early maturity; but it still required insecticide treatment to control the pink bollworm.

Transgenic cotton varieties were developed by Monsanto and released in the early 1990s. Tests by Wilson et al. (1992) established in Arizona that the pink bollworm adult populations were greatly reduced in transgenic cotton plots. The number of pests per 100 bolls was 87.5 for control varieties and only 0.2 for transgenic varieties. Seed damage was similarly reduced in transgenic plots (0.14%) compared with 4.83% damage in control varieties. In summary, Wilson et al. reported that, in transgenic compared with susceptible lines, there was a reduction of 95–99% in rosetted blooms, pink bollworm populations in the bolls, and seed damage.

In the areas applying the SIT against the pink bollworm, the use of transgenic cotton has affected programme execution (Walters et al. 2000a, b); already in 1997, 81% of the plantings in the Imperial Valley were genetically engineered cotton. As a result, Walters et al. (2000a) evaluated the possibility of moving from the exclusion to an expanded eradication programme by integrating engineered cotton with the SIT and other control tactics (eradication defined as 3 years of zero detections after completion of the programme). They divided the components of eradication into five treatments — sterile insect release, genetically engineered cotton, high-rate pheromone release, mid-season pheromone application, and monitoring. This is only possible with the now-enlarged eradication area that includes adjacent growing areas in California, Arizona, and New Mexico in the USA, and in Baja California Norte, Chihuahua, and Sonora in Mexico (NAPPO 2004).

8. ROLES OF SUPPLEMENTARY TREATMENTS IN STERILE INSECT RELEASE PROGRAMMES

Using sterile insect releases in a pest control programme requires that the target population be isolated from adjoining populations, and that the target population is sufficiently reduced so that a high enough ratio of sterile to fertile matings inhibits reproduction. Current programmes achieve isolation by relying on combinations of quarantine barriers, geographic barriers, or treatments at the population margins. To gain the needed overflooding ratio, suppression/eradication programmes usually mention sufficient sterile insect production in conjunction with pest population suppression.

In addition to a lack of immigration and sufficiently high sterile:fertile ratios, other factors are required for suppression or eradication programmes using the SIT to succeed. A key component of successfully applying pest management techniques is the effectiveness of the application in preventing pest damage. Of course, reducing pest populations by reducing their reproductive potential eventually reduces damage. However, sterile matings of the pests reviewed above do not kill the pest. Therefore economic losses continue, arising from reproduction by females mated before sterile insect release, and the continued biting by blood-feeding pests, independent of their sterile or fertile mating status.

The major supplementary treatments reviewed above provide direct and immediate reduction of damage by the pest. Protection from damage by pests is practiced whether the programme releases sterile insects or not, so these treatments are usually widely applied components of pest suppression programmes. However, when these treatments are practiced in areas where the recipients (environmental and human) receive no benefits, as frequently must be done in AW-IPM programmes that integrate the SIT, the programmes can face social and political opposition.

Activities supplemental to sterile insect releases tend to be methods that were developed to control the pest populations or prevent damage from the pests, independent of sterile insect releases. Public acceptance of pest management programmes is best related to the benefits derived directly from the activities, but the success of suppression or eradication is dependent on pest population reduction.

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CHAPTER 4.3.

GENETIC SEXING STRAINS IN MEDITERRANEAN FRUIT FLY, AN EXAMPLE FOR OTHER SPECIES AMENABLE TO LARGE-SCALE REARING FOR THE STERILE INSECT TECHNIQUE

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SUMMARY

Through genetic and molecular manipulations, strains can be developed that are more suitable for the sterile insect technique (SIT). In this chapter the development of genetic sexing strains (GSSs) is given as an example. GSSs increase the effectiveness of area-wide integrated pest management (AW-IPM) programmes that use the SIT by enabling the large-scale release of only sterile males. For species that transmit disease, the removal of females is mandatory. For the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), genetic sexing systems have been developed; they are stable enough to be used in operational programmes for extended periods of time. Until recently, the only way to generate such

strains was through Mendelian genetics. In this chapter, the basic principle of translocation-based sexing strains is described, and Mediterranean fruit fly strains are used as examples to indicate the problems encountered in such strains. Furthermore, the strategies used to solve these problems are described. The advantages of following molecular strategies in the future development of sexing strains are outlined, especially for species where little basic knowledge of genetics exists.

1. INTRODUCTION

The sterile insect technique (SIT) is an increasingly important component of area-wide integrated pest management (AW-IPM) programmes for certain key insect pest species (Table 1). The application of the SIT in operational programmes, and its use against additional species, continues to reveal areas where technology can improve the SIT. Originally, unmodified strains collected from the wild were used for the SIT, and in at least one case, even without laboratory rearing. However, with the advent of genetics, and later on molecular biology, strategies were investigated that would permit the manipulation of certain biological characteristics to generate strains that are more suitable for the SIT. For example, this includes the incorporation of genetic markers to label the released insects, the manipulation of pathogen transmission, and the elimination of females from the released material. An example of using improved technology is the recent transfer of genetic sexing technology to Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) programmes (Rendón et al. 2000, 2004). In future, it is expected that such strains will be required for many other insect species of agricultural or health importance. Beyond application for the SIT, genetic sexing technology has been applied to natural enemies and to the silkworm *Bombyx mori* (L.). In the latter case, males produce more silk. W-autosome translocation sexing strains, based on a cocoon marker, were developed (Nagaraju 2002) and, in India, are reared at a male-only production level of 125 million per week (J. Nagaraju, personal communication).

Based on the reproductive biology of most insects, and on the basic principle underlying the SIT, it is clear that only released sterile males are important for this technique to be effective. Wild females determine the population size of the next generation, but wild males, or more precisely the sperm they produce, are present in such great excess that it would be necessary to remove about 99% of all wild sperm before obtaining a significant reduction in the size of the next generation (Koyama et al. 1984). Sterility is introduced into a wild population only through sterile males, even when they are released together with sterile females. Recognizing this principle, the SIT was initially called the "sterile-male method" (Knipling 1959). However, at that time, the only AW-IPM programme integrating the SIT was against the New World screwworm *Cochliomyia hominivorax* (Coquerel), and both sexes were released. The name was meant to highlight the fact that only the sterile males are the active agent in the SIT. As demonstrated very clearly for the Mediterranean fruit fly by McInnis et al. (1994) and Rendón et al. (2000, 2004), bisexual releases are far less effective than male-only releases in introducing sterility into a wild population, and this will probably apply to most pest species. The obvious reason is that the released sterile males and females tend to mate with each other. As a result, the proportion of matings between sterile males and wild females is reduced, and less sterility is introduced into the wild population.

Table 1. Approximate worldwide maximum mass-rearing capacities for SIT achieved at one time for different species (some data from DIR-SIT (IDIDAS 2004))

Species	Millions per week	Sexing strategy
Mediterranean fruit fly *Ceratitis capitata* (Wiedemann)	3500	Y-autosome translocation, using a temperature-sensitive lethal (*tsl*)
New World screwworm *Cochliomyia hominivorax* (Coquerel)	500	No
Melon fly *Bactrocera cucurbitae* (Coquillett)	280	No (Y-autosome translocation, pupal colour separation[1])
Mexican fruit fly *Anastrepha ludens* (Loew)	240	No
Oriental fruit fly *Bactrocera dorsalis* Hendel	100	No (Y-autosome translocation, pupal colour separation[2])
Pink bollworm *Pectinophora gossypiella* (Saunders)	84	No
Caribbean fruit fly *Anastrepha suspensa* (Loew)	50	No
West Indian fruit fly *Anastrepha obliqua* (Macquart)	50	No
Queensland fruit fly *Bactrocera tryoni* (Froggatt)	40	No[3]
Philippines fruit fly *Bactrocera philippinensis* Drew and Hancock	20	No
Codling moth *Cydia pomonella* (L.)	14	No
Onion maggot *Delia antiqua* (Meigen)	7.5	No
Anopheles albimanus Wiedemann	7	Pupal size, Y-autosome translocation, propoxur resistance
Old World screwworm *Chrysomya bezziana* (Villeneuve)	6	No
Sapote fruit fly *Anastrepha serpentina* (Wiedemann)	5	No
Natal fruit fly *Ceratitis rosa* (Karsch)	3	No
South American fruit fly *Anastrepha fraterculus* (Wiedemann)	2	No
Olive fruit fly *Bactrocera oleae* (Gmelin)	1	No
Gypsy moth *Lymantria dispar* (L.)	1	No
Malaysian fruit fly *Bactrocera latifrons* (Hendel)	< 1	No
Carob moth *Ectomyelois ceratoniae* (Zeller)	0.5[4]	No
Glossina austeni Newstead	0.09	Manual, sex-specific time of emergence, infrared screening of pupae

[1] A sexing strain is available, but is not reared on a large scale (McInnis et al. 2004).
[2] A sexing strain is available, but is not reared on a large scale (McCombs and Saul 1995).
[3] A strain was developed where the females carry a wing mutation (bent wing), and are thereby disabled, so effectively only males are active in the field (Meats et al. 2002). This strain has not yet been used in large-scale programmes.
[4] Quinlan et al. 2002

To address this problem, it was suggested that strains producing only males should be developed (Whitten 1969, Hendrichs et al. 1995). Besides improved effectiveness in the field, it was expected that mass-rearing costs would be reduced, but this is not always the case (Caceres 2002, Caceres et al. 2004). Nevertheless, significant cost reductions can be achieved in the post-production processes. Only half the volume of pupae is handled for marking, irradiation, transport, and release activities, and the cost of monitoring is reduced significantly, especially in combination with female-specific traps (Epsky et al. 1999; Vreysen, this volume).

Another very important consideration is that, in some cases, sterile females simply cannot be released. Fruit fly females may cause damage from "sterile stings" in certain fruit types, females of biting flies reduce meat production in livestock, and females of bloodsucking species may transmit disease (Lance and McInnis, this volume).

For a few species, natural characters can be used for large-scale separation of the sexes, e.g. sex-specific pupal-size differences (Dame et al. 1974), and sex-specific differences in the timing of adult emergence, e.g. tsetse fly *Glossina austeni* Newstead (Opiyo et al. 2000; Parker, this volume). For species where females suck blood, it is possible to feed them with a toxic substance, and then release the males. However, the biology of most species does not permit sex separation on the scale required for the SIT and, therefore, specific strains have to be developed. To date, this has been achieved using Mendelian genetics, specific mutations, and chromosome rearrangements.

Genetic sexing strains (GSSs) have been developed for 19 species (Table 2 in Robinson 2002) using the same basic principle of sex-specific linkage of a selectable marker. However, only for *Anopheles albimanus* Wiedemann and *C. capitata* were GSSs developed to the point where they could be mass-reared at levels required for AW-IPM programmes integrating the SIT (Table 1), and only in *C. capitata* was the sexing system improved enough for truly large-scale application over extended periods of time. As described below, constructing a basic sexing strain is rather trivial. However, such "prototype" strains do not normally show the characteristics that are essential for successful long-term application in an AW-IPM programme integrating the SIT. These characteristics include aspects of feasibility and economy, i.e. using the appropriate type of sex-separation mechanism, as well as productivity and stability, i.e. the genetic structure of the strain. To transfer a sexing system from an experimental laboratory to a mass-rearing facility requires very intensive research into the genetic behaviour of the sexing system. This approach is usually supplemented by incorporating appropriate modifications that provide sustainability for extended periods under the extreme conditions of mass-rearing. This requires basic genetic knowledge of the species involved and, unfortunately, this is the case for only very few pest species.

Since GSSs for operational AW-IPM programmes releasing sterile insects are available only for the Mediterranean fruit fly, and only these strains have been analysed sufficiently to permit general conclusions, this species is referred to extensively in this chapter. It illustrates the problems encountered and modifications introduced to obtain improved sexing strains that are advanced enough to allow a current worldwide production of about 3500 million male flies per week (Table 1).

The lessons learned from this species can very likely be extrapolated to all translocation-based strategies for other species and, to some extent, to new methods using molecular biology. The enormous numbers of insects that must be reared under relatively stressful conditions dictate that even extremely rare genetic or molecular phenomena can threaten the integrity of the sexing system.

2. PRINCIPAL STRUCTURE OF SEXING STRAINS

All GSSs developed to date are based on the same principle, and require two separate components (Fig. 1) (Franz and Kerremans 1994, Robinson et al. 1999):
- Mutation that can be used as a selectable marker for sex separation (a list of selectable markers is given in Table 2 in Robinson 2002)
- Y-autosome translocation to link the inheritance of this mutation to sex

In the Mediterranean fruit fly, and probably also in many other pest Diptera, the Y chromosome carries a dominant *Maleness* factor (Willhoeft and Franz 1996). To construct a GSS, the wild-type allele of the selectable marker is physically linked to this chromosome via a Y-autosome translocation (Fig. 1). In the resulting strain, the males are heterozygous (normal "wild-type" phenotype), and the females
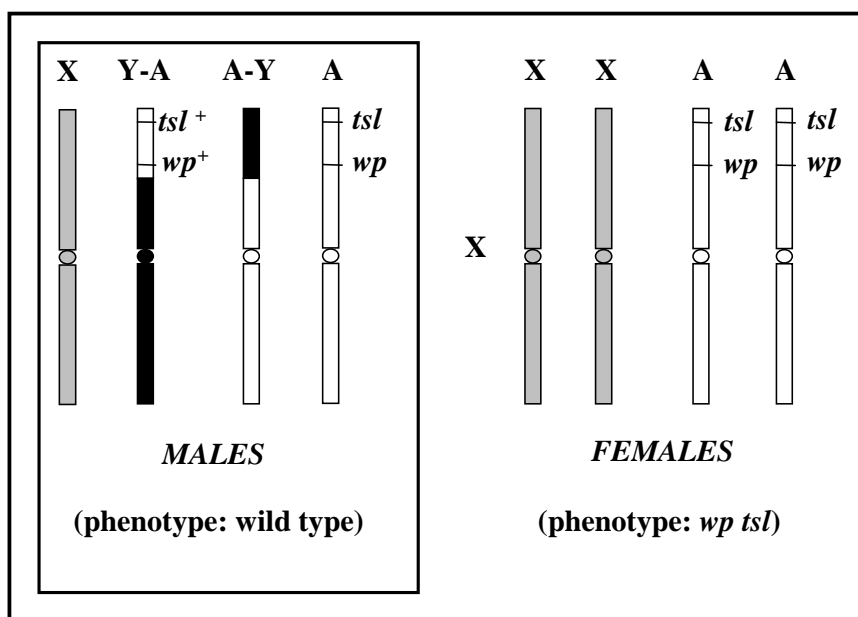
Figure 1. Basic structure of GSS carrying Y-autosome translocation linking normal "wild-type" alleles of the selectable markers white pupae (wp) *and* temperature-sensitive lethal (tsl) *to the male sex. Y-A: translocation fragment carrying Y chromosomal centromere, A-Y: reciprocal translocation fragment carrying autosomal centromere, X: X chromosome, A: autosome.*

homozygous for the selectable marker and thus express the mutation, and can be separated from males. Y-autosome translocations are induced by irradiation, followed by a genetic screen that is based either on the inherited sterility associated with translocations or on a mutation to detect male-linked inheritance. The former method can be used in all species, even where no, or only very limited, genetic information is available. Not all translocations are equally appropriate for use in a GSS, e.g. translocations involving multiple autosomes have a high level of sterility. As outlined below, the stability and productivity of a strain depend crucially on the structure of the translocation.

2.1. Mutations for Large-Scale Separation of Sexes

The choice of a suitable selectable marker involves decisions regarding the feasibility and economics of the resulting sexing mechanism. In this context, several criteria must be considered:

- Sex separation versus female killing. Killing females appears to be the best option, except for special cases where they can be used to rear parasitoids, or where females are needed to maintain the colony (species with a very low reproductive capacity, such as tsetse flies).
- Timing. To minimize rearing costs, females should be killed as early as possible during development. Timing also determines the amount of biomass in which the females must be killed, and this has an effect on the practicality, cost, and accuracy of the treatment, e.g. for fruit flies large numbers of eggs can be treated more easily than large numbers of larvae or pupae.
- Physical or chemical treatment to kill females. The egg stage is the most practical stage, but in most cases chemicals cannot penetrate the eggshell, and a selectable marker that responds to physical treatment (e.g. temperature) is required. If only selectable markers that are sensitive to chemicals are available, the larval stage is the earliest possible stage for sexing. The use of chemicals raises additional points: cost of the chemical, human toxicity (to workers), accessibility to the chemical by feeding larvae (solubility, distribution, and stability in the diet), effect on symbionts or bacteria in the diet, and disposal of spent diet containing the chemical.
- General considerations. It is important also to know the accuracy of the sexing mechanism, the additional costs for equipment, chemicals, etc., and the reduced productivity (in comparison with a standard strain). The accuracy of eliminating females should be close to 100%, but the selectable marker should not negatively affect the viability and productivity of mutant females in the colony.

Choosing the right selectable marker is a vital decision. Several different markers for the Mediterranean fruit fly are available (Robinson 2002). However, most of them are not ideal for large-scale application. Current GSSs carry two mutations, *temperature-sensitive lethal* (*tsl*) (Franz et al. 1994) that is used to eliminate females, and the closely linked mutation *white pupae (wp)* (Rössler 1979) that is used as a visible marker. The *tsl* mutation has certain biological properties that impact on its usefulness, and on the procedures required for mass-rearing or sexing:

- Temperature-sensitive period (in individuals homozygous for the *tsl* mutation). In a GSS, females are homozygous for the *tsl* mutation. The standard protocol to test temperature sensitivity is a 24-hour treatment at temperatures from 31 to 35°C, compared with the control at 25°C. To determine the sensitive period of the *tsl* mutation, eggs are collected for 1 hour, and temperature tests done at different stages of development. As shown in Figure 2A, homozygous *tsl* individuals are very sensitive to temperature during the embryonic stage, and to a lesser extent during moulting from first- to second-instar, and from second- to third-instar, larvae. Figure 2B shows the results of temperature tests with pupae of known age. The pupae are also sensitive to temperature, especially during the first 3 days. Additional tests were done with adults, maintaining the cages at 25, 28, 31, and 34°C, and daily mortality was measured. In the *tsl* homozygous strain, significant mortality was observed already at 28°C (Fig. 3).

  In conclusion, temperature sensitivity is strongest during the egg stage, and elimination of homozygous *tsl* individuals is achieved by incubating eggs at 34°C for 24 hours. Secondly, later stages are also temperature sensitive. Therefore, the temperature during mass-rearing must be carefully controlled to prevent damage to the *tsl* homozygous females. Thirdly, even though the sensitivity of the adults is a complication for mass-rearing, it also has a benefit in that any homozygous *tsl* individuals that escape will not be viable at elevated temperatures. This applies to any flies that escape from a production facility, and to the few remaining females among the released sterile males.

- Maternal effect (individuals heterozygous for the *tsl* mutation). In a GSS, the males are heterozygous for the *tsl* mutation, but does the presence of a wild-type allele completely suppress the sensitivity to temperature? Originally the temperature treatment to eliminate females was done relatively early during embryogenesis, i.e. eggs were collected for 24 hours, followed immediately by the 24-hour temperature treatment. However, this resulted in a reduced recovery of males. Tests of F_1 individuals from reciprocal crosses, between a wild-type strain and a homozygous *tsl* strain, demonstrated that the temperature sensitivity of the F_1 differed significantly, although in both cases the genetic constitution of the F_1 is identical (*tsl/tsl⁺*). Fig. 4B shows that, in this cross, the *tsl⁺* allele is completely dominant over the mutant allele, i.e. the same level of lethality is observed as in a wild-type strain (Fig. 4A). However, in the reciprocal cross where homozygous *tsl* females are used, a significant temperature sensitivity of the F_1 offspring is observed (Fig. 4C).

  This difference is due to a maternal effect. During the early stages of development, the embryo's own genome is inactive, and apparently it utilizes a *tsl*-gene product that it receives in the egg from the mother. Consequently, during these early stages of development, the embryo is dependent on the genotype of the mother, and in a GSS the mother is homozygous for the *tsl*. Only after activating their own genetic material are the heterozygous *tsl/tsl⁺* males protected against elevated temperatures. Based on these findings, the temperature-treatment regime was modified, i.e. after collection, and before the treatment is applied, eggs are maintained at 25°C for at least 24 hours. This led to a significantly improved recovery of males (Fisher 1998).
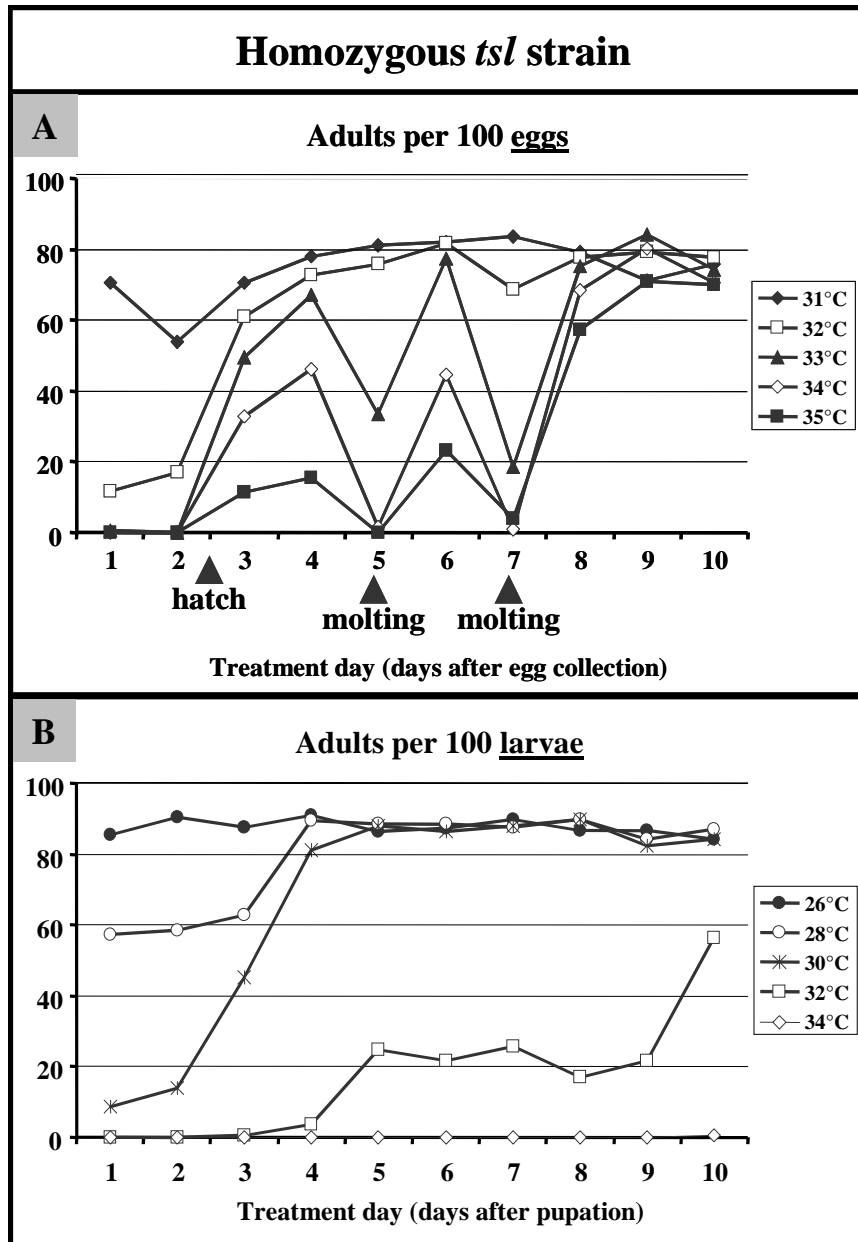
Figure 2. Temperature pulse experiments with homozygous tsl *strain. Eggs (A) or pupae (B) were collected for 1 hour followed by temperature treatment during different days of development with temperatures indicated.*

Homozygous *tsl* strain

Percent adult mortality
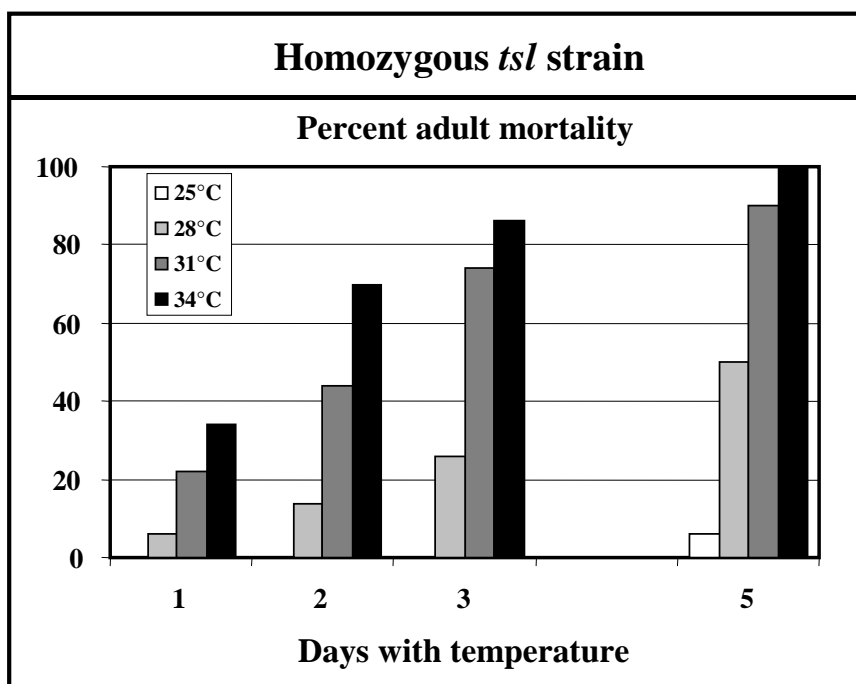
Figure 3. Temperature treatment of adults homozygous for tsl *mutation. After emergence, adults were maintained constantly at temperatures indicated.*

- Delayed development. Individuals homozygous for the *tsl* mutation develop more slowly than those either heterozygous or homozygous for a wild-type allele. Already at 25°C, homozygous *tsl* individuals pupate 1 day later than the wild type. If eggs and larvae are subjected to an extended incubation at elevated but sub-lethal temperatures, e.g. 29°C, the speed of development is affected. As expected, the development of wild-type individuals is accelerated, i.e. pupation occurs 2 days earlier than at 25°C. However, in the homozygous *tsl* strain, pupation occurs only 1 day earlier than at 25°C, i.e. 2 days later than the *tsl*[+] individuals.

  Differences in pupation time are a good indicator of the temperature increase during larval rearing. For example, poor rearing conditions, like excess heat in the larval diet, become apparent as increased differences in the pupation time of *tsl*[+] males and homozygous *tsl* females.

  In principle, the delayed development of the homozygous *tsl* females could be used to separate the sexes at the larval or pupal stages, e.g. for mass-rearing parasitoids. In mass-rearing a *tsl*-based GSS, the first day of pupal collection yields virtually only male pupae, but the collection on the 5th day contains mostly females (Fig. 5 in Caceres 2002). This permits loading production cages with an excess of females (to increase egg production) without using a pupal separator. The surplus of males can be added to the insects to be released.
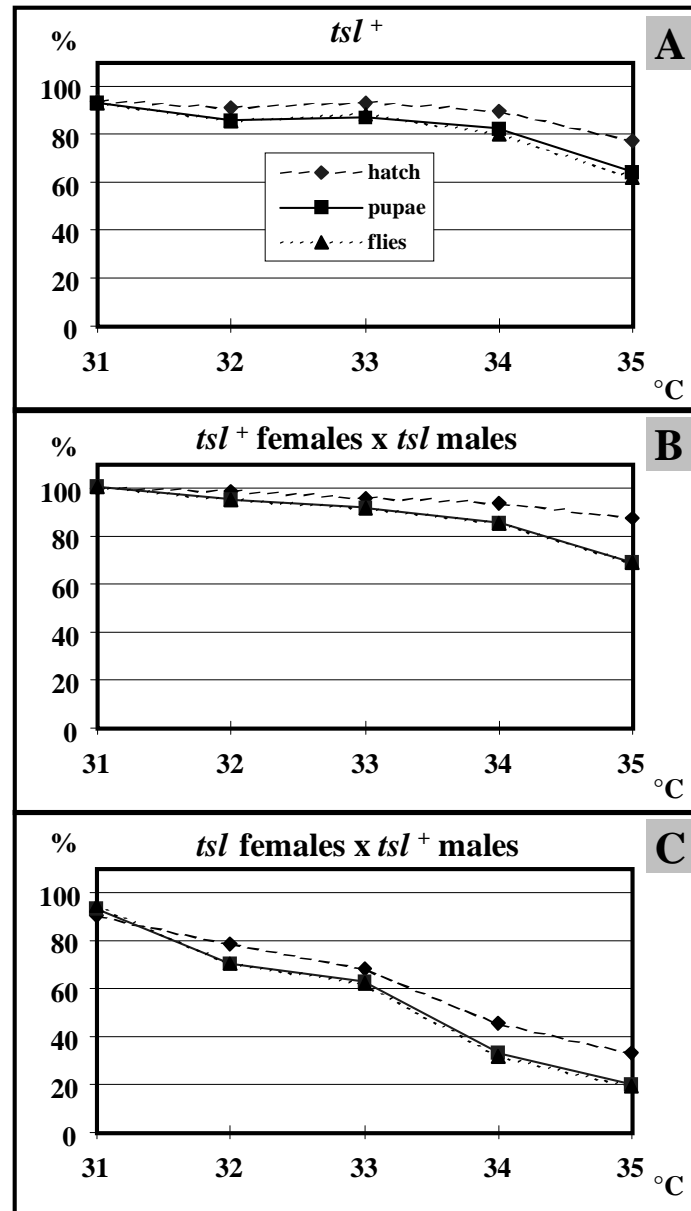
Figure 4. Difference in temperature sensitivity of tsl[+]/tsl *genotypes depending on direction of parental cross (maternal effect). Values shown for hatch, pupae, and adults are given as percentage of values obtained at 25°C. Homozygous* tsl *strain shows no hatch starting at 34°C.*

An additional improvement is the filter rearing system (FRS) (section 4.3.), i.e. the maintenance of a mother colony as a pure GSS where exceptional flies are removed according to their pupal colour phenotype (Parker, this volume). As described below, GSSs can generate unwanted recombinant individuals, some of which cannot be removed by inspection of the pupal colour (Table 2). One type of recombinant female, *wp tsl⁺/wp tsl,* emerges from white pupae, and therefore is not removed by the FRS and will accumulate in the colony, where it becomes evident when an increasing number of white-pupae females survive the temperature treatment. However, such females can be distinguished from the non-recombinant females since the presence of the *tsl⁺* allele causes them to pupate early, together with the males. Consequently, it is recommended for the FRS that new adult cages be set up with only brown-pupae males that have pupated early, and with white-pupae females that have pupated late. It has been shown that this selection scheme significantly improves the accuracy of the FRS.

- General. In mass-rearing, eliminating females requires only a simple and cheap water bath. The temperature treatment is applied during the "egg bubbling stage" when incubating eggs are oxygenated and maintained at a high density in water. The accuracy of the treatment is almost 100%, even in very large facilities where hundreds of millions of eggs are treated every day. Male quality and quantity is not affected significantly by the treatment. In rearing individuals for the colony, temperature must be carefully controlled; if not, female production is reduced, and, as described below, the stability of the sexing system is negatively affected (section 4).

Table 2. Recombinant classes produced in T(Y;5) wp⁺tsl⁺/wp tsl *males*

Recombination type	F₁ genotype	F₁ phenotype	Accumulation in mass-rearing	Detection in filter rearing system
Type-1a	T(Y;5)*wp tsl/wp tsl* males	white pupae, *tsl*, 50% sterile	No	Yes
Type-1a	*wp⁺ tsl⁺/wp tsl* females	brown pupae, *tsl⁺*	Yes	Yes
Type-1b	T(Y;5)*wp⁺ tsl/wp tsl* males	brown pupae, *tsl*, 50% sterile	No	No
Type-1b	*wp tsl⁺/wp tsl* females	white pupae *tsl⁺*	Yes	No
Type-2	Y/*wp tsl/wp tsl* Males	white pupae, *tsl*, 100% fertile	Yes	Yes

2.2. *Sex Linkage of Selectable Marker*

Selectable markers are generally not sex-specific. Therefore, the respective wild-type allele must be linked to the male-determining Y chromosome. This linkage is achieved by irradiating wild-type pupae with 40–50 Gy, and backcrossing individual F_1 males with mutant females carrying the selectable markers. The F_2 is screened for families where the mutation used in the screen is inherited in a sex-specific manner, i.e. males are wild type and females mutant. The choice of the mutation determines which autosome is involved in the translocation.

Several independent experiments to induce Y-autosome translocations in the Mediterranean fruit fly have been performed (Robinson and Van Heemert 1982, Kerremans et al. 1992, Franz et al. 1994, Kerremans and Franz 1995, Delprat et al. 2002). On average, about 7% of F_1 crosses involve male-linked translocations. This frequency depends on the genome size and the size of the chromosomes involved. In total, more than 30 Y-autosome translocations involving chromosome 5 were generated. Chromosome 5 carries both the *wp* and *tsl*. This is a very important prerequisite for the successful development of sexing strains — it allows a choice of the most appropriate translocation (with reference to stability and productivity).

The productivity of a sexing strain is correlated primarily with the segregation behaviour of a Y-autosome translocation during male meiosis. There are two ways in which a Y-autosome translocation can segregate — alternate and adjacent-1 (Fig. 5). In nearly all cases, these two types occur at equal frequencies (Franz 2000; G. Franz, unpublished data). Only alternate segregation leads to genetically balanced offspring, but adjacent-1 segregation results in severe deletions and triplications. Deletions usually cause lethality already during embryogenesis, but triplications can survive, depending on the size and resulting sex, until the pupal or even adult stages. As a consequence of this segregation pattern, only 50% of the offspring produced by males carrying a simple translocation, involving only one autosome, are genetically balanced, i.e. males are 50% sterile. In males with a more complex translocation, where more than one autosome is involved, sterility is increased accordingly (Franz 2000).

The structure of the translocation, in particular the structure of the Y chromosome and the location of the break point on the Y chromosome, affects the segregation behaviour and the viability of the resulting adjacent-1 offspring (Willhoeft and Franz 1996). If the break point is located between the Y-chromosomal centromere and the *Maleness* factor, the triplication-type adjacent-1 individuals will be female, e.g. VIENNA 7 and 8 (sections 4.1. and 4.2., Robinson et al. 1999). It has been shown that females are about 100-fold more sensitive to triplications in most regions of chromosome 5 than males with triplications of similar size (G. Franz, unpublished data), and consequently they do not survive to the pupal stage. Additional rearrangements of the Y chromosome can lead to an increase in the alternate segregation frequency and, thereby, to an increase in the productivity of the strain.
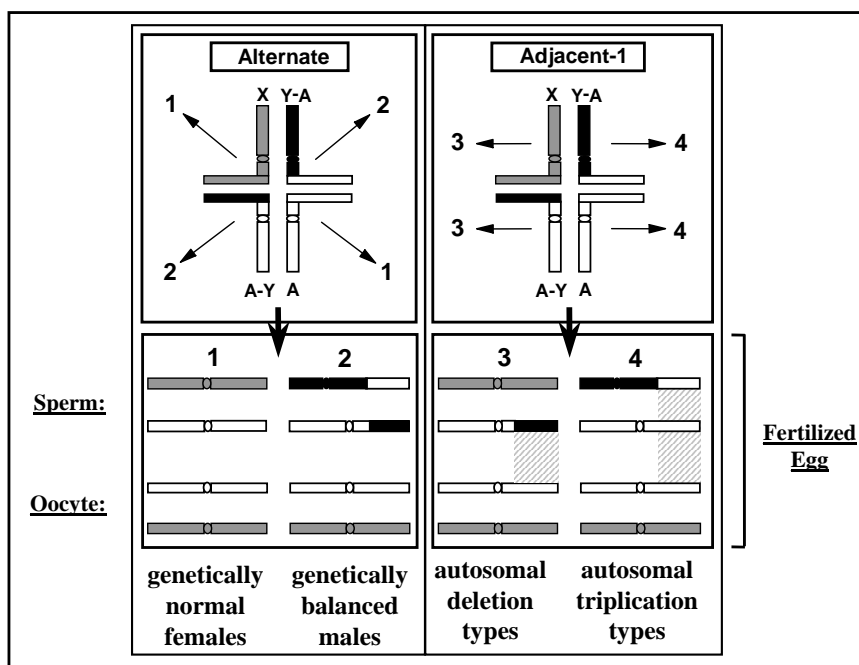
Figure 5. Two different types of segregation of Y-autosome translocations during male meiosis. Y-A: translocation fragment carrying Y chromosomal centromere, A-Y: reciprocal translocation fragment carrying autosomal centromere, X: X chromosome, A: autosome. Adjacent-2 segregation represents a non-disjunction of homologous centromeres, and therefore is not considered here.

3. INSTABILITY IN SEXING SYSTEM

The first Mediterranean fruit fly sexing strain was based on the *wp* mutation and the translocation T(Y;5)101 (Robinson and Van Heemert 1982). In laboratory-scale rearing, no obvious signs of instability were detected. In 1985, this strain was tested in mass-rearing. Although production was rather limited (about 1 million males per week), within a relatively short period the sexing system started to degrade (Hooper et al. 1987). The following actions were taken to remedy the situation:

- Improved cytology, and developing chromosome maps. During the development of polytene chromosome maps, it was discovered that there are two classes of tissues that contain polytene chromosomes with completely different banding patterns (Bedo 1986; Zacharopoulou 1987, 1990, 1991), and the Y chromosome is visible only in polytene chromosomes isolated from trichogen cells in male sub-orbital frontal bristles.

- Induction, analysis, and mapping of new mutations. Many new mutations were isolated and genetic maps constructed (Rössler et al. 1994). To date, seven phenotypic mutations are known for chromosome 5, several of which were mapped on polytene chromosomes by deletion mapping or *in situ* hybridization (Fig. 6).

3.1. Type-1 Male Recombination

The most frequent cause of instability is autosomal recombination in males heterozygous for the selectable marker(s), i.e. recombination between the translocated wild-type chromosome and the free autosome carrying the mutant alleles (type-1 recombination, Franz 2002). In the GSSs being used in operational AW-IPM programmes that apply the SIT, where *wp* and *tsl* are used as selectable markers, genetic recombination in two chromosomal regions is of relevance, i.e. in the region between the translocation break point and *wp* (type-1a), and the region between *wp* and *tsl* (type-1b, Fig. 7). Each sub-type produces two reciprocal recombinants; type-1a replaces the wild-type alleles of both markers on the translocation with the mutant alleles from the free autosome, while type-1b exchanges only the wild-type allele of the *tsl*. This results in the generation of several new combinations of these markers and sex (Table 2).
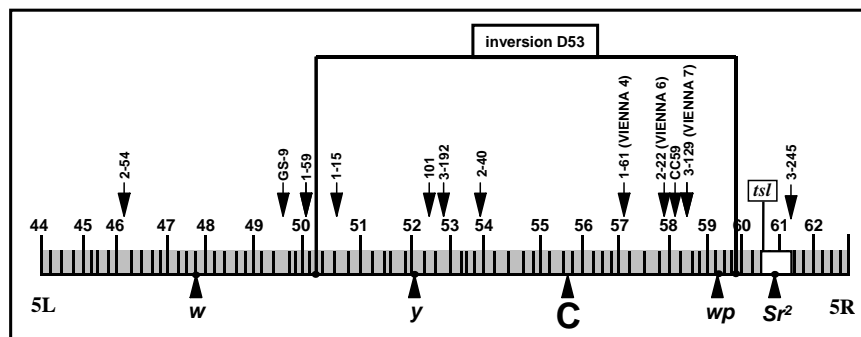
Figure 6. Schematic polytene chromosome map of autosome 5 from trichogen cells: map positions of mutations white (w), yellow body (y) *and* Sergeant^2 (Sr^2) *are shown in addition to location of selectable markers* wp *and* tsl. *Arrows indicate break points of several Y-autosome translocations. Extent of chromosomal region inverted in D53 is shown. C: centromere. (VIENNA 8 includes translocation 101 combined with inversion D53.)*
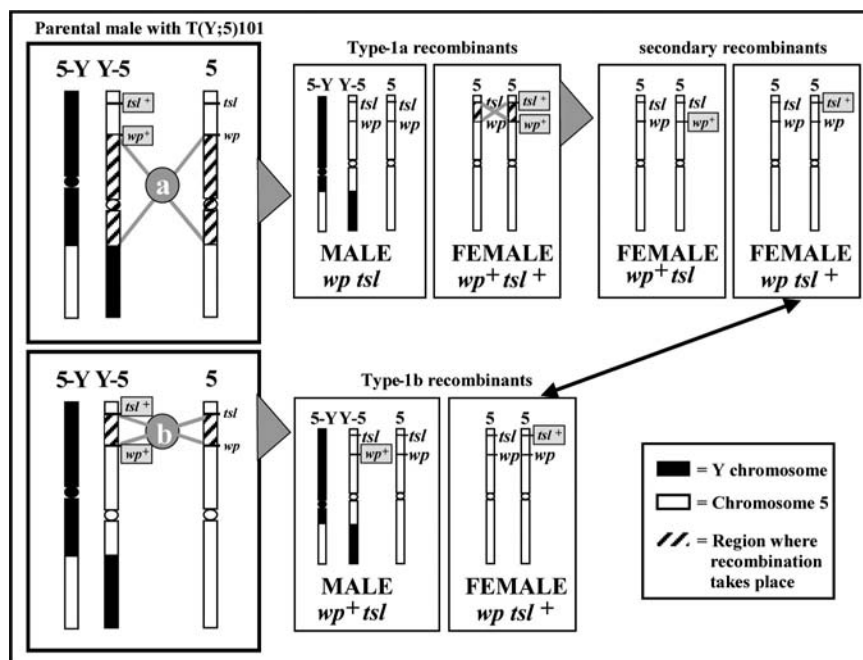
Figure 7. Consequences of type-1 recombination in Y-autosome 5 translocation T(Y;5)101, either in chromosomal region between translocation break point and wp *(type-1a) or between* wp *and* tsl *(type-1b). Also genotype resulting from secondary recombination in type-1a recombinant females is shown. 5-Y: translocation fragment carrying chromosome 5 centromere, Y-5: reciprocal translocation fragment carrying Y chromosomal centromere.*

In Mediterranean fruit fly males, the recombination frequency is very low, especially compared with recombination in females. For example, the recombination frequency between the relatively distant mutations *white* (*w*), on the left arm of chromosome 5, and *wp* on the right arm, is 0.176% in males (Franz 2002) but 48.4% in females (Rössler and Rosenthal 1992), i.e. these two markers behave as if they were unlinked in females. In spite of its rare occurrence, male recombination is nevertheless a threat to the integrity of the sexing system since some of the resulting recombinants have a selective advantage as compared with normal non-recombinant flies. In a continuous rearing system, where exceptional individuals cannot be removed, they will gradually replace the non-recombinant genotypes. To illustrate this, a GSS, based on the translocation T(Y5)101 (Robinson and Van Heemert 1982) and the markers *wp* and *tsl*, was reared in standard small-scale conditions for more than 3 years. In each generation, 34 ml of pupae were taken to initiate the next generation without removing any recombinants; in parallel, flies emerging from 40 ml of pupae were screened for recombinants (Fig. 8). A typical pattern emerges, i.e. from generation 20 onwards, one of the two reciprocal recombinant classes, *wp*⁺
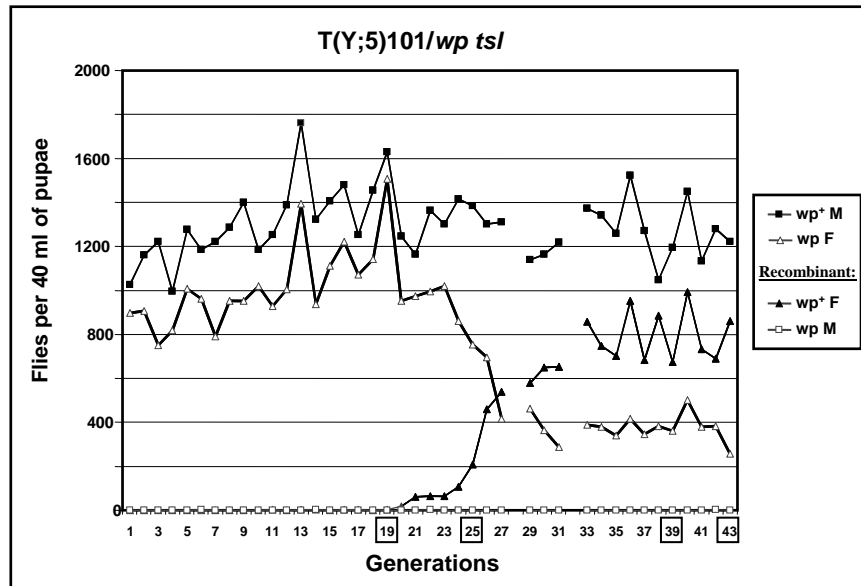
*Figure 8. Breakdown pattern of genetic sexing strain T(Y;5)101/*wp tsl*. In each generation, 34 ml of pupae were taken to initiate the next generation, without removing any recombinants, and a parallel sample of 40 ml of pupae was analysed. Temperature tests were conducted with females from highlighted generations (Fig. 9). F: females, M: males.*

females, accumulates, while the second class, *wp* males, remains insignificant. The accumulation of *wp*[+] females is faster than expected (based on a purely additive build-up of the recombinants occurring in each generation, i.e. the rearing conditions provide a selective pressure in favour of these wild-type females). The reverse argument applies to *wp* males; they have a selective disadvantage compared with normal non-recombinant males, and therefore do not accumulate. Furthermore, the accumulation of *wp*[+] females continues until, in about generation 33, a certain equilibrium is reached, where the wild-type females are about twice as abundant as the non-recombinant mutant females.

Temperature tests were needed to assess the exact nature of the recombinants, with respect to the *tsl* mutation. In generations 19, 25, 39, and 43, eggs from the colony were treated for 24 hours with temperatures between 31 and 35°C, and the phenotype of the resulting adults was scored. Fig. 9A shows that the *wp*[+] females are also wild type for the *tsl* mutation (no reduction in numbers with increasing temperature), confirming that they are type-1a recombinants that resulted from recombination between the translocation break point and *wp*. As expected from the results shown in Fig. 8, such recombinants are not present in generation 19.
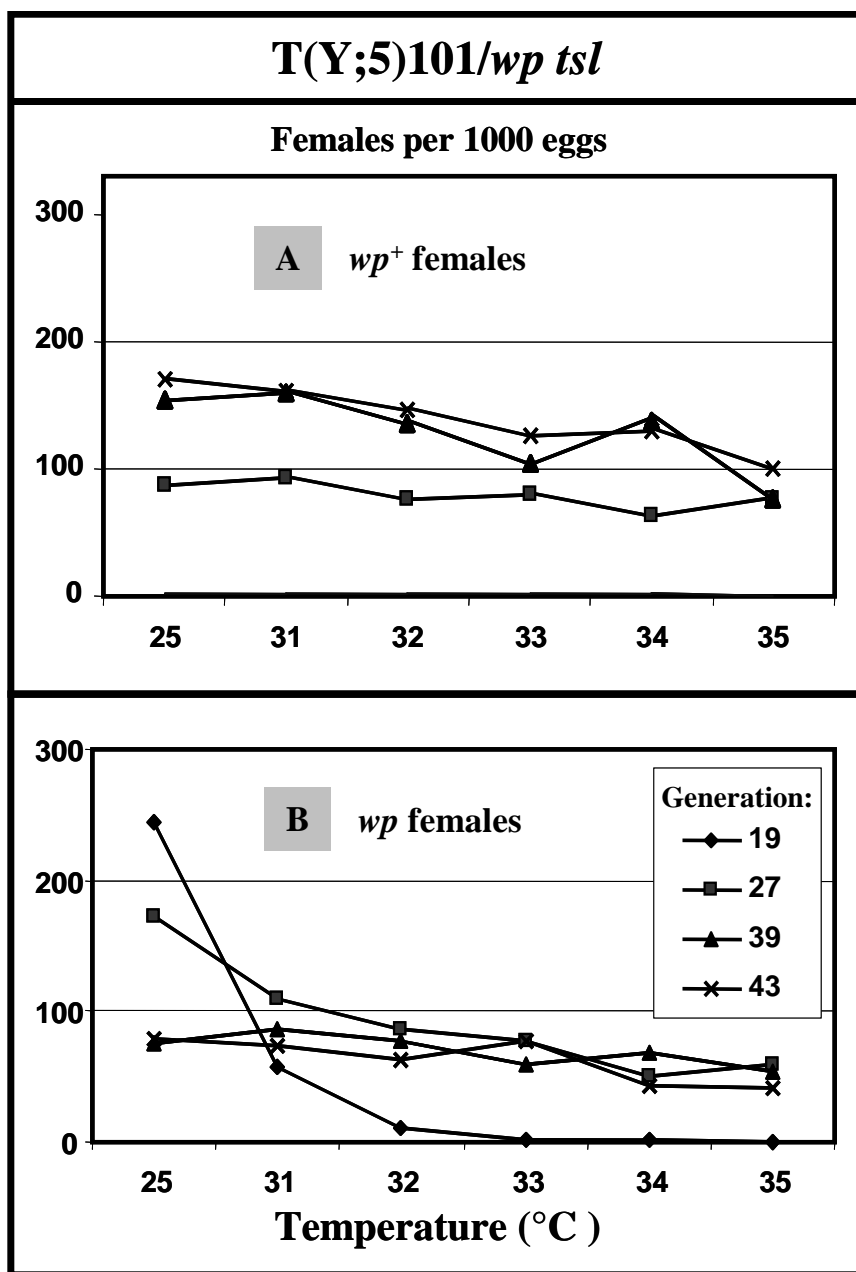
*Figure 9. Temperature tests with females of sexing strain T(Y;5)101/*wp tsl*. Temperature tests were done in generations 19, 27, 39, and 43.*

However, in generation 25, wp^+ tsl^+ females are detected, and their frequency reaches a maximum in generations 39 and 43. Fig. 9B shows the survival of wp females following temperature treatment. In generation 19, all wp females are temperature sensitive, i.e. their genotype is therefore wp tsl. However, already in generation 25, about half of the wp females have lost the tsl (genotype wp tsl^+) (they survive even at 35˚C). In generations 39 and 43, all wp females are tsl^+. As indicated in Fig. 7, there are two ways to explain the occurrence of these wp tsl^+ females, i.e. either by type-1b recombination in the males, or by a secondary recombination in the recombinant wp tsl/wp^+ tsl^+ females. The latter is the most likely explanation since recombination in females is much higher than in males. Furthermore, results with other strains show that type-1b recombination appears to be very rare (see below).

In conclusion, type-1 recombination, in combination with a secondary recombination in females, leads to a strain where the normal wp tsl females are completely replaced by a 2:1 ratio of wp^+ tsl^+ and wp tsl^+ females. The rate at which these recombinant females increase in number cannot be explained by simply adding the recombinants that occur in each generation. A very strong selection is acting on the colony, leading to the rapid accumulation of recombinants that display an advantageous phenotype. The primary increase in selective advantage is caused by the loss of temperature sensitivity and, consequently, no other types of recombinants accumulate. The presence of the wp mutation also causes a selective disadvantage (also noted in other combinations) (Fig. 7 in Franz 2002). The speed of accumulation will depend greatly on the rearing conditions, i.e. if they are sub-optimal, this effect will be accelerated.

3.2. Type-2 Male Recombination

During mass-rearing, a second type of male recombination was detected (G. Franz, unpublished data). It is very rare (estimated frequency about 10^{-5} or less), and generates males with free untranslocated Y chromosomes that are homozygous for wp and tsl (Table 2). Such males accumulate rapidly in the mass-rearing colony (even though they are sensitive to temperature) because they are completely fertile. Their primary effect is on the productivity of the colony, and not on the accuracy of sexing, because together with females they are eliminated during the temperature treatment. The current hypothesis is that recombination occurs between the two translocated Y fragments. Choosing the appropriate Y-autosome translocation, e.g. where the Y-chromosomal break point is close to the centromere, can minimize this problem.

4. STRATEGIES TO IMPROVE STABILITY

Genetic tools are required to improve the stability of sexing strains, and two types of positional information are absolutely essential: (1) the position of the translocation break point, and (2) the position of the selectable marker(s). Also, at least one mutation per chromosome is needed to determine the structure (e.g. which autosomes are involved) and the genetic behaviour of the translocations.

There are two principal strategies to increase the stability of sexing strains. Firstly, by selecting translocations where the break point and the marker are close together, type-1 recombination can be reduced. Secondly, inversions that cover the critical region between the translocation break point and the selectable marker can be incorporated into the strain to eliminate, or at least reduce, type-1 recombination.

4.1. Improved Y-Autosome Translocations

The *wp* mutation is at position 59B on the trichogen polytene map, and the *tsl* is located in the interval 60B–61B (Fig. 6) (Kerremans and Franz 1994; G. Franz, unpublished data). More than 30 translocations were analysed to determine: (1) the position of the break point on chromosome 5 (Kerremans et al. 1990, Franz et al. 1994, Kerremans and Franz 1995), (2) the position of the break point on the Y chromosome (Willhoeft and Franz 1996), and (3) the genetic behaviour (e.g. sterility and segregation behaviour). Based on this information, three strains were selected, i.e. T(Y;5)1-61 (VIENNA 4), T(Y;5)2-22 (VIENNA 6), and T(Y;5)3-129 (VIENNA 7). They were subjected to extensive stability tests, following the same protocol as described above for strain T(Y;5)101 (Franz 2002).

After small-scale rearing for up to 95 generations, no recombinants accumulated in the strains. The overall frequency of type-1a recombination was between 0.017 and 0.021%, and that of strain T(Y;5)101 was 0.084% (if only the generations before recombinants started accumulating were considered). Furthermore, no obvious decrease in sensitivity was shown by the temperature tests, indicating that here also type-1b recombination did not occur at any significant level. Since the distance between the *wp* and *tsl* in the three new strains is the same as in T(Y;5)101, the absence of *wp tsl*$^+$ individuals supports the hypothesis that the occurrence of such genotypes during the rearing of T(Y;5)101 was caused by a secondary recombination event in the *wp tsl/wp*$^+$*tsl*$^+$ recombinant females.

4.2. Inversions

The second strategy is to use the known recombination-reducing properties of chromosomal inversions. Chromosomal inversions render recombinants non-viable if the recombination has occurred within the inversion. The aim is to induce an inversion in the *wp tsl* chromosome. Ideally, it should cover the interval between the translocation break point and the *tsl* mutation, and it must be viable in a homozygous condition. The genotype of the resulting strain would be T(Y;5) *wp*$^+$*tsl*$^+$/Inv *wp tsl* males x Inv *wp tsl*/Inv *wp tsl* females. In a large screen for inversions, using reduced recombination in the interval between the mutation *yellow body* (*y*) and *wp* as indicator, one homozygous viable inversion, D53 (*wp tsl*), was detected (A. Zacharopoulou, C. Caceres, and G. Franz, unpublished data). Unfortunately, it does not cover the *tsl*, i.e. it would not reduce type-1b recombination. However, recombination tests with *wp* and the mutation *Sergeant*^{2} (*Sr*^{2}), located very close to *tsl* (Fig. 6) (Niyazi et al. 2005), showed that inversion D53 also has a strong recombination-reducing effect in neighbouring areas (G. Franz, unpublished data). Based on this finding, D53 was combined experimentally

with the translocation T(Y;5)101, and a new strain VIENNA 8 was constructed. This strain has now been reared for 5 years (60 generations), under the same conditions as T(Y;5)101, without inversion. When the results in Fig. 10 are compared with those in Fig. 8, it is apparent that stability is improved significantly, i.e. no *wp*[+] females (type-1a) were detected. Temperature tests with *wp* females showed also that no *wp tsl*[+] female recombinants (type-1b) accumulated in the colony (Fig. 11), even though the inversion covers only a small proportion of the *wp-tsl* interval.

Both genetic strategies to improve the stability of sexing strains have been successful. Using the appropriate translocation reduces the recombination frequency by 0.65, and if an inversion is added, the frequency is reduced by another 0.80. Inversions have the added potential that they permit translocations based on criteria other than stability to be used. For example, translocation T(Y;5)101 causes less sterility (10–30%, G. Franz, unpublished data) than other translocations, but is not very stable. However, in combination with inversion D53 in VIENNA 8, stability is very high. VIENNA 8 has now been provided to several operational AW-IPM programmes that integrate the SIT.
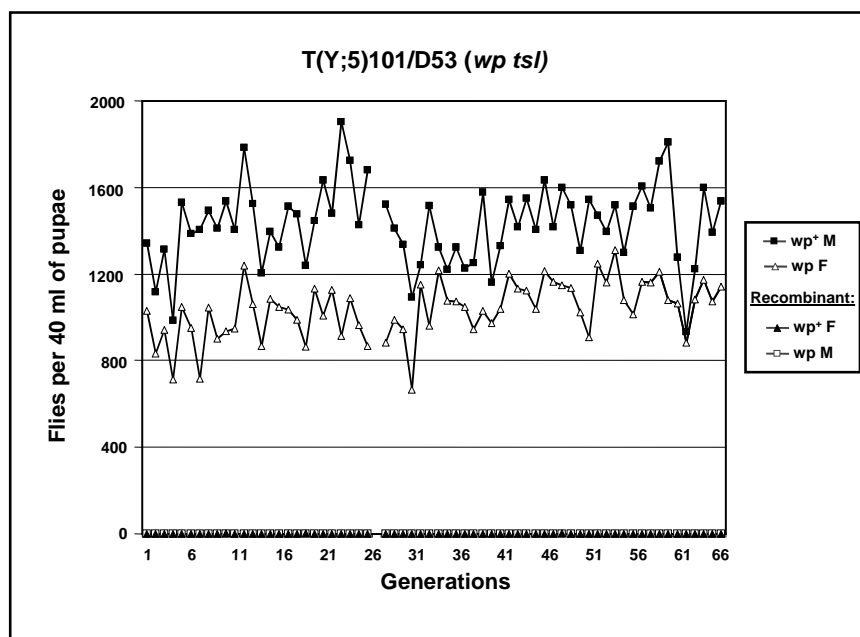
Figure 10. Increased stability due to presence of inversion D53. Neither wp[+] *females nor* wp *males are detected at significant levels. Rearing and analysis were identical to the experiment without inversion. In several generations,* wp *females were tested for temperature sensitivity (Fig. 11). F: females, M: males.*
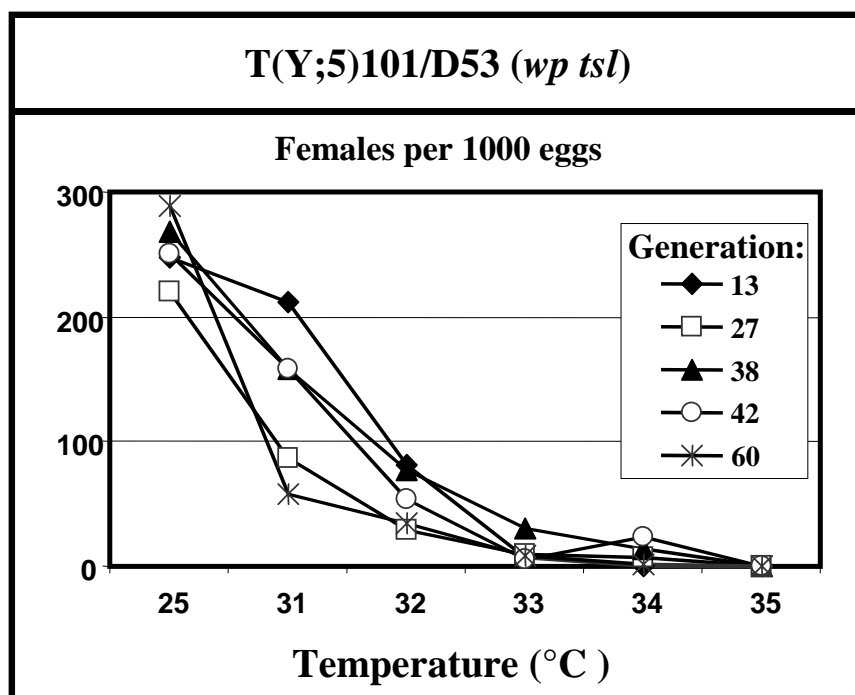
Figure 11. Temperature tests with wp *females from five different generations during rearing of sexing strain T(Y;5)101/D53.*

4.3. Filter Rearing System (FRS)

No GSS will be absolutely stable under conditions of large-scale mass-rearing. Experience with GSSs in different mass-rearing facilities has shown that rearing conditions and the scale of mass-rearing determine whether or not the stability of a strain can be maintained. Up to a certain level of mass-rearing, a facility with well-trained personnel, appropriate equipment, and adjusted operating procedures can maintain the stability. However, if the level of mass-rearing is very high, or any of the aforementioned requirements are not met, stability cannot be guaranteed, and additional measures are required. It is for this purpose that the FRS was developed (Fisher and Caceres 2000). In a systems approach, by avoiding the accumulation of recombinants, the FRS provides a second independent procedure to maintain strain integrity (Parker, this volume). Only with both strategies in place (genetically improved strains and improved mass-rearing procedures) is it possible to produce more than 3500 million Mediterranean fruit fly males per week (Table 1). However, it must be emphasized that the FRS can be used only when a visible mutation is included in the GSS.

5.   ADDITIONAL FEATURES: MARKERS

GSSs should carry markers (internal as well as external) that make them distinguishable from wild flies or other strains. Markers make it possible to: (1) detect the deliberate or accidental contamination of a strain with wild flies or flies from a different strain, (2) determine if non-irradiated flies (especially females) were released, and (3) reduce the biological and economic costs of, and ambiguities in, the currently used procedure for marking released flies (adults emerge from pupae coated with powdered fluorescent dye) (Parker, this volume).

To achieve this goal in the Mediterranean fruit fly, two strategies were pursued. First, a particular type of mitochondrial DNA (mtDNA) haplotype was introduced into the GSS. This mtDNA haplotype was detected in a wild-type strain from Egypt, and it can be used to differentiate the GSS from most field populations where AW-IPM programmes integrating the SIT are being carried out. The mitochondrial genome of the Mediterranean fruit fly has been fully sequenced (Spanos et al. 2000). The second approach is based on the introduction of the dominant mutation Sr^2 (Niyazi et al. 2005) into GSSs. Tests are ongoing to evaluate strains in which the males carry Sr^2, i.e. these males are distinguishable from wild males because they show three instead of two white stripes on the abdomen.

6.   FUTURE PERSPECTIVES

In future, the worldwide capacity to produce sterile males of the Mediterranean fruit fly is expected to increase steadily. In many existing facilities, production will increase, and several new facilities (rearing *tsl*-based sexing strains) are being constructed or expanded, e.g. Peru, USA (Hawaii), Brazil, Israel, and Spain. In particular, the use of the SIT in the Mediterranean area is expected to increase dramatically; several countries want to use the technology for pest suppression to decrease the reliance on insecticides. The Mediterranean region may eventually represent a market of 4000 million sterile Mediterranean fruit fly males per week.

Beyond the Mediterranean fruit fly, there is an increasing need to develop GSSs for other insect species of agricultural or public health importance (Marec et al. 2005; Robinson and Hendrichs, this volume). Since the genetics of many pest species is not well known, using Mendelian genetics takes some time. Part of the reason for this long lead time is that Mendelian genetics involves several unknowns. For example:

- Inducing mutations in a new species is a very unpredictable process, with no guarantee that usable selective markers will be identified.
- Translocation induction is also a random process, and based on experiences with the Mediterranean fruit fly, there are at least two criteria that have to be used to select useful translocations: (1) only one autosome involved, and (2) the positions of the break points on the autosome and the Y chromosome have to be known.
- Inversions will be very important in any GSS based on classical genetics, but they can also be used to facilitate screening for mutations like the *tsl* and for outcrossing of GSSs. Screening for inversions is very time-consuming and

labour-intensive, and requires appropriate mutations and a well-developed cytology.

In contrast to classical genetics, molecular strategies may offer the possibility of developing generic "sexing cassettes" in one species that can be transferred with only minor modifications to other closely related species (Robinson and Franz 2000, Robinson et al. 2004). In this case, much less basic knowledge of the genetics of the target species is required. Several sexing strategies have been identified in *Drosophila* (Heinrich and Scott 2000, Thomas et al. 2000), and they usually consist of three parts — a lethal gene, a female-specific promoter for the gene, and a repression or an induction system to inhibit expression of the lethal gene in females during colony production. Fryxell and Miller (1995) described a variation of this scheme, wherein the induction component is found within the lethal gene itself, i.e. it causes lethality only below a certain threshold temperature. In the Mediterranean fruit fly, an alternative system has been developed that is based on modulating genes in the sex-determining cascade (Pane et al. 2002), making it possible to convert females into males.

However, to date, these very promising systems have been tested on only a very small scale, and thus many important questions, e.g. stability, sexing accuracy, strain productivity, etc., cannot be answered. In the coming years, research will have to provide the answers.

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CHAPTER 4.4.

USE OF GEOGRAPHIC INFORMATION SYSTEMS AND SPATIAL ANALYSIS IN AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES THAT INTEGRATE THE STERILE INSECT TECHNIQUE

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SUMMARY

The advantages that geographic information systems (GIS) and associated technologies can offer, in terms of the design and implementation of area-wide programmes of insect and/or disease suppression, are becoming increasingly recognised, even if the realization of this potential has not been fully exploited and for some area-wide programmes adoption appears to be progressing slowly. This chapter provides a basic introduction to the science of GIS, Global Positioning System (GPS), and satellite remote sensing (RS), and reviews the principal ways in which these technologies can be used to assist various stages of development of the sterile insect technique (SIT) as part of area-wide integrated pest management (AW-IPM) programmes — from the selection of project sites, and feasibility assessments and planning of pre-intervention surveys, to the monitoring and analysis of insect suppression programmes, and the release of sterile insects. Potential barriers to the successful deployment of GIS tools are also discussed.

1. INTRODUCTION

The success of the sterile insect technique (SIT) and other area-wide interventions, aimed at controlling populations of insect pests, depends to a large degree on appropriate project planning and implementation. More specifically, successful programmes depend on an accurate knowledge of pre-existing distributions of insects in time and space, on the appropriate design of insect suppression strategies and sterile insect release programmes, and on the development of suitable frameworks for monitoring and evaluation.

Geographic information systems (GIS), the Global Positioning System (GPS), and remote sensing (RS) are allied technologies that together provide a means of gathering, integrating and analysing spatial data. To date, the application of these tools within area-wide integrated pest management (AW-IPM) programmes has been somewhat limited, but this situation seems likely to change, particularly since GIS and the GPS are already being used extensively in other areas of agroecological management and research (Lefko et al. 1998, Liebhold et al. 1998, Barnes et al. 1999, Letourneau and Goldstein 2001, Nutter et al. 2002). There also is an increasing application within the SIT community, both in terms of the range of spatial tools available and the potential opportunities these may also offer to possible transgenic insect release programmes in the future (Benedict and Robinson 2003). From a practical standpoint it could be argued that there has never been a better time to realize the potential of these tools within AW-IPM programmes that use the SIT. The tools themselves are becoming increasingly accessible to non-specialists, while increases in computing power mean that even high-level GIS systems can now be installed on a standard personal computer. Software costs, once a major disincentive, are now rarely prohibitive, and GIS and RS data are more widely available than ever before.

The principal aim of this chapter is to assess potential areas for the application of GIS and associated spatial tools within AW-IPM programmes. The first part of the chapter constitutes a short primer on GIS, GPS, and RS technologies. Subsequent sections illustrate the use of these tools in ecological and epidemiological studies, and address issues specific to programmes integrating the SIT, particularly with

respect to the potential use of spatial tools in feasibility assessments, planning and implementing pre-intervention surveys, and guiding subsequent programmes of insect suppression and sterile insect release.

2. SPATIAL TOOLS: STATE OF THE ART

2.1. *Geographic Information Systems (GIS)*

GIS can be defined as computer-based systems capable of capturing, cleaning (checking for errors and gaps), integrating, storing, retrieving, analysing, and displaying spatial data. GIS incorporate spatial data (geographical features) in the form of geographical coverages (maps), and descriptive data (attributes) in the form of relational databases linked to the mapped features (Kitron 1998). What makes GIS distinct from other types of databases is their ability to analyse data based on their location and spatial characteristics.

GIS coverages can be developed using information from a variety of sources, including digitized paper maps, field surveys using hand-held GPS receivers, and thematic layers derived from air photographs or satellite imagery. Much of the utility of GIS stems from their ability to combine datasets of different provenances, spatial scales, and data types.

In most GIS packages, geographic data are represented by vector and raster data models. In the vector model, geographical features are represented by points, or as lines and polygons made up of points joined by lines (arcs). In the raster model, spatial data comprise a regular grid of cells in which points are represented as single cells, and lines as strings of connected cells. Depending on the size of the cells that constitute the raster grid, this arrangement can be significantly less precise than the vector model. However, raster data are better suited to storing and modelling variables that vary continuously in space (Bonham-Carter 1994). Topographic data, for example, are commonly stored as raster grids (digital elevation models). Climate data, which vary continuously in space and time, are also commonly stored as rasterized climate "surfaces" (Hutchinson et al. 1995). Today, most GIS software packages can handle both vector and raster data, but relatively few are able to perform analytical spatial operations involving both types simultaneously (Pfeiffer and Hugh-Jones 2002).

While GIS are often used solely for their mapping and visualisation capabilities, their functionality is likely to extend to much more sophisticated forms of spatial and statistical analysis. In this context, spatial analysis refers to the manipulation and transformation of GIS data to extract additional meaning from them. Common examples of spatial analysis include buffering map features (e.g. to define areas of exposure (potential infestation) around insect-breeding sites), interpolating between points (e.g. to produce climate "surfaces" from a network of weather stations), and overlaying a number of individual geographical coverages to produce derivative maps. The latter approach can often take the form of "suitability analysis", in which spatial coverages are weighted and combined to identify and display locations that meet specific criteria (Clarke et al. 1996). Later in this chapter, an example, in which suitability analysis has been used for decision support in trypanosomosis control, is

described. Several introductory texts provide more detail on the range of spatial analytical techniques available in most GIS packages (Bonham-Carter 1994, Burrough and McDonnell 1998).

Traditionally, the statistical functionality built into proprietary GIS software has been relatively weak (limited to the computation of descriptive statistics for single or multiple coverages). This situation is slowly changing; current versions of commercial GIS software now commonly include a range of geostatistical commands, while a number of third-party solutions (software produced by firms/individuals that adds specific functionality to proprietary GIS software) are available for specific analyses, including testing for space-time clustering among point and polygon data (Pfeiffer and Hugh-Jones 2002). This type of exploratory data analysis is particularly appropriate for identifying unusual spatial patterns within large datasets, and is often used as a means of hypothesis generation. GIS are generally less suited, however, to formal statistical modelling and hypothesis testing. For statistical modelling, it is more common for GIS to be used as an "enabling technology", generating data for subsequent use in dedicated statistical software.

The accuracy of the final output from a GIS-based analysis is, to a significant degree, determined by the quality of the data in the GIS. Data need to be temporally as well as spatially accurate (a spatially accurate land-use map is of no use if it is 30 years out of date). The spatial and temporal resolutions of the data used in GIS also need to be appropriate for the application in question. For example, topographic maps at a scale of 1:250 000 would be of little use in a village-scale study. Similarly, a series of annual climate surfaces would be ill-suited to attempts to determine the seasonality of insect populations. Depending solely on secondary data, therefore, may severely limit the range of analyses that can subsequently be carried out. For these and other reasons, hand-held GPS receivers for ground truthing, and satellite remote sensing for updated information on changes in surface conditions, have become increasingly popular sources of GIS data.

2.2. Global Positioning System (GPS)

Hand-held GPS receivers are ideally suited to mapping spatial features where conventional maps are unavailable or inadequate (Thomson and Connor 2000). The basis of the GPS is a constellation of 24 NAVSTAR satellites developed and maintained by the US Department of Defence. These satellites act as reference points, with each satellite transmitting a radio signal in the form of pseudo-random code. On the ground, GPS receivers use this code to determine distances to each satellite ("ranging"), and calculate their position and altitude by "trilaterating" signals from a number of satellites.

GPS receivers typically achieve a horizontal accuracy in the 5–15-m range. Positional errors arise mainly from atmospheric effects on the GPS signals, from clock errors, and as a result of multipath reflection of signals at ground level. Much of the error due to atmospheric effects can be removed using "differential" GPS techniques, in which positions obtained from a roving GPS are corrected using signals received by a static GPS located at an accurately surveyed position. Horizontal accuracy using differential GPS techniques is usually in the 1–5-m range,

although sub-metre accuracy can also be achieved depending on the hardware used. Unless suitable public-domain data are already available, hardware costs associated with installing dedicated differential GPS systems are relatively high (unlikely to cost less than USD 10 000). The cost-effectiveness of such an investment depends on the importance of obtaining positional accuracies in the 1–5-m range. Indeed, some situations may warrant this level of accuracy, e.g. detailed mapping in urban sites, or the registration of sub-metre-resolution satellite data to real-world coordinates. However, in most ecological or entomological survey situations, a positional error of 5–15 m is probably acceptable.

GPS receivers are often used simply to collect spatial data (coordinates) for geographical features, with associated attribute data recorded separately and manually on survey forms. However, in many cases, GPS-receiver software now includes programmable "data dictionaries" which can be used to capture attribute information directly. Alternatively, some GPS receivers can be linked up to personal digital assistant (PDA) devices or tablet computers. Both approaches greatly increase the speed and efficiency with which GPS data can subsequently be incorporated into existing GIS.

2.3. Satellite Remote Sensing (RS)

Satellite RS is the process of gathering information about the earth's surface using electromagnetic sensors on board satellites. The majority of satellite sensors are "passive" — they detect solar radiation reflected from the earth's surface. "Active" sensors, e.g. radar, also exist, but to date have rarely been applied in ecological and epidemiological studies. Data from passive sensors can be used in a relatively raw form, e.g. to derive land-cover classification maps, or can be transformed into indices that constitute direct proxies (substitutes) for environmental variables, such as rainfall, land-surface temperature, and vegetation status (Hay et al. 1996).

The potential value of satellite RS for ecological research has long been recognised, particularly in terms of its ability to offer objective, up-to-date assessments of surface conditions over large, sometimes inaccessible, areas. Moreover, the repeatability of satellite measurements makes RS particularly suitable for monitoring environmental conditions over time. The applicability of remote sensing to different types of ecological study will, however, depend on both the nature of the study and the spatial, temporal, and spectral characteristics of available image data (Box 1).

Images from different sensors vary greatly in terms of spatial resolution, e.g. with pixel sizes for commonly available products currently ranging from under a square metre to several square kilometres. Similarly, the temporal resolution (or revisit time) of individual sensors can be as little as 30 minutes in the case of geostationary meteorological satellites, or as much as 30 days in the case of some polar-orbiting satellites. In a project that requires local, detailed assessments of land cover, spatial resolution will be the prime consideration when selecting satellite data. If the project is more concerned with changing meteorological and vegetation patterns over time, temporal resolution will probably be of greater concern.

In the context of ecological and epidemiological studies, satellite data have been extensively used to model and predict the distributions of insects and/or associated diseases in time and space. Modelling on a large scale has commonly involved using satellite data to delimit specific insect-breeding sites or habitats (Pope et al. 1994, Rejmankova et al. 1995). On a national or regional scale, these distributions are more commonly modelled on the basis of proxies for meteorological variables and/or vegetation status (Linthicum et al. 1990, Rogers et al. 1996, Hay et al. 1998, Brooker and Michael 2000, Randolph 2000). Several general reviews, covering these and other studies, are available (Hay et al. 1996, Thomson and Connor 2000).

Box 1. Resolution in Satellite Remote Sensing

Spatial Resolution (Fig. 1)

The spatial resolution (pixel size) of various sensors varies enormously: 0.61–2.4 m for QuickBird, 5–10 m for SPOT 5, 15–90 m for ASTER, 15–60 m for Landsat, 250–2000 m for MODIS. The width (swath) of images varies accordingly, e.g. about 25 km for QuickBird, 185 km for Landsat. Polar-orbiting meteorological satellites have relatively low spatial resolutions and large swath widths (1.1 km and about 2400 km, respectively, for AVHRR), while images from Meteosat and other geostationary meteorological satellites have 1–8 km pixels, but comprise an entire earth half-disk.

Temporal Resolution

Temporal resolution is defined by the time taken for a satellite to revisit the same point in its orbit (repeat time). Sensors with high spatial resolutions tend to have low orbits and long repeat times, e.g. 16 and 26 days in the case of Landsat and SPOT satellites, respectively. Since over a year's period some satellites sense only a few images for a given locality, obtaining cloud-free data can be problematic. At the other extreme, meteorological satellites have very short repeat times (12 hours for AVHRR, 30 minutes for Meteosat), and obtaining cloud-free data is rarely a problem.

Spectral Resolution

Passive sensors detect radiation from the sun that has been reflected by the earth's surface (as well as, in some cases, radiation emitted directly from earth). The amount of reflected radiation depends on the nature of the surface and on the wavelength of the radiation concerned. For example, vegetation reflects most of the radiation it receives in the green (visible) part of the electromagnetic spectrum, but absorbs much of infrared energy. Dry soil, on the other hand, absorbs large amounts of visible light, but reflects a large proportion of near infrared. The ability to use these "spatial signatures" to infer surface properties depends on the spectral resolution of the remote-sensing data being used. Spectral resolution refers to the number, width and spacing of the spectral "bands" used by the sensor. Traditionally, most sensors have included three to seven bands in the visible and near-to-thermal infrared part of the electromagnetic spectrum (0.3–14-mm wavelengths). However some new sensors, e.g. MODIS and ASTER, have many more, and this improved spectral resolution should increase the ability to distinguish between different land-cover types.

Until relatively recently, researchers have been severely restricted in terms of the range of satellite data available to them. As examples in later sections of this chapter illustrate, traditionally this constituted a choice between two types of product: (1) low spatial/high temporal resolution data from sensors such as the National Oceanographic and Atmospheric Administration's advanced very-high-resolution radiometer (AVHRR), and (2) data from high spatial/low temporal sensors on board Landsat and Satellite pour l'Observation de la Terre (SPOT). This situation is now changing, with the launch of several new earth-observing satellites, and the range of available remote-sensing data has recently expanded significantly (Beck et al. 2000).
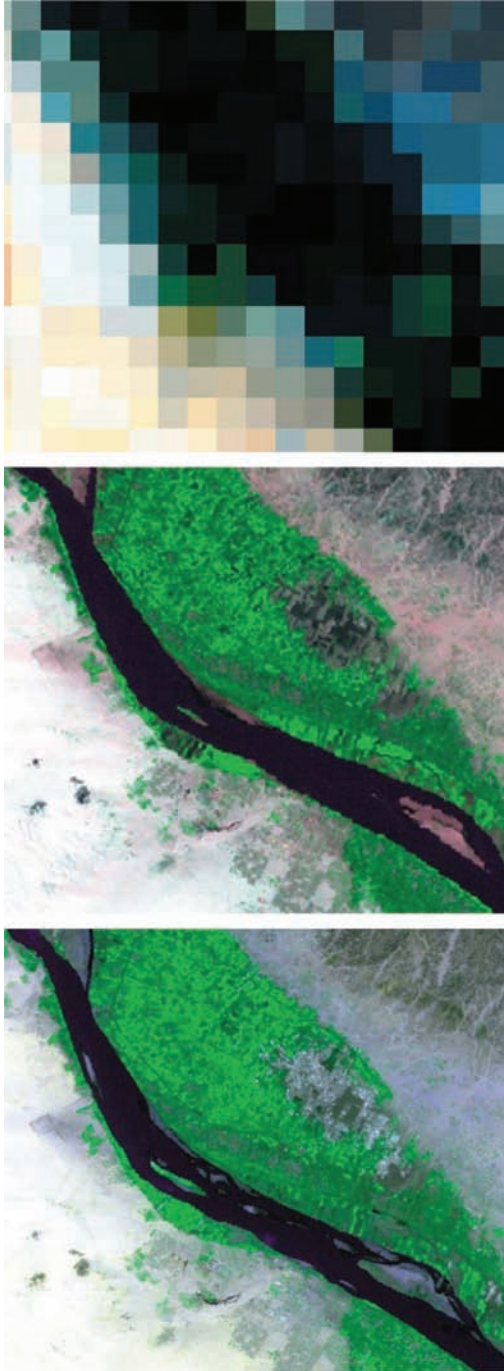
Figure 1. The significance of spatial resolution in remote sensing. These images represent false-colour composites of data from three satellite-borne sensors: (Left) ASTER (bands 2, 3, 1) with a 15-m spatial resolution (pixel size); (Centre) Landsat ETM+ (bands 2, 4, 3) with a 30-m spatial resolution; and (Right) MODIS (MOD09A1 8-day surface reflectance composite, bands 1, 4, 3) with a 500-m spatial resolution. The images represent an area of approximately 7 x 8.25 km, and cover a portion of the Nile Valley in the vicinity of Merowe, northern Sudan. Field boundaries and roads are clearly visible in the ASTER data, but are less apparent in the Landsat data. At a resolution of 500 m, the spectral properties of the Nile channel and surrounding vegetation are not visually distinct. Note that data from meteorological satellites (AVHRR and Meteosat) are commonly available at a resolution of 8 km.

New sensors, such as the moderate-resolution imaging spectroradiometer (MODIS) on NASA's Terra satellite, are helping to bridge existing gaps in data availability by providing data at both moderate spatial and temporal resolutions (250–2000 m and daily, respectively, in the case of MODIS). Another Terra sensor, ASTER, provides imagery with a spatial resolution similar to Landsat and SPOT data (15–30 m in visible and near-infrared bands), but with a vastly superior spectral resolution. Commercial satellites now also provide relatively low-cost data at very high spatial resolutions. For example, data from Digital Globe's QuickBird sensor has a spatial resolution of 0.61 m in panchromatic mode, and 2.4 m in multispectral mode. Since several new satellite sensors are to be launched in the next 5 years, it should become increasingly easy to match the specific data requirements of individual disease or pest suppression programmes with appropriate sources of satellite imagery.

3. APPLICATION AREAS FOR GIS, GPS, AND RS IN OPERATIONAL PROGRAMMES

In the past, the use of GIS and RS in AW-IPM programmes that include the SIT has been limited largely to the "collection of data" and the "production of maps", whereas a key element of spatial decision-support systems — the linking of data with appropriate analytical tools — has been largely neglected. This represents a missed opportunity, but there are many reasons to believe that future programmes will be better placed to use GIS to bring together datasets critical to the planning, implementation, monitoring, and evaluation of the programmes. Certainly there is enormous potential to use powerful analytical frameworks in spatial decision-support systems in AW-IPM programmes, which can take decision-makers beyond the point of simply possessing data, information, and knowledge.

Individually, GIS, GPS, and RS potentially have several important roles to play at various stages of project planning and implementation. The following sections discuss the major areas of application where these technologies, either demonstrated or anticipated, have the most to offer. Where possible, the discussion draws on previous experience in the use of spatial approaches in AW-IPM programmes, but more often the discussion centres on exploring ways in which existing spatial technologies can best be matched with the needs of future operational pest control programmes. The following sections address several key individual stages of project planning and implementation, from the design of pre-intervention surveys to monitoring and analysing data from insect release programmes.

3.1. Planning and Implementing Pre-Intervention (Insect, Disease, Host) Surveys — GIS-Based Modelling of Spatial Distribution of Target Insects

Insect pest control programmes, integrating a combination of suppression techniques, require accurate, up-to-date information on the spatial and temporal distribution of the target insect population. A GIS-based analysis can bring together a wide range of information sources — e.g. climate data, remote-sensing data, land-use and topographic data, historical data on insect distribution and abundance, disease prevalence, etc. — that together can be used to develop modelled or

empirical estimates of the temporal and spatial distributions of the pest or disease. The nature of this GIS exercise, and the data sources used for it, will reflect the stage to which pre-intervention planning has developed. At the very early stages of feasibility assessment and planning, for example, GIS modelling will focus almost certainly on identifying areas of relatively high pest density at the national or regional level, using low spatial resolution data for climate and land cover in combination with available historical information on the insects and/or diseases. These maps may be adequate for planning purposes in cases where insect intervention programmes are implemented at the national or regional scale. In other cases, it may be more appropriate to use these broad assessments for directing more detailed modelling efforts, using higher-resolution geographic datasets (and possibly prospective sampling of insects) to specific areas of interest.

3.1.1. Mapping Pest Distribution on a Regional Scale

Several published research studies have explored using GIS and RS for predicting the distribution of insects on a regional scale, although none has had a specific SIT focus. Hay et al. (1997) and Thomson and Connor (2000) provided useful reviews of much of this work. The discussion here is limited to work most pertinent to using the SIT in the context of AW-IPM programmes, most of which has focused on the spatial prediction of tsetse flies *Glossina* spp.

The use of low-spatial-resolution satellite data to predict insect distributions dates back to attempts in the early 1990s to correlate the distribution of tsetse and the incidence of trypanosomosis to spatial variations in climate and the normalized difference vegetation index (NDVI) (Rogers 1991, Rogers and Randolph 1991, Rogers and Williams 1993). Later models also incorporated surrogates of land-surface temperature from AVHRR satellite data, and a proxy variable for rainfall (cold-cloud duration) from Meteosat data. Rogers et al. (1996), for example, used Fourier-processed satellite data for climate and NDVI in combination with digital elevation data to predict the presence/absence of eight tsetse species in Côte d'Ivoire and Burkina Faso, with an accuracy of 67–100%.

A similar approach, using logistic regression, has also been used to model ranges of tsetse species in East Africa. Fig. 2 shows the distributions of *Glossina fuscipes fuscipes* Newstead and *Glossina pallidipes* Austen. The distributions were produced by modelling the "known" presence and absence of the flies, using the Ford and Katondo maps (Ford and Katondo 1977), but then modified with more recent information obtained from national and international agencies and researchers. The modelling process relies on the logistic regression of fly presence against a wide range of predictor variables for a large number of regularly spaced sample points for each area. The predictor variables include remotely sensed (satellite image) surrogates of climate — vegetation, temperature, and moisture, which have been subjected to Fourier processing to provide an additional set of season- and timing-related measures for each parameter. Demographic, topographic, and agro-ecological predictors are also used. These models are then applied to the predictor imagery to produce predicted probabilities of fly distributions at 1-km resolution.

In southern Africa, Robinson et al. (1997) used climate surfaces, together with NDVI and elevation, to model the distributions of three tsetse species in the

common fly belt. Maximum-likelihood classification techniques yielded overall correct predictions of 92.8 and 85.1% for *Glossina morsitans centralis* Machado and *Glossina morsitans morsitans* Westwood, respectively. In Togo, Hendrickx et al. (2001) found that discriminant models, based on satellite data, were generally less successful at predicting disease outcomes in cattle (trypanosomosis prevalence or packed-cell volume) than tsetse abundance.
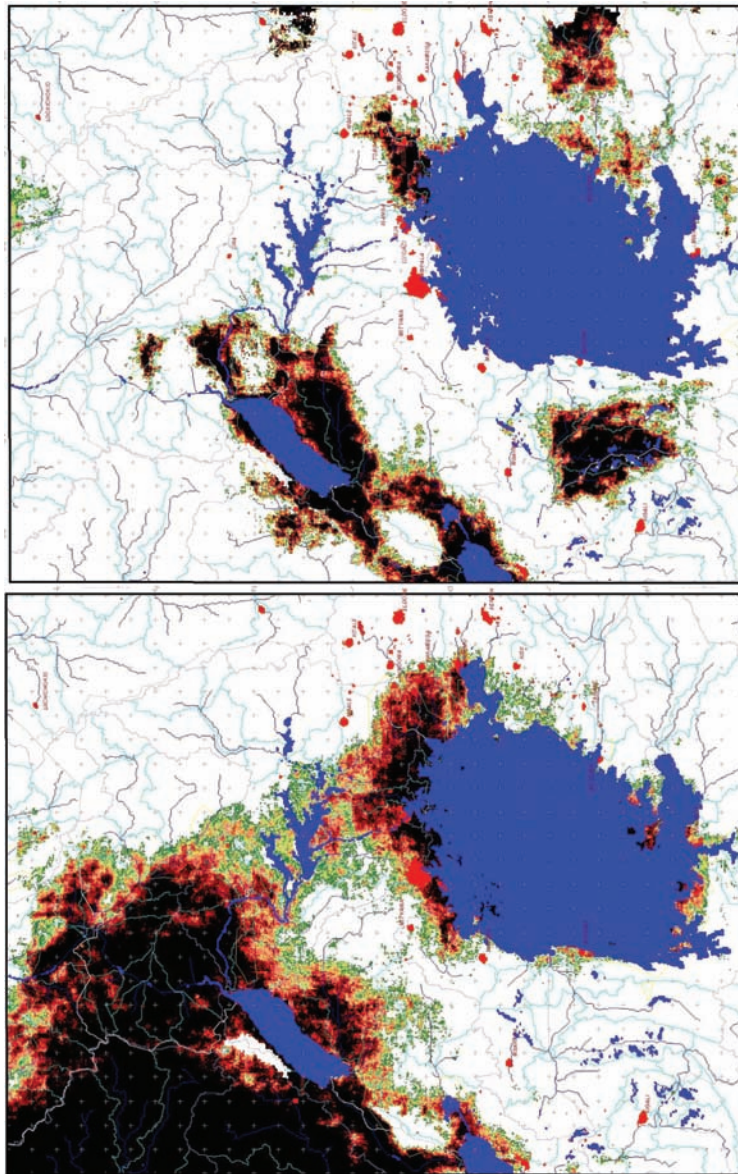
Figure 2. Presence-absence prediction model of the tsetse flies Glossina fuscipes fuscipes (left) and Glossina pallidipes (right) in Uganda, and in parts of Kenya and Tanzania. Colour and number (%) = probability of presence: black (+90), brown (80–90), red (70–80), pink (60–70), orange (50–60), yellow (40–50), light green (30–40), dark green (20–30), white (<20). Red polygons with names = urban centres. Blue areas = lakes. (Maps produced by Environmental Research Group Oxford for the FAO/IAEA, reproduced with permission.)

The high predictive accuracy of these models belies two fundamental limitations. Firstly, much of the insect data used to train the prediction process, and assess the accuracy of the model output, date back several decades — which raises questions about the relevance of model outputs to current insect distributions.

Secondly, historical data for insect abundance — arguably a more meaningful indicator of disease risk than simple insect presence — are relatively rare, and have not been widely used for disease-risk modelling. At the continental or regional scale, it could be argued that the relative risk of insect presence is an adequate indicator for abundance. However, this assumption is not valid for small areas, where models require up-to-date data from abundance surveys. It is probable that the principal role of RS and GIS, in these situations, is to make prospective surveys more cost-effective (Hendrickx et al. 1999).

3.1.2. GIS for Decision Support

Mapping pest distribution on a regional or national scale is an important first step in terms of assessing the feasibility and spatial targeting of the SIT and other area-wide control actions. However, technical and resource constraints may make large-scale operations over the whole of the identified area of potential pest distribution to be impractical or uneconomic. Therefore more specific environmental information is required, either to identify parameters that are linked to pest presence, or guide future sampling efforts to address specific questions including levels of genetic variability, similarity, and diversity among target insects.

This scenario is well illustrated by recent experiences in Zambia, where the chief mode of tsetse control has changed from widespread eradication towards local, community-based suppression. This has been accompanied by a shift in approach from simple vector control to disease management targeted at specific priority areas. As a first step in identifying these priority areas, Robinson (1998) used GIS to highlight areas where continuous tsetse suppression would be most appropriate, either in terms of disease reduction or facilitating agricultural expansion from areas of high land pressure. GIS coverages for land designation, percentage agriculture, net cattle-stocking rate, and relative arable potential were combined using a decision-tree approach, and overlaid with the estimated spatial distribution of tsetse. Significantly, model output highlighted areas of high-absorption capacity (low stocking rates and high relative arable potential) in close proximity to areas currently experiencing very high land pressure. Subsequent analysis (Robinson et al. 2002) for eastern Zambia included additional decision-making criteria (proximity to existing control operations, human population density), and used weighted linear combinations of these variables to prioritize areas for trypanosomosis control. Spatial decision-support systems have also been used to develop cost/benefit "layers" for trypanosomosis control in Togo (Hendrickx et al. 2001).

3.1.3. Mapping Pest Density on a Large Scale

The spatial distribution of most insect pests is not uniform but patchy, and there can be localized pockets of high insect density, in spite of a potentially misleading low-overall population density. It is of prime importance, for the development and

implementation of an insect control programme, that these pockets or "hot spots" be located (Shiga 1991).

Depending on the spatial scale of the heterogeneity of insect density, the climate and remote-sensing datasets used to predict insect distribution over wide areas may not be appropriate for work on larger scales. AVHRR data, for example, have a native resolution of 1.1 km, but are commonly resampled to derive images with 4×4- or 8×8-km pixels. However, several studies have successfully used high-resolution spatial data from Landsat and SPOT satellites to identify habitats associated with high insect density (Rejmankova et al. 1995, Roberts et al. 1996). For tsetse, Kitron et al. (1996) analysed trap data in the Lambwe Valley in western Kenya during 1988–1990, and found that multiple regression using seven Landsat TM bands explained 87% of the variance in fly density. Landsat TM band 7 (near infrared), which is a particularly good indicator of soil moisture status, was consistently highly correlated with the trap data.

Since the spatial distributions of insect populations are not constant, but tend to change over time, it is important that, where possible, risk maps be dynamic rather than static. For example, populations of riverine tsetse flies in West Africa commonly expand and contract seasonally along the river vegetation and perpendicular to the tributaries. The use of spatial analysis incorporating multi-temporal remote-sensing data should assist with the development of a "dynamic population distribution model" to predict these temporal and spatial population dynamics, and to link spatial patterns with heterogeneity of habitat. This would allow for a more efficient and guided (rather than *ad hoc*) deployment of sampling devices during subsequent surveys (i.e. the sampling devices can be deployed in those areas where there is a high probability of trapping or, alternatively, in areas of low probability to confirm the model). The implication is that, assuming an adequate geo-spatial model exists, an efficient survey strategy can be developed largely from the office, and detailed implementation guidelines, regarding where, how, and when to deploy the sampling devices, can be elaborated for the field teams. This would not only ensure adequate sampling coverage in all ecosystems, but also prevent the deployment of too many sampling devices in unproductive or unrepresentative sites.

The availability of temporal and spatial distribution models of the target insects on a large spatial scale has implications beyond the design of efficient sampling frames. In particular, such models should facilitate a more efficient deployment of suppression tools, as well as a better-targeted release of sterile insects. This increased efficiency should also translate into considerable economic savings in terms of logistics, personnel and sterile insects.

3.2. Development and Implementation of Appropriate Insect Suppression and Sterile Male Release Programmes

3.2.1. Selection of Appropriate Suppression Methods

Since the release of sterile insects is only efficient when they sufficiently outnumber the native insects, the SIT becomes more cost-efficient with reduced density of the target population (Dame 1971). The density of untreated insect populations is in most instances too high, and needs to be reduced prior to the mass release of sterile

insects. Depending on the target insect, a variety of pre-release population suppression methods are usually available (Mangan, this volume), but their usefulness, appropriateness, and effectiveness will be determined by the characteristics of each target area or local situation. Pending the availability of suitable data layers (demography, land use/land cover, vegetation classification, distribution of target insect, etc.), spatial analysis could assist with the decision to select the most appropriate suppression method for a given target zone. This is demonstrated by the following examples for the suppression of tsetse fly populations in AW-IPM programmes:

- Sequential aerosol technique (SAT). The SAT involves the application of non-residual ultra-low-volume insecticides by fixed-wing aircraft or helicopter (or from vehicles via hot and cold fogging). The goal is to kill adult tsetse flies in the first spraying cycle by direct contact with insecticide droplets, and then kill emerging flies in five subsequent application cycles before the emerged flies can deposit larvae (Allsopp 1984). In view of the sensitivity and susceptibility of the SAT technique to topography, wind velocity and direction, temperature inversion, etc., a spatial analysis can provide information on the suitability of the target zone in terms of: (1) topography (the application of the SAT becomes problematic when the terrain becomes mountainous), (2) habitat and vegetation cover (correlation analysis between the vegetation density and the propensity of insecticide droplets to penetrate the tree canopy, to make predictions on the vertical dispersal rate of insecticide droplets in various vegetation types), and (3) wind velocity (using climatic models to predict the dispersal and distribution patterns of insecticide droplets in each particular situation).

- Live-bait technology. In this technique, residual insecticides are applied to host animals that attract tsetse flies, which are killed on contact with the insecticide (Bauer et al. 1982). The technique is efficient and cost-effective, provided the density of the livestock population in the target area (if livestock is the main host) is sufficiently high. A spatial correlation analysis between livestock and tsetse population distribution/density can provide an indication of the suitability of this "pour-on" technology in the target zone, and make predictions regarding its efficiency.

- Stationary-bait technology (insecticide-impregnated targets and traps). Tsetse populations can also be suppressed by deploying artificial stationary devices, which attract tsetse flies (Green 1993). The flies will be killed either upon making contact with the surface area of the target/trap (which is impregnated with a residual insecticide) (Laveissière et al. 1980), or when retained in a no-escape device (Dransfield et al. 1990). The stationary-bait technology is usually promoted in the context of "community participation", and the efficiency of such a programme will be affected by the extent of the overlap of the distributions of village communities and tsetse flies (Holmes 1997). A spatial correlation analysis, using demographic data layers and tsetse population distribution models, can indicate how efficient or suitable this technology is in specific areas.

3.2.2. *Models to Predict Outcome of Different Suppression Scenarios*
Each area targeted for control will in most cases be heterogeneous, in terms of composition of the habitat (land use and land cover), vegetation cover and species composition, host (e.g. livestock and agricultural crops) distribution, demography, topography, etc., which will demand an integration of different suppression methods (Vreysen 2001). Spatial analysis can be used to model the effect of various combinations of methods on the insect population, and assist in the decision to select the best combination.

3.2.3. *Implementation of Suppression and Sterile Male Release Programmes*
Aside from decision support in the development and implementation of surveys, selection of the most appropriate suppression method, and modelling the outcome of various suppression scenarios, GIS tools can provide assistance in the actual implementation of suppression programmes, including sterile insect releases. The geo-spatial model, which was developed for the distribution and density surveys, can be exploited to provide detailed guidelines on how the suppression strategy can be implemented. For example, spatial analysis can provide decision support in the selection of appropriate sites in which to deploy insecticide-impregnated traps and targets in tsetse suppression programmes, and indicate the required target/trap density per surface unit in relation to vegetation type, topography, insect distribution, and other relevant factors (Box 2).

The uniform application of certain suppression measures (e.g. bait sprays for fruit flies, SAT for tsetse flies) over a heterogeneous pest distribution target area can have negative implications, both in terms of cost and environmental impact, since habitats may be contaminated by unnecessary applications of insecticide (Papadopoulos et al. 2003). New navigation/recording systems (such as Trimble's TrimFlight 3 Ag-GPS system, or SATLOCK's AirStar system), which guarantee the correct application of insecticide during aerial application, and ensure that fuel and materials are used efficiently, have been used for years in several fruit fly AW-IPM programmes that release sterile flies. Using these systems facilitates precise guidance, automated recording of covered areas (maximum efficiency, minimum overlap, and skips (omission of areas)), identification and remedy of skips before leaving the operational area (avoiding costly call-backs), waypoint navigation, mapping capabilities, and control of insecticide-flow and sterile insect release rates (depending on pest distribution and density) based on ground speed. Thus there is no need for time-consuming and costly ground markers, such as beacons or flaggers. The system is compatible with a wide range of GIS software packages, and enables applications to have better than 1-m accuracy.

In many programmes that include the release of sterile insects, these are released at a constant dispersal rate (i.e. a uniform density per surface unit). As a consequence, insufficient overflooding ratios might result in areas of high native-insect population density, whereas more sterile insects than necessary might be released in areas of low native-insect population density (Vreysen, this volume). GIS and spatial analysis can provide guidance on spatial sterile insect requirements and dispersal patterns, in relation to wild insect population densities, habitat, elevation, etc. in a dynamic way (i.e. data layers on the target insect distribution are

updated regularly). This will result in a much more efficient use of sterile insects. In addition, commercially available satellite navigation/flight recorder systems, e.g. Ag-Nav, provide real-time tracking, and can visually display the areas that were treated with sterile insects (or have been left out), and at which altitude insects were released (to ensure a proper spread). This will permit proper feedback to programme managers (Dowell et al., this volume).

Box 2. Past and Current Applications of GIS in Area-Wide Integrated Pest Management Programmes that Include SIT

The use of GIS/RS, as a decision-support tool in AW-IPM programmes integrating the release of sterile insects, has thus far been limited largely to the spatial display of data, and it has seldom been applied to the planning, implementation or evaluation of programmes because of the paucity and limited accessibility of spatial data, duplication in efforts to develop fundamental and useful databases, little sharing of databases, and the use of non-uniform GIS models and non-compatible methods. AW-IPM programmes using the SIT against fruit flies are among the most successful programmes against major insect pests, and these programmes display the most advanced use of GIS. In programmes against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), such as the programme in Argentina, the "Moscamed" programme in Guatemala/Mexico, and the prevention programmes in California and Florida (Enkerlin, this volume), GIS/GPS/RS are mainly used:

- To provide navigational guidance in the release of the sterile insects, and to provide "real-time tracking" using commercially available satellite navigation/flight recorders.

- To map and visually display the various trapping sites and monitoring routes. An example from an AW-IPM programme on the oriental fruit fly *Bactrocera dorsalis* Hendel in Thailand is shown in Fig. 3. To better understand the actual distribution of the insects, in some programmes a "live" connection is created between trap-capture data and the trap locations to display updated captures on a map. Likewise, the recapture rate of sterile males is displayed on a map to monitor the appropriateness of the sterile-release procedures.

- To select trapping sites, using a grid layer over topographical maps or satellite imagery, and associating the trapping site with host and topographical features and their attributes.

In addition, the "Moscamed" programme in Guatemala/Mexico is using GIS as a major component of various studies on: (1) fly performance in relation to altitude, wind velocity, habitat, etc., (2) "hot-spot" areas (where the pest persists over time despite intense efforts to suppress it), (3) insect behaviour in relation to the timing of release (afternoon, night, or morning), and (4) changes in the dispersal behaviour of sterile insects over time (P. Gomes, personal communication).

In AW-IPM programmes against the New World screwworm *Cochliomyia hominivorax* (Coquerel), GIS/RS have been introduced only in the last few years. In a recent study in Panama, GIS/RS were used to identify different vegetation types, and correlate the spatial and temporal distribution of screwworms with the various vegetation covers in a tropical environment. After classifying the forests based on tree height, structure, and species composition, the highest screwworm population density was found during the transition period from the wet to the dry season, and in forest habitats as opposed to open areas (Phillips et al. 2004). These data were used to more efficiently deploy monitoring tools in habitats favoured by screwworms, which could lead to earlier detection of low densities of screwworm populations, or possibly earlier control of outbreaks. The study clearly showed that GIS/RS can be used to improve trap placement by identifying areas of high screwworm activity. The method was applied in early 2003 to develop a trapping strategy after the accidental outbreak of the screwworm at the screwworm facility in Chiapas, Mexico (Phillips et al. 2004). Using GIS/RS techniques, optimal trapping sites were selected, which represented a reduction of 79% of the original trapping sites, i.e. considerable savings in terms of personnel and logistics.

In AW-IPM programmes against other insect pests (Lepidoptera, mosquitoes, and tsetse flies), GIS have been used only sporadically, e.g. in the programme against the tsetse fly *Glossina austeni* in Zanzibar, GPS were used for accurate navigation during the sterile male release operations, and the trapping sites in the extensive monitoring programme were selected using satellite imagery and land-use/land-cover maps.
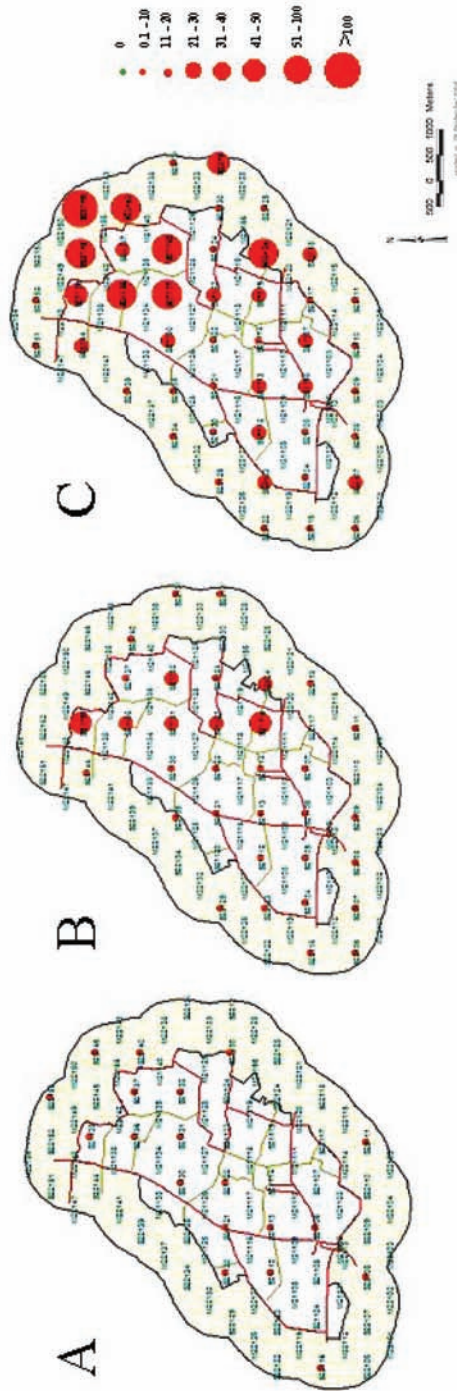
Figure 3. Use of GIS to display the spatial and temporal dynamics of an uncontrolled oriental fruit fly population sampled with Steiner traps baited with the male-specific attractant methyl eugenol. Pichit province, Thailand, from A: 31 December 2003–7 January 2004, B: 14–21 January 2004, and C: 28 January–4 February 2004. The filled circles indicate the location and number of flies caught per trap per day. (Maps from Watchreeporn Orankanok and Sirilux Noiakeaing, Department of Agricultural Extension, Ministry of Agriculture and Cooperatives, Chatuchak, Thailand; reproduced with permission.)

3.3. Monitoring Suppression or Release Programmes

Monitoring is an essential component of any AW-IPM programme (Vreysen, this volume). However, monitoring is time-consuming, and requires considerable funds for materials, logistics, and personnel. A careful balance must be found between "cost-efficiency" and collecting "reliable data". In most AW-IPM programmes, in view of the size of the target areas, monitoring (direct and indirect) must be restricted to carefully selected representative sites. Spatial analysis can assist in identifying and selecting appropriate reference or fixed monitoring sites (FMS). The concept of FMS was developed and used during the monitoring activities of the AW-IPM programme in Zanzibar, Tanzania, against the tsetse fly *G. austeni* Newstead, albeit without any GIS support (Vreysen et al. 2000; Vreysen, this volume).

From a pragmatic standpoint, it is important that all avenues of increasing the efficiency of trap-based monitoring systems are explored. Technologies that assist in the rapid transfer of data from the field to GIS are, in future, likely to be a key element of such systems. For example, in the codling moth AW-IPM programme in British Columbia, Canada, a GIS-based system is used to monitor a network of geo-referenced traps. A bar-coding system is employed, in which the time and date that traps are monitored is automatically recorded, along with data on trap catch entered manually during a trap check (Dyck et al. 1993). These data could then be uploaded to the main project database via modem, facilitating the rapid output of electronic maps showing trap data (Fig. 3).

It is also likely that future area-wide pest control activities will use geostatistical analysis routines to get "better value" out of available trap data. A range of spatial analysis techniques, employing both geostatistics and GIS, may be valuable for analysing insect population processes at a landscape scale (Liebhold et al. 1993). Of these, probably the most widely used analytical technique is "kriging" — an interpolation procedure that relies on an autocorrelation function (the variogram) to provide weighting of nearby points used in the estimates. Kriging is ideally suited to the analysis of trap data, with interpolated output taking the form of contour maps or density surfaces of insect densities.

Such an approach has recently been used to monitor and predict populations of the spruce budworm *Choristoneura fumiferana* (Clemens), an important defoliator of trees in boreal forests in North America. Using kriging as a basis, Lyons et al. (2002) developed a set of software tools to produce interpolated estimates, using data from a pheromone-trapping network covering much of Canada and north-eastern USA. Output from the software system can be reclassified in a variety of ways, using GIS to provide maps that address particular management concerns. For example, maps are routinely produced that display areas where moth densities exceed a critical threshold, and hence where conventional larval sampling activities need to be initiated. Change-analysis approaches are also used to create difference maps for consecutive years which, when re-classified, highlight areas of increasing, decreasing and stable moth populations. Kriged map surfaces have also been used as variables which, when combined with historical data on defoliation and defoliation frequency, have been used to successfully predict levels of defoliation in the following year.

In the wider world of commercial crop decision-making, this type of approach has been taken a step further. In several states in the USA, integrated systems of insect and weather monitoring have been developed, providing estimates of insect and disease risk in near real-time. These systems, described by Thomas et al. (2002), use a network of geo-referenced automated weather stations, which utilize radio telemetry to send data — on temperature, relative humidity, precipitation, and other meteorological parameters — every 15 minutes to a central processing centre. These data form the basis of Internet-based, daily estimates of weather and disease-risk related parameters (including insect degree-day maps), in addition to risk maps for specific diseases such as powdery mildew and *Botrytis* spp.

3.4. Data Analysis

Area-wide insect pest control programmes generate a large amount of entomological and other related data, not only during baseline data collection and feasibility surveys but also during the monitoring of suppression and release activities (Dyck, Reyes Flores et al., this volume; Vreysen, this volume). It is a real challenge to manage data efficiently, analyse and interpret the results in a timely manner, and provide programme managers with data in a suitable format. The GIS unit of any pest control programme provides an ideal medium for the storage and analysis of data, and it can greatly facilitate their interpretation. Adequate feedback to programme managers is a prerequisite for sound decision-making. The complexity and diversity of data accumulated during a control programme, requiring proper spatial analysis, are typified in the following examples:

- Data derived from initial surveys
  Temporal and spatial fluctuations in:
  o Population distribution
  o Population density
  o Population structure (composition)
  o Disease prevalence and infestation levels in hosts
- Data derived from monitoring suppression activities
  o Temporal and spatial changes in population distribution due to the application of the suppression methods
  o Temporal decline in the density of the target pest population in relation to different ecosystems
  o Temporal changes in population structure (due to increase in mortality rates) in relation to different ecosystems
- Data derived from monitoring sterile insect releases
  o Spatial and temporal fluctuations in the ratio of sterile to wild insects
  o Spatial and temporal fluctuations in the rate of induced sterility in the native insect population
  Spatial correlation analysis using these variables will contribute to modelling the competitiveness of released insects in relation to habitat, host abundance/distribution, climatic variables, etc. Obtaining spatial and temporal data on sterile insect competitiveness is one of the most important features of any AW-IPM programme that releases sterile insects.

o Spatial and temporal fluctuations in the recapture rate of insects in traps, damage to hosts, disease patterns, etc.

These data can be correlated with the release rate of sterile insects over the release grids, and then models developed on the mobility, dispersal characteristics, and spatial occupation of the sterile insects in different vegetation types, etc.

The results of a spatial analysis of a suppression and release programme can provide answers on issues such as the adequacy of deployment of suppression devices (sufficient number, adequacy of spread, efficiency, level of damage, timeliness of replacement of the suppression methods, etc.), and the appropriateness of sterile insect release operations (e.g. coverage of the proper block, flight at the proper altitude, possibility that insects were blown into nearby bodies of water, influence of wind, delivery of sterile insects so that they find and concentrate at the same locations as wild insects, etc.).

3.5. Support for Sterile Insect Quality Control (QC)

Frequent monitoring of the performance of sterile insects (i.e. insect quality) in the field is an important, although often neglected, component of AW-IPM programmes that integrate the SIT (FAO/IAEA/USDA 2003). Parameters such as survival, mobility, dispersal characteristics, and spatial occupation of the habitat significantly influence the field performance or the competitiveness of released insects (Calkins and Parker, this volume; Lance and McInnis, this volume; Vreysen, this volume). The values of these parameters may change over time, and could be affected by host availability, vegetation cover, vegetation species, altitude, etc. GIS and RS can be used to predict these temporal and spatial changes in insect quality, and thus assist in developing remedies and timely adjustments in an intervention programme. As an example, swaths of flight release lanes are inherently linked to the dispersal capacity and mobility of the released insects, whereas their average survival determines the release frequency (Hendrichs et al., this volume). Models can be developed to correlate sterile insect survival with host availability, vegetation cover, etc. to assist in regularly adjusting the dispersal rate of insects in relation to space (e.g. more frequent releases in those areas where survival is low). In addition, a spatial and temporal analysis of the dispersal characteristics offers opportunities to assess whether this parameter is changing with the length of time that the insects have been colonized in the rearing facility (Parker, this volume).

There is great potential for GIS to support the assessment of the quality control of released insects, particularly with regard to: (1) comparing the performance (competitiveness, mobility, dispersal, survival) of sterile insects derived from different strains, (2) studying the effects of releasing flies using different release systems, or at different altitudes, over different topographies (e.g. canyons), at different wind velocities, etc., and (3) analysing hot spots or reservoir areas where the pest persists in spite of intense actions to suppress the population.

3.6. *Barriers to Using GIS*

In previous decades, developing a GIS component within a broader operational programme involved overcoming a number of potential hurdles. Cost was a major concern, in terms of prices for both software and hardware (the cost of proprietary software packages, in particular, could be notoriously high, as could the workstations required to run them). The cost of acquiring primary data (such as satellite remote-sensing data), or third-party value-added data (data modified and improved by another firm or organization), was also generally much higher than today; also the choice of available data was often rather limited. Most of the RS data available in the 1980s and 1990s were supplied "as is", and there was little effort on the part of data providers to supply them either in the form of corrected ready-to-use imagery, or as derivative products (vegetation indices, rainfall indices, etc.) that could readily be used by non-experts.

Today, the barriers to successful GIS development are significantly lower than they were in the past. The costs of hardware, software, and data are now relatively low, while the availability and choice of GIS and RS data on the market are now rather large. Perhaps most significantly, GIS software is becoming increasingly accessible to non-specialists, which means that non-geographers can now carry out many GIS procedures without specialist support (although this does not completely eliminate the need for expert input; note comments below under "GIS expertise").

These advances, however, do not mean that potential barriers or pitfalls no longer exist. Several considerations need special attention, particularly when working in the context of a developing country, if a GIS endeavour is to be successful (BESR 2002). These considerations include:

- Cost limitations. Cost may still be relevant, particularly with respect to the purchase of satellite imagery over large areas. Some third-party datasets can also be very expensive.
- Data limitations. Although data availability is becoming less of an issue, there may still be instances where up-to-date data, at an appropriate spatial resolution, are not readily available. Costs can spiral upwards when customized datasets, e.g. of climate, elevation, etc., have to be produced from the beginning.
- Technical limitations. Limits in the accessibility of spatial data, such as inadequate telecommunications infrastructure, limited bandwidth, and low Internet connectivity, may hamper efforts to rapidly process and output GIS data.
- GIS expertise. Trained and experienced experts in GIS are often in rather short supply, particularly in developing countries. The increasing accessibility of GIS software does not obviate the need for input from appropriately trained operators. Technical issues around data capture and integration can be non-trivial, as are procedures for image processing. Decisions regarding accuracy and permissible levels of data generalization also require relevant experience on the part of the GIS operator.
- Maintaining consistency. In many instances, AW-IPM programmes are implemented over large geographical areas, i.e. on a national or regional scale, employing various field teams in a series of sub-programmes, each attached to a particular country, zone or region. Agreements must be reached on compatible

GIS formats, standardized methods of data collection, and data resources. These need to be kept consistent throughout the life of a project.

- Administrative challenges. It can be a real challenge to easily gain access to available data because of unawareness about data request procedures on the part of government officials, complicated protocols for requesting government data, a variety of data standards making the sharing of data difficult, and complications due to copyright and distribution issues.

It is important that, at all stages of GIS development, a dialogue between those responsible for designing and maintaining a GIS system, and those whose principal interest is the output of the system, begins at the start of the planning process, and continues throughout the life of a project. GIS operators need to realize that many decision-makers, in developed and developing countries, have no experience with GIS and other spatial decision-support tools, and thus do not appreciate the potential in using geographic information, or the technical issues involved in setting up and maintaining GIS.

In this context, one essential message must be conveyed — the development of GIS requires careful planning, and often a substantial time-commitment. In a previous section (2.1.) of this chapter, GIS were defined as systems for capturing, cleaning, integrating, storing, retrieving, analysing, and displaying mapped information and data. These functions can be broadly seen as a series of operations that describes the data stream from the original source to a final map or output. The initial stages of this process (up to the point of storing the data) can be extremely time-consuming, and therefore it is important that GIS are designed to meet the needs of the various end-users, and to include as little redundant information as possible. Once data have been integrated into GIS, it is possible to perform a wide range of spatial analyses on them at very little cost.

4. CONCLUSIONS

In ecology and epidemiology, GIS, GPS, and RS are increasingly being used as a complementary set of spatial tools for project planning, implementation, and evaluation. In spite of the fact that a GIS-centred approach offers many potential advantages to the area-wide application of the SIT and other control methods, it is still under-used as a decision-support tool in the day-to-day management of AW-IPM programmes. In the past, GIS have been applied mainly by academics as an end in itself, or at best as a research tool analysing correlations between different parameters to select priority areas where pest elimination would result in the highest socio-economic impact. Insufficient attention has been given to using GIS as a tool to make planning, implementing, and evaluation of SIT-based operations more efficient.

GIS also facilitate the overlaying of a variety of data coverages, e.g. climate, land use, drainage, etc., to identify factors that may explain the spatial and temporal patterns of insects and/or disease. Using appropriate spatial analytical approaches, GIS can be used to identify and map the habitat of insect species and their relationship to cropping areas or human and animal populations. In this way, it is possible to generate maps indicating the risk of disease on a variety of spatial scales,

and to monitor, in space and time, integrated information on insect population dynamics, ecological and meteorological conditions, and the incidence of disease or crop damage.

AW-IPM programmes that include the release of sterile insects are, because of the interdependence of their many linked components, inherently complex; the collapse of one component might jeopardize the successful outcome of the entire programme. The success of such a programme depends mainly on aspects related to: (1) the quality of the insect (e.g. sexual competitiveness of the gamma-sterilized and released insects, survival, mobility, dispersal characteristics, etc.), (2) the management of the release programme (e.g. timely delivery of insects, appropriate placement of the insects in the natural habitat, uninterrupted supply of sufficient sterile insects, etc.) (Dowell et al., this volume), and (3) the implementation of related programme components (e.g. adequate suppression of the native-insect population, relevant public relation campaigns, ample collaboration with the livestock/crop industry, etc.) (Dyck, Regidor Fernández et al., this volume; Mangan, this volume). Programme managers need to keep a comprehensive overview of all these essential programme components and their outcomes, almost on a daily basis, and GIS provide an ideal tool to analyse and display data from these multi-facetted programmes. Close collaboration between programme entomologists, and SIT and GIS experts, will be a prerequisite to fully exploit the potential of GIS as a decision-support tool, and to render AW-IPM programmes using the SIT much more efficient and cost-effective.

To make GIS/RS more applicable, programme managers must get access to all available data layers (administrative boundaries, soil types, crops, meteorological data, satellite imagery, vegetation cover, etc.). GIS technicians can, in many instances, produce data layers that are not available or are missing, for example, by digitizing topographical maps. Also the establishment of global networks to enhance research collaboration, data sharing, and the pooling of common resources (e.g. via the development of special websites), can greatly facilitate the development potential of GIS. Regarding the day-to-day implementation of the various programme components, all data sets on the target insect (survey data, monitoring data, etc.) and related aspects (crop damage, disease incidence, etc.) must be geo-referenced and entered into databases that are compatible with, and can easily be linked to, GIS software (e.g. using ACCESS-based databases rather than EXCEL spreadsheets), allowing straightforward summaries and queries. The Disease and Vector Integrated Database (DAVID), and the newly developed Tsetse Intervention Recording and Reporting System (TIRR system) which is tailored specifically for tsetse SIT operations, are fine examples of compatible ACCESS-based databases. Finally, standardized data collection, continuous flow of data files to a central location, and increased understanding of the basics of GIS by programme managers, are additional prerequisites to exploit GIS to their full potential in AW-IPM programmes.

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PART V

ECONOMIC, ENVIRONMENTAL, AND MANAGEMENT CONSIDERATIONS

CHAPTER 5.1.

APPLICATION OF BENEFIT/COST ANALYSIS TO INSECT PEST CONTROL USING THE STERILE INSECT TECHNIQUE

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SUMMARY

Before embarking on area-wide integrated pest management (AW-IPM) programmes involving eradication, exclusion, or suppression of insect pests using the sterile insect technique (SIT), and/or other area-wide control measures, not only their technical but also their economic feasibility needs to be assessed. They may require significant initial capital investments to achieve long-term returns in subsequent periods, and may raise questions about the distribution of benefits or the justification of public or private pest control efforts. A consistent and transparent system is needed to analyse the benefits and costs of such programmes and to demonstrate their value, or in some cases to assess appropriate contributions to the costs by the various stakeholders who gain the benefits. Benefit/cost analysis (BCA) provides such a framework, and has been applied to many AW-IPM programmes that integrate the SIT, in

which it has been used to demonstrate the expected value of area-wide eradication, exclusion or suppression. This chapter outlines the process of BCA in which itemized future costs and benefits are compared in terms of present values. It also provides a review and examples of the application of BCA to the SIT. A checklist of BCA inputs, and some examples of benefit/cost outputs, are also presented.

1. INTRODUCTION

The principle of the benefit/cost analysis (BCA) is to provide a model framework in which all costs and benefits, applicable to an area-wide integrated pest management (AW-IPM) programme such as pest suppression, exclusion, or eradication that incorporates the sterile insect technique (SIT) (Hendrichs et al., this volume), can be compared with alternative management options over a specified period of time. The analysis informs decision-making by structuring estimates of all costs and benefits, including externalities such as environmental and social impacts, but it does not prescribe choices. Ultimately decisions depend on social, political and commercial values and judgements. The BCA is a very helpful tool for making the decision process transparent for governments, investors and beneficiaries.

The BCA model provides a common framework for assessing and comparing the overall flow of benefits and costs from different management options over time. This is very important for comparing area-wide programmes using the SIT (which generally have substantial initial costs, but which provide long-term benefits through subsequent suppression, exclusion or eradication) with individual and short-term control (such as by conventional pesticide application). In the BCA the monetary value of all identifiable benefits and costs are estimated as objectively as possible over the expected period during which the programme will operate. Since the benefits and costs are in the future, there is inevitably some uncertainty in these estimates. The BCA model needs to be flexible so that the various management options and expected scenarios can be tested, taking into account uncertainties, and demonstrating the sources and influence of the uncertainty. For example, Vo (2000) presented two scenarios for an assessment of the New World screwworm *Cochliomyia hominivorax* (Coquerel) in Jamaica, in which the major uncertainty was programme cost. For some species, for example the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), rearing costs are well known (Quinlan et al. 2002), but there may be considerable uncertainty about other variables such as reinvasion frequency in the case of exclusion or eradication programmes.

Sensitivity analysis, the process of testing the model with a realistic range of values, is important in BCA to indicate how risks associated with the programme could affect decisions. Ideally the economic framework should be prepared in parallel with the technical feasibility assessments of a programme so that each can inform the other. In this way the final analyses can be efficiently directed to the technically most effective and economically viable plans. The BCA may be needed both before and after programme implementation, first to decide on how to proceed, and later to evaluate performance and suggest operational improvements.

1.1. Timescale and Geographic Scope

The initial steps in the BCA include defining the timescale and the likely geographic scope of the programme. The time period may include: (1) a preparatory phase (research, baseline-data collection and feasibility studies, construction of the insect production facilities, etc.), (2) a control phase (which could include a series of zones through which treatments are applied in succession as suppression, exclusion or eradication is achieved), and (3) a reasonable period beyond the control stage. This post-intervention phase should be long enough for benefits to establish before there is the inevitable time-related increase in uncertainty about reinvasion (but not in the case of a suppression programme), new pest entry or other circumstances that could affect the expected benefits or costs. The first two phases are determined largely by technical constraints, although there may be opportunities to reduce them by spending more money. The geographic scale may also be determined by technical considerations (islands, topography, limit of host range) or by economic factors (too little return in areas of marginal productivity or lower pest attack).

A cost function is likely to be composed of three parts: (1) variable costs per area to be treated for control, (2) variable costs for all other related management activities (surveillance, follow-up treatment, etc.), and (3) fixed costs associated with operating the programme. The benefits would include a function based on replacing current costs and losses in the area to be controlled, plus any additional market opportunities that may arise through the pest control achieved, and the reduction or elimination of pesticide applications and residues. Costs and benefits may need to be attributed to particular production sectors or uses (for example, production of meat, milk and draught power in tsetse fly *Glossina* spp. control programmes) or to geographic areas (for example, selection of individual regions where benefits might be greatest). Environmental costs and benefits, discussed later in this chapter, should be included along with direct monetary values from improved production and cost savings. An increasingly important issue in pest management BCAs is how much of the cost can be recovered from stakeholders and how this can be achieved.

1.2. Available Benefit/Cost Analyses

Many suppression, exclusion and eradication programmes have been undertaken or proposed, and most of them have had either formal or informal BCAs (Mumford 2004; Dyck, Reyes Flores et al., this volume). Examples of BCAs for AW-IPM programmes that integrate the SIT include:
- Mediterranean fruit fly: California (Dowell et al. 2000, CDFA 2003), Florida (FDACS 2003), the Maghreb (IAEA 1995), eastern Mediterranean (Enkerlin and Mumford 1997), South Africa (Mumford 1997), Portugal (Mumford and Larcher-Carvalho 2001, Larcher-Carvalho and Mumford 2004, IAEA 2005), Western Australia (Fisher et al. 1994, Mumford et al. 2001) (Box 1), Chile (UN 1997), Tunisia (Knight 2001), and Argentina (De Longo et al. 2000)
- Tsetse: Kabayo and Feldmann (2000), Msangi et al. (2000), Knight (2001), Kamuanga (2003), Shaw (2003), and Shaw (2004)
- New World screwworm: Vo (2000), Wyss (2000, 2002)

Box 1. *Mediterranean Fruit Fly in Western Australia*

The Mediterranean fruit fly has been a pest of commercial and backyard fruit throughout much of Western Australia since it was introduced to the state around 1900 (Fig. 1). It imposes costs on fruit growers through insecticide treatments, fruit losses, and the presence of insecticide residues and the insects themselves. Backyard growers also suffer and get less enjoyment from their fruit trees. It causes problems for the international export of Western Australian fruit, and also to other Australian states. South Australia, in particular, is faced with the costs of quarantine and frequent eradication of contained Mediterranean fruit fly outbreaks originating from Western Australia.

A pilot eradication project using the SIT was conducted at Broome, Western Australia, and showed that eradication of the Mediterranean fruit fly in Western Australia is technically feasible. In an analysis of the eradication of the fly in Western Australia (Mumford et al. 2001), it was clear that eradication, in a series of geographical zones, would take several years. The model was therefore based on the concept of summing the individual costs and benefits across each zone, allowing for a phased extension of the eradication across the state with a rolling quarantine to protect the eradication frontier as it progressed. The principal inputs within each zone affecting costs and benefits were the total areas to be treated and the values of losses that would be prevented with eradication. The selection of zone boundaries was based on:

- Climate (mainly the effect of winter temperature on Mediterranean fruit fly development)

- Phased increase in the treatment area to build-up expertise and capacity

- Treatment areas determined using satellite imagery of likely host presence and agricultural census data

- Maximum annual treatment area of 1000 km^2 (reflecting managerial capacity)

- Phased decrease in the treatment area as the programme winds down through lower-risk areas, to maintain capacity in the event of renewed outbreaks in any fly-free area

- Existing local government administrative districts to be used as the basis of both statistics and management

- Old World screwworm *Chrysomya bezziana* (Villeneuve): Tweddle and Mahon (2000)
- Codling moth *Cydia pomonella* (L.): Canada (DeBiasio 1988, Dyck et al. 1993, Bloem and Bloem 2000, Bloem et al. 2000), Syria (Mumford and Knight 1996), and South Africa (Mumford 1997)

For comparison with control using the SIT, some other non-SIT control cost and general loss estimates for fruit flies exist for Egypt (Joomaye et al. 2000), islands in the Indian Ocean (Price and Seeworoothun 2000), and Pakistan (Stonehouse et al. 1998).

The economic conditions that favour area-wide management include an efficient and effective integration of a technique such as the SIT, a clearly articulated demand by stakeholders, good management capacity, homogeneous risks so that benefits are fairly evenly distributed, a mechanism to capture benefits and recover costs, and since the SIT is species-specific it also relies on there being a single dominant pest species (Klassen 2000, Lindquist 2000, Mumford 2000).

Ceratitis capitata
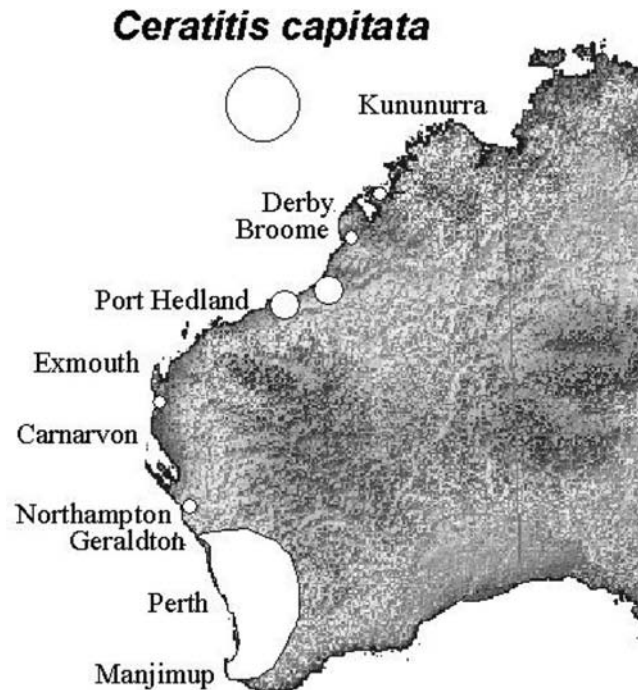
Figure 1. Map of western part of Australia showing areas infested with Mediterranean fruit fly (white circles). Large infested area in the south is around Perth, and six small areas occur farther north along the coast. (Map from A. J. Jessup and B. Woods, reproduced with permission.)

1.3. Economic Benefits of AW-IPM Programmes that Integrate SIT

The benefits of programmes using the SIT have been widely described, and vary enormously with the species concerned, and the scale and objective of the programme (Enkerlin 2003; Bloem et al., this volume; Enkerlin, this volume; Vargas-Terán et al., this volume). For example, it has been estimated that, if all species of tsetse flies and the diseases they transmit were eradicated in all of sub-Saharan Africa (which is not feasible or even appropriate since most species are not of economic importance (Feldmann et al., this volume)), the overall benefit would total USD 4500 million per year (of which USD 1200 million per year are the direct losses from trypanosomosis-affected cattle and associated current control costs) (OAU 2000). However, critics of the SIT for tsetse eradication cite higher costs in areas with multiple species, and the high costs of reducing initial populations to levels at which the SIT can work efficiently (Hargrove 2003). Such analyses could also be used to set target cost levels for more efficient sterile insect production and release technologies to achieve returns comparable with conventional control.

Establishment of the Mediterranean fruit fly in California would threaten losses estimated at between USD 1000 million and 3600 million per year (CDFA 2003), but the release of sterile insects in the preventive programme has maintained the fly-free status. The successful eradication of the Mediterranean fruit fly in Chile in 1995 opened up approximately USD 100 million per year in additional fruit markets (IAEA 1999). On a much smaller scale, in South Africa grape growers on 4000 hectares in the Hex River Valley were estimated to save over USD 150 per hectare per year in conventional insecticide costs, plus the added value of entering low-residue markets, through using the SIT to suppress the Mediterranean fruit fly (Mumford 1997). Suppression with the SIT offers similar advantages for Mediterranean fruit fly control in Israel, Portugal, Spain, and other countries where ecological circumstances indicate that at present continual reinfestation is likely, and the cost of quarantine may be relatively high. While suppression does not have the finality that gives eradication such political appeal, it also does not have the high costs of certification and quarantine. Furthermore, because there would be an ongoing need for sterile insects, there may be greater potential interest for private investment in SIT production facilities and delivery services (Mumford 2000, Quinlan et al. 2002).

For a practical decision on the merits of eradication, exclusion, or suppression using sterile insects, these benefits must be set against expected costs, which for many AW-IPM programmes are now well documented in a range of national circumstances. Issues remain, however, about how to capture the benefits within the various economic sectors that gain from control, and to transfer some of this to the public or cooperative sectors that provide the service. AW-IPM programmes that integrate the SIT have traditionally been public or largely public programmes, but may increasingly be partly or wholly funded directly by beneficiaries (Dyck, Reyes Flores et al., this volume).

2. BENEFIT/COST ANALYSIS FORMAT

The output of the BCA is likely to appear as a spreadsheet-based time-profile indicating inputs and outputs by year, location and sector (which could be crop/livestock type, urban/rural, public/private, etc.) depending on the needs of the commissioning agency. The spreadsheet models the flows of inputs and benefits as each area reaches the year assigned for particular management actions. An example output appears in Table 1 and Fig. 2.

The model structure has a set of cost and benefit components specified for each area and each year. Each of these refers to a standard set of cost, price and production parameters per area to give the model consistency, while allowing the flexibility to analyse different SIT control plans. Different plans could, for instance, include changing the sequence of zones to be controlled, or the number or size of zones targeted for eradication in each year. Some values may need to be expressed with specified levels of uncertainty associated with them, e.g. the cost of sterile flies may not be known before a factory is built, but costs from similar factories give a good approximation. Sensitivity analysis would demonstrate the range of outcomes using input values with some variation around the most likely expected values.

Table 1. Output scenario for a possible multi-zone 6-year Mediterranean fruit fly eradication in Western Australia (Mumford et al. 2001), including a 2-year pre-eradication phase, 6 years of eradication starting from the south-western districts of the state, and a continuing extra quarantine post-eradication (additional cost beyond present quarantine). Two years of pre-eradication demonstrations and survey cost USD 0.66 million per year. After Year 10 it is expected that post-eradication costs would continue at USD 0.13 million per year, mainly for quarantine and marketing to maintain the advantage of eradication. Benefits would increase as the area under cultivation is assumed in this scenario to double from Year 1 to Year 20 (Fig. 2 and Box 2)

	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9
Zone/phase	South-west	South	Perth East	Perth	Central	North	Post-eradi-cation
Fly challenge	Low	Medium	High	High	High	High/Medium	
SIT area (km^2)	260.0	554.4	1019.6	978.4	73.0	69.1	
Costs (USD million)							
Bait (pre-SIT)	0.208	1.173	2.930	2.925	0.194	0.248	
Environ. costs pre-SIT control	0.125	0.704	1.758	1.755	0.116	0.149	
Direct SIT costs	1.378	2.885	5.234	5.021	0.391	0.362	
Quarantine post-eradication		0.055	0.115	0.209	0.201	0.016	0.014
Monitoring		0.156	0.333	0.612	0.589	0.044	0.042
Misc. expenses	0.303	0.635	1.151	1.105	0.125	0.125	0.125
TOTAL	2.014	5.609	11.521	11.628	1.615	0.944	0.181
Benefits (USD million)							
No spraying	0.010	0.139	0.375	0.393	0.482	0.574	0.606
No residual loss	0.020	0.257	0.632	0.662	0.767	0.987	1.043
Extra local sale	0.075	0.223	0.232	0.295	0.312	0.330	0.348
Export access/ residue benefits	0.000	0.032	0.158	0.248	0.383	0.617	0.782
Garden fruit	0.014	0.039	0.067	0.094	0.072	0.102	0.102
Environmental benefits	0.015	0.108	0.269	0.282	0.342	0.423	0.447
No S. Australia quarantine	0.000	0.066	0.206	0.206	0.464	0.483	0.500
No Kununurra fly-free zone	0.000	0.007	0.021	0.021	0.046	0.048	0.050
No post-harvest research						0.125	0.125
TOTAL	0.134	0.870	1.959	2.203	2.868	3.688	4.003
Net (Benefit/Cost) (USD million)							
Total	-1.880	-4.738	-9.561	-9.426	1.253	2.744	3.822
Cumulative	-3.205	-7.943	-17.505	-26.931	-25.678	-22.934	-19.112
NPV 20 years	7.969						
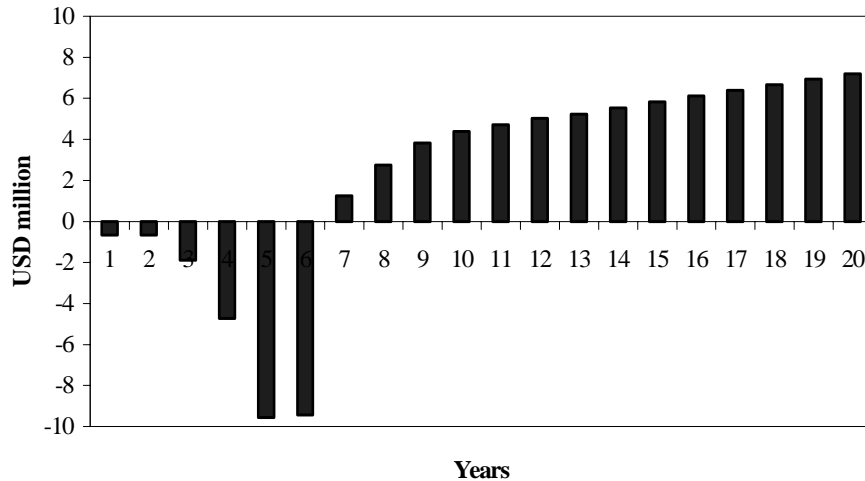
Figure 2. Annual net projected benefit/cost for one scenario of the Western Australia Mediterranean fruit fly eradication programme before any discount rate is applied (Mumford et al. 2001). The itemized values of costs and benefits for years 3 through 9, and the net present value with discount rate applied, are shown in Table 1. The benefit/cost analysis demonstrates that, under the assumptions applied in this scenario, the net present value is positive, and the initial investment could be justified by the later returns.

Many of the BCA models referred to in this chapter are related to the general format of a generic Mediterranean fruit fly BCA model under development at the International Atomic Energy Agency (W. Enkerlin, J. D. Mumford, and A. W. Leach, unpublished spreadsheet models). While each case has specific elements of geography, ecology or market conditions, there are many common principles and a growing globalization of SIT infrastructure. For instance, large and efficient production facilities (Quinlan et al. 2002) can ship sterile insects to an international market at competitive prices — New World screwworms from Mexico to Libya, Jamaica, and Panama, and medflies from Guatemala to the USA, Israel, and South Africa. Many items of equipment used in rearing and aerial release are now common across many programmes. Unfortunately, components of costs for sterile insects and their application are not standard throughout the world; there are large local cost and salary differences. In addition, economies of scale may also play a role. Major advances continue to be made in insect mass-rearing processes, which should reduce production costs of many SIT-targeted species (IAEA 1999; Parker, this volume).

The time dimension for BCA predictions is very important, since there is a trade-off between adding the extra benefits over a longer term after most of the cost has been completed, and adding greater uncertainty of further pest invasion, market changes, etc. While eradication may be seen by many as a once-and-for-all achievement, with an indefinite stream of benefits, experience shows that, for some pests under some situations, reinvasion occurs frequently (for example, the Mediterranean fruit fly has reoccurred in California and Florida following

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Box 2. Case Example — Western Australia Mediterranean Fruit Fly Eradication Plan

An economic study was conducted to look at the overall costs and benefits of a potential technical programme to eradicate the Mediterranean fruit fly in Western Australia (Mumford et al. 2001).

The study developed a benefit/cost framework for the analysis, and collected data and subjective estimates of costs and benefits that could be applied to the programme. It was estimated that the total treatment area would be approximately 2000 km². It was assumed that an eradication effort would be a phased programme over six years, with a maximum control area of around 1000 km² per year in the peak years (Table 1). This would require the release of about 100 million sterile male flies per week. A phased programme would require considerable publicity and internal quarantines to protect the fly-free areas as the eradication moved on to new areas. Given the scale of the operation, much of this would need to rely on public cooperation, rather than very expensive physical quarantine barriers, such as manned roadblocks. Intensive monitoring would be needed to prove that eradication had taken place. The overall cost of eradication was estimated to be about USD 35 million. The likelihood of success for such an eradication programme would be very high, given the well-established technology. The phased programme would move from less climatically suited areas into Perth, leaving the smaller infestations in the north to be treated last (Fig. 1).

The principal benefits of Mediterranean fruit fly eradication would be: (1) reduced conventional pesticide and application costs, (2) reduced pest losses to growers (current insecticide control is not perfect), (3) reduced pesticide residues (increasingly important in international markets), (4) improved market access, (5) community benefits to the environment, backyard production and enjoyment, and (6) lower costs to government for quarantine, emergency control and continued research on conventional control improvement. Approximately 68% of the direct benefits arise from reduced production costs and new market opportunities, the remainder from reduced community/government costs. Both growers and the public would benefit, and could be expected to contribute directly or indirectly to an area-wide eradication programme, as well as participating in its management.

The analyses indicated that, if horticultural areas double over the next 20 years, the net benefits at present values for Mediterranean fruit fly eradication are likely to be positive (almost USD 8 million net present value (NPV) for 20 years). Even if the area were to increase only slightly (at least 18%), a break-even result is likely. Uncertainty analysis indicates that additional research on the presence of non-commercial hosts in riverine and urban areas, and on the extent of residual losses despite current conventional control, may make the analyses both more precise and more positive (as some non-host areas are likely to be eliminated from control). Increasing demands for residue-free produce in export markets, and the withdrawal of many conventional fruit fly insecticides, may make SIT-based eradication essential, rather than merely desirable, to the future of export horticulture in Western Australia.

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eradications, although the benefits of even short-term eradication exceed the costs in such major exporting areas). So while longer timeframes would give a greater apparent return to an eradication programme, assuming the costs of maintaining quarantine or preventive control are not prohibitive, the probability of losing the benefits through a new outbreak increases as more years are added to the anticipated flow. In any event, future discounting reduces the impact of extending the time horizon.

Benefits and costs that arise in future years should be compared in terms of their equivalent present values, so that all the values are directly comparable, since the same nominal value further in the future is worth less in present terms. The net benefit (benefit minus cost) over the whole programme period being considered would be expressed as a net present value (NPV). The discount rate is used to calculate these net present values. The discount rate is a measure of the value that people place on having money now rather than later. It is generally considered to be

equal to the interest rate on savings minus the inflation rate. In relatively stable economies the discount rate ranges from about 5–9%; it is likely to be higher in less stable economies (Mumford 2000). The US government guideline on the discount rate suggests a central value of 7% for public benefit programmes, with sensitivity analysis using the wider values above (OMB 2003). While a common discount rate, such as 10%, could be used in all analyses, this would not demonstrate the differences in future values that actually occur in different economies. To calculate present value, the following formula is used:

$$\text{Present value} = \text{Future value}/((1+\text{Discount rate})^{\text{Number of Years}}) \qquad (1)$$

At a discount rate of 0.07 (7%), this formula indicates that a value of USD 100 in 10 years has a present value of only USD 51, and USD 100 in 20 years is worth only USD 26 in present terms. Therefore long time frames do not add as much benefit as may be imagined, particularly where discount rates are high, while they add greatly to the uncertainty of the estimates.

3. MODEL INPUTS

3.1. Costs

The following cost items must be predicted:
- Pest management treatments (the combination of the SIT and related technology to be applied, based on technical selection and specification of control activities and locations; variable costs to be determined per unit area, plus initial and subsequent annual fixed costs).
- Management area (this is the main driver for costs, since most costs are variables based on treatment application per unit area).

Cost categories (examples are given for some of the important cost categories, mainly based on values for the Mediterranean fruit fly, one of the most commonly controlled pests using the SIT, but some of the categories are too site-specific to give meaningful examples):
- Pre-treatment preparation (demonstrations, trials to prove the effectiveness of techniques, and to build technical capacity and public confidence).
- Surveys (pest population, host areas, current control practices and losses).
- Population reduction needed prior to the SIT (by bait or other treatment) to bring populations to a low-enough density for effective SIT control:
  - o For the Mediterranean fruit fly, approximately USD 6000 per km^2 (Mumford et al. 2001).
- Environmental costs (mainly pesticides used for pre-SIT population reduction):
  - o Pimentel and Lehman (1993) suggested that social and environmental costs from pesticide use in the USA amounted to approximately USD 2 for every USD spent on pesticide active ingredient. This is an average figure calculated for all US agriculture, and included damage to operator health, public health through residues and run-off, and losses of wildlife and domestic animals.

- o Mumford et al. (2001) used a variable figure for environmental costs based on expenditure for pesticide active ingredient to control Mediterranean fruit flies in Western Australia of USD 1 per USD of pesticide active ingredient. The lower figure, compared with Pimentel and Lehman (1993), was based on smaller areas with relatively low-toxicity treatment, often distant from major urban areas and water supplies.
  - o Kovach et al. (1992) proposed an environmental impact quotient (EIQ) for pesticides, which gives an economic value for the environmental damage of many individual pesticides, particularly those used in horticulture.
  - o Environmental and health impacts can be quantified in monetary units using willingness-to-pay methods, or by establishing aspiration levels (Farnsworth 1986).
- SIT costs (approximate figures for the Mediterranean fruit fly, as of 2004):
  - o Production: About USD 250–500 per million irradiated male pupae at the factory. The current cost of purchasing irradiated male pupae from Moscamed Guatemala, with the largest Mediterranean fruit fly rearing facility, is about USD 200 per million pupae (Hendrichs et al. 2002). (The release rate would be 100 000–150 000 (pupae) per km^2 per week; numbers may depend on host density.)
  - o Shipment: The current cost of air-freight shipment of pupae from Guatemala to Israel is about USD 250 per million pupae (J. P. Cayol, personal communication). Of course this cost would be lower if the production facility were located nearer to the release site. Intercontinental air shipment may cause mortality and loss of vigour, reducing effective numbers by 50% by the time flies are released.
  - o Local fly emergence, handling and aerial release: Cost is about USD 150–200 per million male pupae (Mumford et al. 2001; J. P. Cayol, personal communication).
  - o Local fly release alone: Cost is about USD 1100–2000 per km^2 per year of application (assumes 52 weekly releases) (Mumford et al. 2001).
- Quarantine (only for eradication or exclusion — prevention of re-entry, and management of outbreaks post-eradication).
- Monitoring (during eradication activities and post-eradication) and certification (only for eradication — intensive monitoring post-eradication to prove pest free status):
  - o For the Mediterranean fruit fly, a certification trap grid of 10 traps per km^2, inspected fortnightly, costing USD 2 or 3 per trap per inspection (Mumford et al. 2001); monitoring during exclusion or preventive programmes may cost less, e.g. 4 traps per km^2 in the preventive release programme in California (Dowell et al. 2000).
- Miscellaneous costs (administration, publicity, marketing the improved pest free quality produce from the area).
- Additional management and infrastructure costs to cope with possible increased pressure on land use after pests are eliminated.

A time-profile of the inputs, with changes over time, a time limit for analysis, and an agreed discount rate, are also needed.

The management operations are specified according to the technical needs of the programme, for example pre-SIT bait applications. The economic analysis can be used to choose between different technical options, e.g. the order in which zones are treated in a phased eradication may have significant economic implications. There may be technical or managerial limits on the size of treatment zones, which affects the pace of eradication. The release rate of sterile insects, pre-SIT control regimes, and standards for monitoring, are all based on previous experience gained in successful eradication programmes, on the ecological circumstances in the area, or, for new SIT species, on field research in pilot programmes.

The treatment area in each year consists of all the pest-host areas within a zone, along with some additional areas along the edges of host areas. Depending on the pattern of hosts, it may be necessary to include areas that do not contain the pest but for practical reasons must be included in the treatment area. Treatment areas may be predicted by land-use images from satellite or aerial photos, and/or from ground surveys. Crop areas, livestock densities, and production levels are often available from agricultural statistics, and households can be obtained from census records. Local surveys of vegetation, animals, and households may be needed where information is scarce.

Prior to the SIT treatments, there may be a need for trial runs to evaluate procedures, or to provide some stakeholders with demonstrations of operations and impacts. All subsequent operations can be treated as either direct area functions in the BCA spreadsheet, or as indirect area functions (for instance, environmental costs are likely to be determined by the volume or value of pesticide used, which will itself be area-related).

The timescale for the analysis should include the preparatory phase, the operational phase, and the ongoing period during which benefits and any further costs can be confidently expected to accrue. The endpoint for the analysis should be chosen after consideration of ecological, market and quarantine uncertainties, which increase over time, and the effect of future discounting, which makes long-term future values relatively less significant in present terms.

3.2. Benefits

The following groups of benefits are likely to accrue:
- Reduced direct and indirect costs of current control (this requires technical specification and information on the proportion of users for each current practice, obtained by survey).
- Reduced residual losses to crops or livestock due to target pests that an SIT treatment would eliminate (such losses occur despite current control efforts, either because little or no control is applied in many low-input farms, or control is often not completely effective even when fully applied). The lack of fully effective controls is often a substantial motivation for the SIT, whether eradication, exclusion or suppression.
- For livestock — reduced veterinary, surveillance and treatment costs.
- For livestock — shorter time for animals to reach market weight.

- Reduced environmental impacts from pests that affect natural vegetation or wildlife, which would be prevented through control: NPB (1999) and Pimentel and Lehman (1993) discussed the environmental impacts of invasive insect species. Mumford (2001) described the ways in which economic values can be put on non-crop losses in natural environments.
- New market opportunities or improved retention of existing markets (e.g. due to reduced pesticide residues on produce) or certified disease- or insect-free status.
- Greater impetus to invest in agriculture in areas in which pests have been controlled.

A time-profile of benefits and their distribution (geographical, sectors, etc.) is needed, along the same lines as for costs.

3.3. Input Format

A typical spreadsheet for the BCA would consist of the following example data pages: a delineation of areas; a catalogue of data on the number of square kilometres per district (total area, and area of particular pest hosts, or density of hosts); SIT-treatment areas by zone (excluding areas the target pest would not inhabit due to climatic conditions or a lack of hosts); potential and residual losses due to the pest (affected by productivity in the area, climate, susceptibility of hosts to pest); current control costs, including social and environmental costs of current control practices, lost market opportunities (due to residues, residual pest damage or quarantine exclusion), SIT-treatment costs, including additional monitoring and quarantine costs and pre-treatment preparation, and a discount rate for the country; and the input summaries for each scenario (e.g. Table 1).

4. MODEL OUTPUTS

4.1. Net Present Value and Internal Rate of Return

Model outputs should indicate: summaries of costs and benefits over a timeline agreed to for the analysis of the strategy, and economic indicators (such as net present value, pay-back period, and internal rate of return) for each proposed strategy. The net present value is the sum of the present values of future net returns, using the discount rate to calculate back to the present from the expected future nominal values. The pay-back period is the number of years before the cumulative benefits exceed the cumulative costs, which is a measure of the riskiness of a programme. The internal rate of return is the discount rate that would give a net present value of zero to the stream of net benefits resulting from the programme. The programme would exceed break-even if actual discount rates were expected to be below this value.

The output of the BCA provides a comparison of the stream of net benefits, expressed in present values. Strategies with higher net present values are preferred, although sensitivity analysis may indicate that some high returns are associated with greater risk. Cases of eradication require a long-term commitment to ensure that the

investment in eradication is protected, and this can add considerable cost. The net present value is based on the calculation based on the average of each input value, but each of these inputs may be uncertain. Where probability ranges have been estimated for various input values, it is possible to use simulation software such as Crystal Ball® or @Risk® to calculate the range and frequencies of output values.

4.2. Interpretation and Apportioning of Benefits

The distribution of benefits can be apportioned by sector, e.g. commercial versus backyard, by geographical zone, by public/private finance, etc. This has important implications for the political desirability of a programme, the relative role of various stakeholders, and the potential for cost recovery. The initial benefits are likely to go to commercial producers for programmes involving fruit flies, codling moths, or other agricultural pests. Consumers may subsequently benefit from lower prices if production becomes more efficient later and residue levels are reduced.

Many countries now have policies that require the government to seek to recover costs, wherever possible, from public programmes such as insect eradication, e.g. the New Zealand Biosecurity Act 1996. The BCA could form the basis for determining not only what expenditure will be needed for a successful programme, but, through the assessment of the benefits, how that expenditure should be shared. However, identifying benefits does not directly indicate who should pay. Some costs, e.g. non-monetary environmental costs, are difficult to recover, and may only be practical for society as a whole to bear, or to claim compensation from government (Mumford 2001). In other cases, too many beneficiaries may be involved from which to collect individually, e.g. where urban householders benefit. Any area-wide insect management programme will encounter the issue of free-riders who do not contribute directly (Lindquist 2000). Where these benefits contribute to the broader public good, it may be more efficient to fund programmes centrally from government and thus spread the cost through general taxation. Where the benefits are geographically isolated, and beneficiaries few in number and well organized, such as in the current Hex River Valley Mediterranean fruit fly control programme in the South African table-grape industry (ARC Infruitec 2003), a levy on growers is a practical and fair way to pay for part of the costs (Dyck, Reyes Flores et al., this volume).

5. BENEFIT/COST ANALYSIS CHECKLIST

- Planning and feasibility studies (technical and economic) that provide initial descriptions of inputs, and estimates of costs and effectiveness
- Current pest losses (without control and in spite of control, for crops or livestock, over several seasons)
- Market exclusion due to pest presence, damage or pesticide residues
- Current control practices (area treated, effectiveness, cost, environmental impact)
- Area to be treated overall

- Areas within the overall area in which pest hosts occur (by management units, which would be a minimum area in which the pest population may be controlled using the SIT, e.g. the unit for aerial application may be 500 m^2)
- Pre-programme monitoring for hosts and populations
- Publicity to make the public aware of the area-wide programme
- Regulatory controls (such as hygiene, local quarantine inspections, reporting of pest occurrence)
- SIT costs:
  - Pre-release insecticide baiting (or alternative population reduction practices)
  - Sterile insect production
  - Sterile insect storage and transport
  - Sterile insect release
  - Field monitoring (for operational management)
- Field monitoring for pest free certification
- Post-control area quarantine
- Marketing to capture benefits of pest and pesticide reduction/elimination
- Agreed programme timescale for analysis and applicable discount rates

This checklist provides guidelines on the basic information that ideally would be used in the BCA. More precise information will give more confidence in the analysis, but may be expensive to obtain. Therefore some compromises between uncertainty and cost may be required. The goal is to provide transparent comparisons of specified strategies, with as much objectively agreed information as possible, and with any uncertainties explicitly identified and included.

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CHAPTER 5.2.

ENVIRONMENT AND THE STERILE INSECT TECHNIQUE

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SUMMARY

The sterile insect technique (SIT) is an exceptionally promising pest control method in terms of efficacy and environmental compatibility. Assessments of environmental risks vary according to the status and origin of the target pests. The suppression or eradication of exotic pest populations with the SIT raises few environmental concerns, and these are related mainly to pre-release suppression techniques. However, the elimination of native species, or at least populations of native species, requires more detailed and complex assessments of ecological effects and consequences for biodiversity conservation. Eradication programmes provide opportunities to study these topics within the scope of both environmental impact assessments and operational monitoring programmes.

1. INTRODUCTION

The sterile insect technique (SIT) is a specific control method that may be applied in the area-wide integrated pest management (AW-IPM) of insect pests of medical, veterinary, and agricultural importance. Contrary to chemical and biological products, sterile insects are non-invasive agents rather than intrusive toxic, pathogenic or otherwise destructive entities. Therefore, the SIT alone poses *a priori* an exceptionally low risk to the environment (Müller and Nagel 1994, Hendrichs 2001). Ecologically, the release of sterile insects represents an input into ecosystems of allochthonous, living yet non-reproductive biomass readily integrated into and processed within food webs. Thus, if there are any direct adverse impacts of sterile insects on non-target biota, they are most likely related to changes in interactions among species. However, the SIT is not a stand-alone technique, and in most situations, to be effective and economically viable, the SIT requires pre-release population suppression with conventional control techniques. These encompass simple devices such as sticky traps, as well as large-scale aerial treatments with insecticides. Therefore, environmental hazards range from negligible to high, depending on the actual type and combination of control agents and tactics.

For example, malathion, used solely to control the boll weevil *Anthonomus grandis grandis* Boheman in the Rio Grande Valley, USA, caused serious secondary infestations of the beet armyworm *Spodoptera exigua* (Hübner) because of a strong decline in the number of natural enemies (Klassen 2000). However, when target population densities are low, less hazardous control techniques can be employed to reduce target populations. For example, the initial population densities of the tsetse fly *Glossina austeni* Newstead in Unguja Island, Zanzibar, were already low due to habitat destruction. Thus, suppression was achieved with pour-on pyrethroids sprayed on cattle (living targets) in pastoral areas, and with insecticide-impregnated blue cotton screens (targets) in areas where cattle were scarce (Vreysen et al. 2000), posing a low risk to the environment (Nagel 1995). An even lower risk is anticipated from the use of chemical larvicides to control the New World screwworm *Cochliomyia hominivorax* (Coquerel); they are applied directly to the wounds of infested livestock, posing no hazard to treated animals or components of the environment (USDA 2001a).

Area-wide control targets the entire population of a specific pest in a particular area (Lindquist 2000; Klassen, this volume). Previous AW-IPM programmes that integrate the SIT, against pests such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and the New World screwworm, demonstrated that area-wide control

is technically and economically feasible (Klassen 2000, Feldmann and Hendrichs 2001). Most suppression or eradication programmes were directed against populations of introduced (exotic) species, and none aimed explicitly at eradicating a pest species from its entire natural range. The risk assessment of full eradication strategies requires in-depth investigation of the ecological role of target pests in ecosystems and food webs, and the possible consequences of their disappearance.

This chapter discusses environmental issues related to different steps in the SIT strategy and life cycle, from sterile insect production to release, and from direct to indirect ecological effects. Given that suppression and/or eradication programmes may eventually extend from area-wide to range-wide, in other words from population eradication to species extinction in the wild, trophic and other ecological interactions among target insects and non-target fauna and flora will also be addressed. While evaluating environmental risks associated with the SIT in a more general sense, particular emphasis is placed on the environmental effects of tsetse fly *Glossina* spp. control in Africa.

2. MASS-REARING AND STERILIZATION

2.1. Mass-Rearing

Insect-rearing facilities pose a low environmental risk if designed using current biosafety principles, and if managed according to best practices and standard operating procedures (Quinlan et al. 2002; Parker, this volume). However, given that large numbers of insects are reared at a single site, they are a potential source of unintentional release of fertile insects into the environment. This might cause some damage if facilities are located within the natural range of the native species, but serious hazards if environmental conditions are suitable for the establishment of exotic species. Therefore, setting up facilities within the potential habitats of the target insect requires adherence to strict quarantine and security guidelines during all steps of insect production and shipping. To reduce this risk, sterile fruit fly production in the United States is only allowed in areas where the respective species is established or where environmental conditions are not suitable for establishment (USDA 2001b). Thus, production facilities may be located far away from the release area, but in many programmes that release sterile insects, insects are mass-produced in their vicinity.

Insect mass-rearing facilities can pose occupational health risks. Exposure to frass, hairs and scales may induce allergenic responses in humans (Parker, this volume). Another risk arises from exposure to microbials (fungi, bacteria). Therefore, rearing facilities must be equipped with effective air filtration systems to minimize the aerial concentration of allergenic particles and potential pathogens, and staff frequently exposed to these agents should wear protective clothing and masks. This may be problematic in countries with insufficient occupational health standards and capacities.

Another problem is the disposal of large quantities of liquid and solid organic wastes (Parker, this volume) which can carry immature stages of the insect as well as various microbial species, e.g. *Escherichia coli* (Migula). Effective waste-

treatment facilities are needed to prevent the release of insects and pathogens into the environment, and to prevent the direct infection of livestock if solid waste is processed further into animal feed.

Insects for the SIT are reared on natural or artificial diets. For most species, no problems arise from using these techniques. However, in the early days of tsetse mass-rearing, living mammals were used as hosts to feed the flies. This procedure was neither practical nor compatible with animal welfare. It was eventually replaced with a silicone-membrane feeding system simulating the skin of a host, and using blood collected from abattoirs (Bauer and Wetzel 1976).

2.2. Irradiation

Radioactive isotopes such as ^{60}Co are used for the sterilization of pupae or adults of target insects (Bakri et al., this volume). Therefore, insect sterilization facilities have to be earthquake-safe and operated according to nuclear safety standards. These are part of the standard operating procedures for sterile insect production (Quinlan et al. 2002). Further guidance is given by the International Atomic Energy Agency (IAEA), which is involved in most AW-IPM programmes that use the SIT. Thus, in countries with a proven record of the safe use and correct disposal of hazardous substances, a risk associated with the regular use of irradiation sources is not anticipated. According to IAEA norms, irradiation facilities cannot be set up in countries with insufficient nuclear safety legislation and infrastructure. In situations of political instability, contingency plans must foresee that irradiators and associated technology are secured.

2.3. Autosterilization

Autosterilization in the field has been proposed as a complement to the release of reared sterile insects. This technique relies on attracting target insects to baits or trapping devices and exposing them to chemosterilants. Various chemosterilants have been tested for tsetse control, including metepa, bisazir and insect growth regulators (IGRs) such as triflumuron, a chitin synthesis inhibitor, and pyriproxyfen, a juvenile hormone mimic (Oloo et al. 2000). Metepa and bisazir are carcinogenic, and may pose a risk to operators and trespassers. These chemosterilants have, therefore, never been used in operational control programmes. The other agents are less harmful, but lack clear evidence of efficacy. The environmental risk of chemosterilants such as IGRs is similar to the risk associated with stationary attractive devices (section 3.3.). Recent field work in Spain has indicated some potential for this technique (Navarro-Llopis et al. 2004).

3. PRE-RELEASE SUPPRESSION OF TARGET POPULATION

3.1. Overview

Although large numbers of sterile insects can be produced in modern rearing facilities, the pre-release suppression of wild populations remains a prerequisite for many AW-IPM programmes that release sterile insects (Mangan, this volume). Depending on the target species, minimum ratios of sterile to wild fertile insects during the first release cycle vary from 2:1 to 15:1 (Feldmann and Hendrichs 2001), but may reach 100:1 (Drew et al. 1982).

The most common methods to diminish target populations fall into two broad categories, insecticide spraying and artificial attractive devices. Other methods are employed against particular insects, for example, insecticide treatment of livestock against tsetse flies (pour-ons), wound treatment against screwworms, and soil-drench treatment against fruit flies. Cultural control can often play a significant role by ensuring, for example, that hosts are removed from the area at the end of the season. Integrated control strategies combine different techniques to optimize control efficacy and minimize adverse environmental effects.

3.2. Insecticide Spraying

Insecticides are applied with ground sprayers or aircraft, depending on the type, accessibility, and size of the target area. Aircraft are often used to apply larvicides to bodies of water to control mosquitoes and black flies. Ground applications include spot as well as small-scale, full-cover treatments, and are more focused than aerial applications. Many AW-IPM programmes, however, extend over large areas where ground access is very limited or even impossible, and reliance has then to be placed on aerial spraying as the main tool to reduce a population. Chemical pesticides are selected mainly because of their efficacy. Furthermore, spraying techniques and cycles are adapted to specific behavioural and life-history traits of the target. Thus, environmental effects depend not only on the type of insecticide but also on the actual use pattern. Two examples of the environmental impact of aerial spraying for population suppression are given below.

Over the last decades, aerial malathion-based bait-spray applications have been the most common and effective control tool against exotic fruit flies (USDA 1993, 2001b). The spray consists of the organophosphorous insecticide malathion mixed with a protein hydrolysate acting as an attractant and feeding stimulant. The use of a bait improves efficacy because then the insecticide uptake is higher (via the integument and the oral route). Therefore, malathion can be used at much lower dose rates than necessary for contact applications. The spray is used alone, or in combination with the SIT, to suppress or eradicate fruit flies. Malathion is highly toxic to terrestrial and aquatic non-target invertebrates. Aquatic fauna are exposed via direct over-spray of water bodies or via run-off. Terrestrial insects, which are also attracted to the bait, are particularly at risk, including ground beetles (Carabidae) and many other beneficial organisms. In fact, a malathion bait spray may disrupt a substantial portion of natural biological control agents (USDA 1993,

2001b). Native fruit flies may also be adversely affected, resulting in shifts in community structure and perturbations of ecological services (section 5.5.). Possible hazards further up the food chain include secondary poisoning or food deprivation of insectivorous vertebrates. New insecticides, e.g. spinosad (naturally produced bacterial (actinomycete) compounds) and SureDye® (xanthene dye) (Adán et al. 1996; Vargas et al. 2002; Edwards et al. 2003; Revis et al. 2004; Mangan, this volume), have been tested as substitutes for malathion in bait formulations. The toxicity of these agents to non-target biota is less than that of malathion, and fewer adverse effects are expected. Acceptable spinosad formulations, certified as organic, have now been developed and are being used extensively in the Mediterranean fruit fly programme in Guatemala (USDA/APHIS/PPQ 2000) and Chiapas, Mexico.

The second example is from tsetse fly control. The pre-release suppression of tsetse, in some cases, has involved sophisticated aerial spraying techniques. Sequential aerosol drift application with fixed-wing aircraft, which previously relied on endosulfan with sometimes negative ecological impact especially on fish in shallow water, is now based on pyrethroids, in particular deltamethrin. This insecticide is one or two orders of magnitude more toxic to tsetse than to house flies and honey bees, respectively (SWRC 2001). Therefore, field rates are exceptionally low, posing a low risk to terrestrial non-target invertebrates. Furthermore, application parameters such as height, time and sequence of spraying are adapted specifically to behavioural and life-history traits of tsetse flies, reducing the risk to terrestrial organisms even further. Finally, as deltamethrin has low persistence, it does not accumulate in food chains. Nevertheless, adverse effects may occur temporarily in terrestrial environments, e.g. effects on certain spiders, and in particular in aquatic environments (Peveling and Nagel 2001). For example, diving beetles and decapod crustaceans are highly susceptible to pyrethroids. A reduction of aquatic and semi-aquatic macroinvertebrates may translate into food shortages for fish and reduce the survival of fry, which in turn may adversely affect local fisheries.

Another possible risk arising from broad-scale sequential applications of low-dose pyrethroids is that the selection for resistance in other insect pests may be enhanced. For example, bednets impregnated with pyrethroids are widely used for mosquito control to prevent malaria in tropical areas. Selection for resistance in malaria vectors, through insecticide use in AW-IPM programmes that release sterile insects against agricultural pests, would reduce the efficacy of this important control method. This externalized consequence of population suppression for the SIT would be expected mainly in rural areas with a high human population density, where pesticides may be used for domestic and/or agricultural purposes.

Treatments with deltamethrin, using the sequential aerosol technique (SAT), have been used mainly in combination with trapping and baiting devices for tsetse suppression (section 3.3.). In a recent SIT tsetse eradication programme in the Okavango Delta, Botswana, the technique was applied for pre-release population suppression (Allsopp and Phillemon-Motsu 2002; Feldmann et al., this volume). Preliminary assessments showed that adverse effects on terrestrial invertebrates were minimal (Biotrack 2003). A synthesis of the results from the environmental monitoring is pending. Another insecticide widely used for sequential aerosol

applications in the past is endosulfan, a chlorinated hydrocarbon. Tsetse flies are three orders of magnitude more sensitive to this chemical than honey bees (SWRC 2001). However, endosulfan is highly toxic to fish and other non-targets, including the Salvinia weevil *Cyrtobagous salviniae* Calder and Sands, a classical biological control agent introduced successfully to contain the alien invasive aquatic fern *Salvinia molesta* Mitchell (SWRC 2001). In view of these risks, the endosulfan option for Okavango was discarded in favour of deltamethrin.

All available evidence suggests that the effects of the SAT on most terrestrial non-target arthropods are of short duration. Nevertheless, environmental monitoring in Zimbabwe revealed long-term adverse effects on populations of certain lycosid spiders, *Hippasa* sp. (SEMG 1993). Given that little is known about the resilience of affected populations in natural or semi-natural ecosystems, the SAT should be used only temporarily for pre-release suppression. More selective techniques, such as stationary targets (section 3.3.), should be given priority unless they are logistically too demanding for large-scale operations or do not yield sufficient pest population suppression. Using a four-scale risk classification scheme (negligible, minor, serious, severe), Grant (2001) classified the environmental impact of sequential aerosol spraying as "minor". In principle, this classification is acceptable, but should be taken with caution. The resilience of small and often highly fragmented non-target populations, to tolerate incremental insecticide stress in a multiple stressor environment, declines (Peveling 2001). Therefore, environmental impact assessments should be conducted with respect to site-specific features, such as the prevailing land-use patterns or the presence of vulnerable species. As one measure to mitigate adverse effects, Nagel (1995) proposed patchwork applications of different insecticides. This tactic would also reduce the above-mentioned risk of resistance development in other pests (Hoy 1998).

Low-flying spray aircraft may disturb domestic animals and wildlife, visually and by noise. Nesting birds are particularly vulnerable to this combination of disturbances. Although adverse effects such as increased susceptibility to predation should be temporary, nest abandonment may occur in the more sensitive species (USDA 2001b). A critical situation may arise in tsetse spraying, which relies on nocturnal treatments for optimal efficacy, using low-flying aircraft equipped with bright spotlights. Scared birds are unlikely to recover their nests during the night, leading to increased egg or nestling mortality. In Botswana, for example, there was concern that the wattled crane *Grus carunculatus* (Gmelin), an endangered species breeding in seasonally flooded marshes in the Okavango Delta, might experience reduced breeding success following perturbations by spray aircraft and insecticide-induced food shortages. A pilot study found high levels of nest attrition due to predation and other factors in both sprayed and unsprayed areas, but no indication of breeding failure due to tsetse spraying (Allsopp and Phillemon-Motsu 2002, BBCWG 2002). However pre-spray observations were lacking, and follow-up studies are necessary before final conclusions can be drawn.

3.3. Artificial Attractive Devices

A variety of traps and targets (devices attracting insects without necessarily trapping them) have been developed for the detection, monitoring or control of insects. Tsetse flies are attracted by odours (ketones, octenols, phenols, acetone, carbon dioxide) and by visual cues (colour, shape). Some odours induce host searching behaviour while others promote landing on the device or entering the trap (Vale 1993). Fruit flies, screwworms, and boll weevils respond to olfactory cues, such as pheromones, parapheromones, host odours or food baits. Devices such as odour-baited targets for tsetse flies, and coloured baited traps for fruit flies, combine visual and olfactory stimuli to enhance trapping efficacy. Insects lured to traps or targets become stuck to glue (sticky traps), or are exposed to insecticides applied to the material. Sticky panels for fruit fly control use synthetic lures (trimedlure, ceralure, cuelure) applied directly to the panels or to wicks attached to the panels (USDA 2001b). These devices are deployed from the ground by nailing to trees or posts. Alternatively, insecticide-treated baited panels (wood chips) or wicks (so-called cordelitos) are released from aircraft. The parapheromone methyl eugenol is particularly attractive to males of some species of *Bactrocera* fruit flies (Shelly and Villalobos 1995), and can be used in male-annihilation programmes prior to the release of sterile males. Specific sex attractant and aggregation pheromones are used for mass trapping boll weevils. Autosterilization techniques for tsetse control aim at female flies using stationary targets treated with chemosterilants such as IGRs (section 2.3.), leading to the sterilization of females and a gradual suppression of the tsetse population (Knipling 1999, Oloo et al. 2000). Baited traps for screwworm flies are employed only for detection and monitoring purposes (USDA 2001a); population suppression is achieved effectively by treating livestock.

The densities of traps or targets vary depending on the insect species and its potential growth rate (Weidhaas and Haile 1978), the type of trapping device, and features of the landscape. In the case of fruit fly suppression, trap densities must be very high to be effective (USDA 2001b), and mass trapping is, therefore, not practicable over larger areas. For example, more than 1000 traps per square kilometre were deployed in an oriental fruit fly *Bactrocera dorsalis* Hendel eradication programme in Mauritius (Seewooruthan et al. 1997). In contrast, suppression of certain tsetse fly populations can be achieved with as few as 2–4 targets per square kilometre (Dransfield and Brightwell 1992).

The deployment and maintenance of trapping devices requires a network of tracks and roads. Servicing activities may disturb wildlife, especially in wilderness and/or sensitive areas. Thus, two issues have to be addressed with respect to the risk assessment of insect trapping techniques: (1) direct effects on non-target animals, and (2) indirect effects related to trap placement and servicing.

3.3.1. Direct Effects on Non-Target Animals
Traps using species-specific pheromones pose a low risk to non-target organisms. Certain species may be attracted coincidentally, especially potential predators and scavengers of target insects. However, local populations are unlikely to be affected. Ecologically relevant adverse effects are most likely to occur with non-target

animals from the same genus or family as the target insects, even though other non-target species may sometimes be collected in high numbers. For example, mass trapping of fruit flies, using parapheromones as attractants, may also have an effect on non-pest fruit flies. Some species are important pollinators, thus, there may be a risk of reduced pollination and hence fruit-set of certain plants at high trap densities (section 5.5.). Obviously the risk to non-target organisms rises with increasing trap density, yet the risk is expected to be lower than that associated with aerial bait spray or with the application of insecticide-treated cordelitos or panels dispensed in high numbers from aircraft (USDA 2001b). Suda and Cunningham (1970) reported that lacewings (beneficial predators) were caught unintentionally in methyl eugenol traps; the attractant methyl eugenol has been used in some programmes applying the male annihilation technique (MAT) (Koyama et al. 1984).

Contrary to fruit flies, tsetse fly trap densities are much lower, and the chances of incidental encounters of non-target organisms with stationary targets deployed for tsetse suppression are scarce. The degree of species-specificity depends on the combination of visual traits (shape, colour) and odours (octenols, phenols), as well as on the mode of placement. Blue and white targets attract more insects, including pollinators, than odour-baited black screens, which attract mainly stable flies (Stomoxyinae), non-biting Muscidae, and horse flies (Tabanidae). Some of these flies are possible vectors of livestock and human diseases, including trypanosomosis (Sumba et al. 1998), and can be considered as pests, but others such as male tabanids are beneficial flower-visitors and pollinators. Even though the number of studies is limited, there is as yet no clear indication that these taxa are adversely affected on the population level (SEMG 1993, SWRC 2001). Likewise, insecticides such as deltamethrin and α-cypermethrin, used to impregnate the fabric of targets, pose a low risk. During the rainy season, some active ingredient may be washed off, but the contamination is negligible compared with full-cover spraying in agriculture and vector control, and the effects are restricted locally (Cuisance et al. 1984, Müller et al. 1984).

3.3.2. Indirect Effects on Non-Target Animals

The deployment and servicing of large numbers of traps and targets are logistically feasible and environmentally sound in agricultural areas with existing road networks. These conditions prevail in some areas subjected to fruit fly suppression or eradication. Therefore, disturbances of wildlife through the placement and maintenance of traps are expected to be minimal when compared with those induced by other agricultural activities such as planting, irrigation and harvesting. However, disturbances may become more critical in landscapes composed of mosaics of agricultural and natural land.

This is particularly true for tsetse control, which is often conducted in protected and/or wilderness areas. Opening tracks for the deployment and maintenance of stationary targets can cause erosion, and provide access, not only to control operators and park rangers, but also to poachers (Grant 2001). It also facilitates unauthorized logging activities and firewood collection. Such illegal activities may disturb wildlife. A study in Kasungu National Park, Malawi, found significantly reduced numbers of small antelopes and certain birds on transects with odour-baited

targets (Cheke et al. 1997, De Garine-Witchatitsky et al. 2001) caused by the insecticide-impregnated cloth, rather than the odour (acetone), acting as a repellent. For birds, the effect was linked to the possible decline in the number of horse flies, affecting the insectivorous little bee-eater *Merops pusillus* Muller, and, as a consequence, reduced pollination and fruit-set of flowering plants, affecting nectarivorous sunbirds and the frugivorous bulbul *Pycnonotus barbatus* (Desfontaines). However, as no pre-treatment observations were made, follow-up studies are needed to validate these results. In West African gallery forests, insecticide-impregnated blue screens along river courses are sometimes deployed at distances of only 100 m. Studies in West Africa on invertebrates and insecticide residues in soil and wildlife showed at most negligible ecological effects (P. Müller et al., unpublished data). It is not known if there are further studies on indirect ecological effects resulting from such high screen densities, but assumedly the effects are of minor importance compared with other anthropogenic disturbances encountered in West African river systems.

3.4. Other Methods

A range of alternative methods to suppress fruit fly populations in AW-IPM programmes has been reviewed in environmental impact assessments, including physical, cultural, biological and biotechnological methods (USDA 2001b). A detailed discussion of these methods is beyond the scope of this chapter, but one method, soil treatment with organophosphorous insecticide, deserves mention because it may cause serious hazards. It consists of drench treatments within the dripline of infested host plants to control larvae entering the soil and adults emerging from the soil. Soil drenches with organophosphates, such as chlorpyrifos, fenthion and diazinon, are highly toxic to soil fauna, and may adversely affect key ecological processes, such as the breakdown of organic matter and nutrient cycling. Individual vertebrates are also at risk, even though the limited use of soil drenches (usually less than 1% of the total surface), as a whole, provides protection of populations.

Pour-on treatments of livestock (living targets) with insecticide, to control tsetse flies and other parasites or for population suppression, are widely used in Africa. Livestock are treated in dip tanks or by applying pour-on formulations. Pyrethroids, such as deltamethrin, α-cypermethrin and flumethrin, are used for this purpose. Originally, pyrethroids were applied mainly to control ticks, but it was found that they were equally effective against tsetse flies.

Dip and pour-on techniques may have adverse effects on non-target insects, including blood- and dung-feeders, as well as on livestock-associated insect-eating birds. Insect species attracted to livestock are often the same as those attracted to stationary targets, but there is an elevated risk since livestock densities are often much higher than stationary target densities. Adverse effects to other dipteran pests are highly desirable, from an animal health perspective. However, a risk to birds cannot be dismissed. For example, the decline or local extinction of the oxpecker *Buphagus africanus* L. in South African rangelands has been associated with the use of arsenic pesticides and organophosphates in cattle dips for tick control (Mundy

1991). These compounds were eventually replaced with insecticides posing a low risk of acute poisoning in birds. Nonetheless, little is known about sub-lethal effects or indirect effects due to food deprivation.

Dung beetles (Scarabaeidae) are important decomposers of animal dung, and benefit soils and plants by improving soil fertility. They are also important food items for a wide range of invertebrate and vertebrate predators. Similar to parasiticides such as avermectin (Petney 1997, Steel and Wardhaugh 2002), faecal residues of pyrethroids are toxic to dung beetles and other dung fauna such as muscoid larvae (Grant 2001, Kruger et al. 1999, Vale 2002), thereby impeding the decomposition of pats and depriving predators of their prey.

Inappropriate disposal of used pesticides is a serious risk of the insecticide-dipping technique. Dips are often disposed of on open ground, resulting in soil contamination. In other cases, dips are dumped in pits and covered with soil; this may lead to the contamination of groundwater.

3.5. Environmental Effects of Suppression Techniques — Conclusion

Each population suppression technique incorporated into AW-IPM programmes is associated with particular environmental risks. The optimum combinations of control methods, that result in the lowest cumulative risk but achieve the highest possible effect on target populations, vary among species, regions and ecosystems. Therefore, environmental assessments may come to different conclusions in different environmental settings. Nevertheless, some general conclusions can be drawn. Aerial spraying, depending on the form of application, is usually more harmful to non-target biota than ground spraying (which is often more focused and limited in scale) or attraction techniques using traps, lures or targets. One exception is residual ground application against tsetse flies; due to relatively high insecticide doses and persistence, it may be more harmful than aerial sequential aerosol applications as shown by the environmental recovery monitoring in Botswana, which indicated that most non-target species recovered to pre-spraying levels (Perkins and Ramberg 2004). Stationary attractive devices are normally the least harmful method (Nagel 1995, 1996), but the direct and indirect impact of generating and maintaining access (tracks or transects) to service them over large areas is usually underestimated. In general, treatments in protected and/or wilderness areas require more careful approaches and risk mitigation measures than those conducted in croplands. This is because protected/wilderness areas are more complex in terms of biodiversity and interactions among biota, and hence less predictable in their responses to disturbances. Also natural systems lack the adaptation of agroecosystems to human impact manifested in the dominance of ubiquitous or euryoecious species.

4. STERILE INSECT RELEASE

In AW-IPM programmes integrating the SIT, sterile insects are released from vehicles or aircraft (Dowell et al., this volume). Ground release requires appropriate road networks. This may be critical in sensitive areas, posing similar risks to non-

target organisms as trap or target deployment and maintenance (section 3.3.2.) (Vreysen, this volume). Likewise, sensitive species, or life stages such as nesting birds, may be disturbed by the sight of, and noise from, aircraft dispensing sterile insects (SWRC 2001, USDA 2000b). However, such disturbances are expected to be lower than those caused by low-flying spray aircraft because sterile insects are released at higher altitudes, e.g. greater than 200 m for tsetse flies (Vreysen et al. 2000) and about 600 m for Mexican fruit flies *Anastrepha ludens* (Loew) (SIRF 2001).

Aerially applied sterile insects are released directly into the air from temperature-controlled containers (chilled flies) or indirectly from cardboard boxes or paper bags that open once thrown out of the aircraft. In the latter cases, debris from the releases may be a visual disturbance, but such disturbance is transient since the materials used are biodegradable.

Sterile insects released in large numbers may become a nuisance to potential host animals, or may damage plants or fruit. Moreover, natural trophic interactions within communities, such as host or prey detection, may be disturbed by the sheer abundance of sterile insects (although, in the case of the Mediterranean fruit fly, the insect with the highest release rates, approximately only one sterile male per 10 m^2 is released). However, little research has been done in this field. In the case of tsetse control, released at the lowest rates (approximately 20–100 sterile insects per km^2), the release of flies increases the vector load in the target area, leading to a temporary increased risk of trypanosomosis transmission. This can be countered by releasing only sterile males pre-fed with a trypanocide-treated blood meal (Dyck et al. 2000). Lastly, biosecurity concerns may arise in the case of releases of sterile insects of species outside their natural range (section 2.1.). Nevertheless, adverse effects are short-lived because of the high natural mortality of the released insects, and because they cannot reproduce. No such concerns exist if AW-IPM programmes using the SIT are directed against native species. In this case, the SIT can be compared with augmentative biological control, in which native species are mass-produced and released in their natural range.

5.   ECOLOGICAL CONSEQUENCES OF ERADICATION

Many area-wide SIT applications aim at suppression or containment, not eradication (Hendrichs et al., this volume). However, if eradication is the goal, the following issues are relevant.

5.1. Risks and Benefits

Populations living on remote islands are more vulnerable to extinction than those living on continents. This also holds for pest species targeted by area-wide control programmes. For example, the coconut moth *Levuana iridescens* Bethune-Baker, a widespread local pest in Fiji, is thought to have been eradicated by the tachinid fly *Bessa remota* (Aldrich) during a classical biological control programme in the 1920s (Howarth 1991). However, in the history of economic entomology, there is only one known case of a continental agricultural insect pest becoming extinct. The Rocky

Mountain grasshopper *Melanoplus spretus* (Walsh) was a major pest of crops in North America in the nineteenth century. The demise of this species was the result of the anthropogenic destruction of breeding habitats in river valleys (Lockwood and DeBray 1990). This shows that numerical abundance alone does not assure survival (Lockwood 2002). On the other hand, even the most intensive use of chemical insecticides has not threatened the survival of any major insect pest. Only area-wide control strategies incorporating the SIT offer the opportunity to eradicate entire pest populations from selected areas. Eradication is most effective when directed against isolated populations on islands and in habitats separated by natural barriers from the main range. However the eradication of the New World screwworm populations in North and Central America shows that a pest can be eliminated even from continuous continental areas.

Thus, the extinction of whole pest species from the wild seems possible in the longer term. It follows that ecological consequences must be evaluated thoroughly and impartially. What are the benefits and risks associated with the area-wide elimination of pests? As for the benefits, these are beyond dispute. An immediate economic as well as environmental benefit is the enormous reduction in pesticide use (e.g. Kinley 1998, CDFA 2001). For example, to contain permanent Mediterranean fruit fly infestations with conventional methods, more than 2 million kg of active ingredient would be needed annually (CDFA 2001; Enkerlin, this volume). The benefits arising from the eradication in 1991 of the New World screwworm in Libya are immeasurable (Reichard 2002, Vargas Terán et al., this volume). Its spread over the entire African continent would have been an environmental, social and economic disaster, possibly comparable to the impact of rinderpest in the 19th century.

Environmental risks are much more controversial (Allsopp 2001, Feldmann and Hendrichs 2001, Grant 2001). In view of the devastating economic and/or medical impacts of the prime targets of the SIT, most notably the huge economic losses caused by fruit flies, moths, screwworms and tsetse flies (Mumford 2000, Wyss 2000), the suffering of people and livestock from tsetse-transmitted trypanosomosis in Africa (Grant 2001), and the threat to the biodiversity of island faunas by exotic invasive species which could be effectively controlled with the SIT (Suckling 2003), one might even question the justification for raising the risk issue at all. Nonetheless, it is important that possible risks, however low, should be brought into perspective too, irrespective of the overwhelming environmental, economic and health benefits; only then can risks be managed adequately. The specific goal of this chapter is to elucidate ecological roles of certain target insects in their respective ecosystems, and to identify research themes and topics that should be addressed when embarking on future eradication programmes.

5.2. *Loss in Genetic Diversity of Target Species*

An area-wide programme that targets the eradication of populations of the pest insect leads inevitably to a decline in the genetic diversity of that pest species. For various reasons (section 5.3.) this may not be generally acceptable. Therefore, in the unlikely case of eradication from the whole range, a certain level of genetic diversity

of the target insect can be maintained in laboratory colonies. These could be used, and their genomes explored, for future research in the biological and life sciences. It would be impractical, of course, to rear strains from all populations representing discrete demes within metapopulations. Yet, as a minimum requirement, voucher specimens from all target populations must be deposited in scientific collections, including all development stages and both sexes. One part should be mounted, and others preserved deep-frozen and in alcohol (various concentrations between 60–100%). The preservation of insects will be greatly facilitated by using the cryopreservation techniques for embryos (long-term storage at liquid-nitrogen temperatures), which are already available for a number of insects (Leopold et al. 1998; Parker, this volume).

5.3. *Eradication versus Conservation*

The prospect of the eradication of native species from their complete natural range is largely theoretical at this point. Nevertheless it raises some general questions with respect to conservation. On the one hand, the planned extinction of a limited number of pests appears to be a negligible issue in view of the scale and dynamics of the speciation and extinction processes in the evolutionary history of the earth. However, in modern times, human activities have become the major cause of extinction, leading to a strong imbalance of speciation/extinction rates towards extinction. Given the dramatic decline of species worldwide, biodiversity conservation — as outlined in the Biodiversity Convention — has become a global priority. This was motivated partly by the prospect that biodiversity will provide enormous resources for future uses, as well as by fears that ecosystems might be destabilized if extinction continues unabatedly. The latter notion leads to the introduction of the precautionary principle into decision-making. Precautions must be taken whenever we are uncertain about the ecological consequences of our activities. This principle, however tentatively, also applies to pathogens and pests. Only if ecological consequences could be assessed and predicted conclusively would we dare to signal the deliberate elimination of a species. Such safety of judgement did not even exist for the smallpox virus, which is still being preserved for possible future medical research, including drug development (Joklik et al. 1993). In view of our limited knowledge about the consequences of eradication, strategies that control native pests, without necessarily eradicating them from their entire natural range, are preferred. Furthermore, alternative approaches, such as transgenic technologies, should not be ignored (Hao et al. 2001). These options obviously require that genetic resources be preserved in a viable state.

The precautionary approach does not apply, in the same way, for the elimination of populations of exotic species that have invaded areas outside their native range. Application here of the precautionary principle implies the imperative that outbreaks of exotic pests be eliminated to prevent adverse effects on native communities (CBD 2000, 2001). Even then, care should be taken to minimize destabilizing impacts on other species as the exotic one is progressively removed.

It is beyond the scope of this chapter to discuss these hypothetical possibilities in more detail; doing so would involve ethical, philosophical, biological, and

ecological discourses. Nevertheless, it is important to point out that these aspects should be considered when designing strategies that attempt the eradication of native species throughout their whole range.

5.4. Release of Wildlife from Disease

Diseases induced by tsetse flies, screwworms, and other insect vectors can adversely affect the health of wildlife, and reduce their survival rates. Conversely, free-ranging wild animals may benefit from the eradication of disease vectors and/or parasites, and increase the size of their populations.

If tsetse flies were eliminated, the immediate benefit to wild mammals would be only moderate, since African wildlife is trypanotolerant to at least those trypanosomes to which they are continuously exposed (Reichard 2002). Yet wildlife could benefit indirectly by spending more time in habitats previously avoided when fly densities were high. For example, the presence of tsetse can deter elephants from riverine forests (Bond 1993). These habitats would become fully available after tsetse eradication, but to the possible detriment of the woody vegetation. Nevertheless, experience has shown that forests are exploited and devastated whenever elephant populations exceed the carrying capacity, irrespective of the presence or absence of tsetse flies (Nagel 1995).

The impact of screwworms on wildlife can be much more pronounced. Prior to the eradication of the New World screwworm from the southern United States, a wide range of mammals was attacked by this parasite. White-tailed deer *Odocoileus virginianus* (Boddaert) populations were highly susceptible, with fawn mortality reaching 80% (Reichard 2002). White-tailed deer are hosts to more than 100 disease agents (Schaefer and Main 2001). While these agents are seldom, by themselves, fatal to deer, interactive effects may, in periods of stress and malnutrition, weaken animals and cause substantial mortality. After the eradication of the screwworm, a dramatic surge in deer numbers was observed, and this was welcomed by hunters and the game-ranching industry (Reichard 2002). However it caused a new problem for cattle — they became heavily infested with the deer-parasitizing Gulf Coast tick *Amblyomma maculatum* Koch (Kettle 1993). Moreover, in marginal production areas, increased numbers of deer increased competition for pasture. Overall, however, both domestic and wild animals benefited tremendously from screwworm eradication. This includes the endangered Florida or dwarf key deer subspecies *Odocoileus virginianus clavium* Barbour and G. M. Allen. Its population has stabilized to several hundred animals from a low of fifty in the 1930s (Schaefer and Main 2001). Likewise, the endangered Florida panther *Puma concolor coryi* (Bangs), the bobcat *Lynx rufus* (Schreber), and other predators profited from the increase in the numbers of deer and wild boar *Sus scrofa* L.

Similar effects may occur in other areas subjected to screwworm eradication, yet with different ecological consequences and management implications. The endemic Jamaican hutia or cony *Geocapromys brownii* (J. Fischer) is highly endangered due to predation by the exotic small Indian mongoose *Herpestes javanicus* (E. Geoffroy Saint-Hilaire), as well as stray domestic cats and dogs. Stray dogs, with an infestation rate of 40% (Hoelscher 1999), are to some extent kept in check by the

screwworm. Releasing cats and dogs (mongoose seems to be less susceptible) from the disease may translate into increased predation on conies, and, unless predator control measures are intensified, upset conservation efforts. Likewise, free-ranging introduced deer, pigs, and goats are expected to increase in numbers, threatening crops and remnants of natural vegetation. These examples show that the risk of area-wide control differs among ecosystems. Island ecosystems are highly susceptible to the addition or elimination of elements of native food webs. Pest management solutions must, therefore, be adjusted to recognize these specific risks.

5.5. Pollinator-Plant Interaction

Pollination is the most important ecological service provided by insects to flowering plants. Pollinator assemblages comprise a wide range of insects, including blow flies (Calliphoridae) and fruit flies (Tephritidae) (section 3.3.1. regarding male horse flies). Most species in these families are opportunistic, non-specific pollinators. Therefore, the removal of the population of a single species is unlikely to impede pollination. Among the main dipteran pests targeted by the SIT, only some fruit flies comprise obligate pollinators. A mutualistic relationship has coevolved between species of *Bulbophyllum* orchids and certain *Bactrocera* spp. fruit flies, including pest and non-pest species (Tan 2000). Male *Bactrocera* are attracted to orchid flowers by synomones, i.e. chemicals benefiting both the plant and the fly. These are consumed (pharmacophagy), and used further as aggregation and sex pheromones to attract females. Apart from orchids, some species of the Araceae and Lecythidaceae are pollinated by male fruit flies. It is evident that perturbation of species-specific flower-pollinator systems may result in reproductive failure of the plant. In this case, applying the SIT against pollinator fruit flies within their natural range may not be an environmentally acceptable control option. However, apparently, no specific flower-pollinator relationships exist for the principal SIT targets. The Mediterranean fruit fly, the oriental fruit fly, the Solanum fruit fly *Bactrocera latifrons* (Hendel), and the melon fly *Bactrocera cucurbitae* (Coquillett) are controlled as exotic pests mainly outside of their natural range.

5.6. Predator-Prey and Host-Parasitoid Interaction

There are apparently no obligate predators of insects targeted by the SIT (Laird 1977; Nagel 1988, 1995). In fact, most predators have opportunistic feeding habits. Therefore, ecologically relevant effects on predator populations are unlikely. The role of parasitoids, however, is much more specific, and needs to be discussed in more detail.

Many hymenopteran larval and egg parasitoids attack fruit flies. Their potential as biocontrol agents has been explored on a limited scale in several classical and augmentative biological control programmes (Montoya and Liedo 2000). The host range of parasitoids among tephritids varies, but none seems to be host-specific. Therefore, a risk to parasitoids of fruit flies, resulting from an AW-IPM programme using the SIT, is not anticipated.

Contrary to the species-rich Tephritidae and Calliphoridae, the Glossinidae comprise only 23 species. The most frequent parasitoids of tsetse puparia are *Chrestomutilla* spp. (Mutillidae) and *Exhyalanthrax* spp. (Bombyliidae). In tsetse flies, about 40 parasitoid species have been reported, some of which appear to be hyperparasitoids (Greathead 1980). *Exhyalanthrax* spp. have a wide host range among tsetse flies and other dipterans. Some species have been reared from only one tsetse species (Greathead 1980). *Chrestomutilla glossinae* (Turner) appears to be restricted to *Glossina* spp., while *Nesolynx glossinae* (Waterston) (Eulophidae) is believed to have hosts other than tsetse (Greathead 1980). The rate of natural parasitism rarely exceeds 20%, and often is considerably lower. Although some speculation remains with regard to host specificity, experience from other taxa suggests that several parasitoids are specific to certain tsetse fly species (but only about 5–7 tsetse species are of economic importance). Therefore, the possibility remains that parasitoid species would be eliminated along with their hosts resulting in a loss of biodiversity and, possibly, in a diminution of natural control in case of tsetse fly reinvasions.

Females of *C. glossinae* are long-lived, and have exceptional host-finding ability. These traits can be used in biological control approaches. Knipling (1999) proposed tsetse eradication tactics that combine augmentative releases of *C. glossinae* with the SIT. The logical end, however, should the eradication of all hosts from their whole range be achieved, would be the demise of the parasitoid. Even though, at this stage, this scenario is merely hypothetical, measures to conserve mutillid parasitoids should be components of full-range eradication programmes. Conservation can be achieved by establishing and maintaining combined tsetse/parasitoid rearing facilities. This would also provide insurance against the possibility that mutillids or other parasitoids are eliminated but not their hosts. Such undesirable effects have been observed in previous tsetse programmes targeting eradication at the population level (Fiedler et al. 1954). In this case, parasitoids could be released to re-establish natural biological control.

5.7. Host-Vector Interaction

Tsetse flies are the main vectors of African trypanosomes. Metacyclic (infective) forms of the parasites colonize the salivary glands, and are transmitted during blood meals. Apart from trypanosomes, saliva also contain viruses (Sang et al. 1999), bacterial symbionts (Beard et al. 1998), and rickettsia-like organisms (Weyda et al. 1995). This suggests that tsetse could also transmit pathogenic bacteria and viruses, or antigenic fragments of them, to their hosts, evoking concomitant immuno-responses. Therefore, wildlife immunization against bacterial or viral diseases may be enhanced by exposure to pathogens and/or antigens transmitted by tsetse (and other blood-sucking insects), thereby improving the health and fitness of wildlife. However there is as yet no evidence on which to base such a hypothesis. Some salivary viruses are known to be pathogenic to tsetse flies (Sang et al. 1997), but their pathogenicity in vertebrates is unknown. Even if wildlife pathogens and/or antigens were transmitted by tsetse, their contribution to the overall load of transmission would probably be low compared with more abundant blood-sucking

species such as horse flies or stable flies *Stomoxys* spp. (Muscidae). So "vaccination services" provided by tsetse may be seen as an odd and far-fetched scenario. Nevertheless, as long as epidemiological data are insufficient to unambiguously dismiss this hypothesis, this topic should be put on the research agenda.

Pre-release immunizations against viral and bacterial diseases are important steps in wildlife translocation and release programmes. If the animals are to be released in high-density tsetse areas (Woodford 2000), existing protocols even suggest moderate pre-release exposure to vectors of trypanosomes and other parasites to enhance immunization. Allsopp and Phillemon-Motsu (2000) predicted that the eradication of a tsetse population in a given area may cause a temporary rise in endemicity of trypanosomosis. If tsetse disappear from areas in which trypanotolerant breeds were reared, the more productive trypanosusceptible Zebu cattle might be introduced and the genetic trait of trypanotolerance lost. In the event of a reintroduction of tsetse, this loss would seriously disrupt livestock production. These examples show that host-vector interactions should be evaluated beyond established views.

This is substantiated by experiences in the temperate zone, showing that management traditions may be ill-founded. In areas in the UK, with high bovine tuberculosis infection rates, it has been a long-standing practice to cull Eurasian badgers *Meles meles* (L.), the main non-bovine reservoir of *Mycobacterium bovis* Karlson and Lessel. Surprisingly, field trials revealed that culling increases rather than controls the incidence of bovine tuberculosis in cattle, indicating that transmission pathways and dynamics are highly complex and not fully understood (Donnelly et al. 2003).

These arguments are not raised against livestock-insect pest eradication programmes. Instead they aim to encourage and extend epidemiological research to non-haemoparasitic diseases in eradication zones, and to emphasize the importance of trypanotolerance in wildlife populations and livestock breeds.

5.8. Effects of Tsetse Eradication on Land-Use

The area-wide control of disease vectors and parasites may cause a strong increase in the number of domestic and wild herbivores, resulting in significant changes in land-use dynamics and patterns. This issue is particularly controversial with respect to tsetse flies (Grant 2001, Peveling and Nagel 2001). On the one hand, it has been suggested that tsetse flies provide insurance against human encroachment of wilderness areas (Jordan 1986, Chater 2003), and against environmental degradation caused by cattle overgrazing in the ecologically vulnerable Sahel zone (Ormerod 1990). Grzimek and Grzimek (1960) wrote about the tsetse fly:

> It is the only true friend the elephants and zebras have left, for it makes their homeland uninhabitable for men.

Other authors dismiss these notions on the grounds that it is the enforcement of regulations and not tsetse that protects wildlife areas (including gallery forests and dense woodland), and that new areas are opened up to cultivation as a result of human population pressure, independent of tsetse presence or absence (Nagel 1994,

Kinley 1998, Feldmann and Hendrichs 2001). Furthermore, African landscapes now plagued with tsetse were thriving pastoral lands rather than wildlife havens until the rinderpest pandemic swept over the continent in the 19th century, depriving once prosperous pastoral societies of their livelihood (Pearce 2000, Feldmann and Hendrichs 2001). From this perspective, fighting tsetse flies means reclaiming land that had already been used for thousands of years. Arguments can be raised in favour of either standpoint.

Pearce (2000) holds rinderpest responsible for the spread of tsetse flies in the 19th century — the demise of cattle led to the transformation of pastures into savannah woodland, providing new habitats for tsetse flies. This mechanism, however, is hypothetical. Typically, cattle promote bush encroachment by selectively exploiting the grass biomass, which in turn favours the growth of tree seedlings and shrubs. Furthermore, because the fuel load of the standing grass crop in heavily grazed savannahs is low, seasonal bushfires are less destructive to the woody vegetation. This idea, that tsetse flies benefited from land-use changes, was already put forward by Ford (1971). In his view, these changes resulted from activities of cultivators and pastoralists who transformed forests into savannah woodland long before trypanosomosis and rinderpest struck the area.

Historical evidence suggests that tsetse flies prevented the migration of early pastoralists into the tsetse "belt" of Africa. Surely tsetse flies and trypanosomosis were a constraint to the colonization of African landscapes for animal husbandry and agriculture, but their presence did not halt this process. For example, a study in Côte d'Ivoire found no indications that recent land-use changes and deforestation were governed by tsetse control (Erdelen et al. 1994, Nagel 1994). Studies in Ethiopia found expanding agriculture in areas where tsetse suppression was successful, but overall changes in vegetation cover and structure were low (Reid et al. 1997, Wilson et al. 1997). In other parts of Africa, tsetse suppression may have had a greater impact on rural development (Jordan 1986, Stevenson 1988, Bourn et al. 2001). Conversely, rural development may affect the distribution of tsetse flies. Some species have disappeared due to the expansion of cropland, and the destruction of woodland and riverine forest habitats. Others have adapted to rural landscapes, where they feed mainly on domestic animals (Jordan 1986, Dye 1992).

It has been proposed that the eradication of tsetse flies may enable a more-even distribution of livestock, thereby reducing over-grazing and erosion in the labile Sahel and highlands, and may also reduce the poaching intensity in national parks and wilderness areas since eliminating wildlife reservoirs of trypanosomes would no longer be practiced (Feldmann and Hendrichs 2001). This is a desirable but unlikely scenario. The following issues are considered to be equally important determinants of resource allocation and exploitation: food supply and security, land tenure, access to regional and global markets, and political and socio-economic stability. Decisions by people are based on the specific combination of these factors (Jordan 1986, 1992). The key to the sustainable use of natural resources is strategic land-use plans that mitigate deleterious changes (Grant 2001). This is true irrespective of the severity and extent of tsetse infestations.

In conclusion, it is true that wilderness and protected areas in Africa may harbour dense populations of tsetse flies. However, the conservation of many of

these areas is also due to the fact that they are unsuitable as arable land. Moreover, some tsetse species have become resident in rural areas. Thus, the notion that tsetse flies are key protectors of wilderness areas cannot, as a general rule, be maintained. Finally, human/livestock health and wildlife conservation should not be traded against each other. Both are objectives in their own right, and they require concerted efforts to realize one without hampering the other.

6. CONCLUSIONS

In this chapter the principal environmental risks of the SIT and related activities have been reviewed. Some of them have been substantiated by reliable scientific evidence, while others are merely hypothetical and warrant further investigation.

SIT-related activities *sensu stricto* comprise the mass-rearing, sterilization, and release of sterile insects. Few environmental risks are associated with these activities, as long as safety standards and good field practices are guaranteed. However, the SIT involves other activities, and some of these may have adverse effects on ecosystems.

The first relates to pre-release population suppression. Insecticide applications may be harmful to the environment. In many situations, suppression can be achieved with more selective and ecologically sound techniques, such as traps and targets. These can be used alone or in combination, and may provide an environmentally acceptable alternative or supplement to spraying non-persistent insecticides. However, adverse effects on selected biota or ecological processes and interactions cannot be ruled out for either method.

Secondly, area-wide control leading to the suppression or eradication of populations may have adverse indirect effects. These are often related to insufficient land-use planning and/or the lack of appropriate means of implementation, which in turn may lead to the unsustainable use of natural resources.

The third aspect is of particular importance if native species are targeted. Eradication of a species throughout its range may lead to a loss in species diversity of target and non-target species, including parasitoids and hyperparasitoids. Thus, the SIT can adversely affect host-parasitoid systems. Another aspect refers to the risk to pollinators, which has to be taken into account in all AW-IPM programmes that integrate the SIT. Finally, the immunological and epidemiological consequences of the transmission of non-haemoparasitic disease agents by vector insects, and the consequences of a loss of trypanotolerance in wildlife and livestock populations in tsetse areas, are open questions.

Optimum combinations of different control methods, resulting in the lowest cumulative risk while achieving the highest possible effect on target populations, vary among species, regions and ecosystems. Thus, environmental assessments may reach different conclusions in different environmental situations, and pest management solutions must be adjusted accordingly.

7. ACKNOWLEDGEMENT

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CHAPTER 5.3.

MANAGEMENT OF AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES THAT INTEGRATE THE STERILE INSECT TECHNIQUE

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SUMMARY

Effective management of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT) is key to success. Programme planning includes collection of baseline data and a feasibility assessment. The optimal management structure is where the programme can be implemented effectively and flexibly, independent of government politics, bureaucracy, and even corruption that impede timely goal achievement. Ideally, programmes include both public and private management, and require strong and steady financial support. Governments and donors are the most common sources of funds, but a mixture of public, community, and private funds is now the trend. Interrupted cash flow severely restrains programme performance. Physical support of programme operations must be reliable, and led by a maintenance professional. It is essential to have full-time, well-paid, and motivated staff led by a programme manger with technical and management experience. Programme failure is usually due to poor management and inadequate public support, and not to poor technology.

1. INTRODUCTION

Even though the technical elements of area-wide integrated pest management (AW-IPM) programmes that include the sterile insect technique (SIT) are critical to their success, the management components are of equal importance. However, more emphasis is usually placed on getting the "science right" than getting the "management right". In fact, the failure of area-wide programmes is usually due to poor management and inadequate public support and not to poor technology.

Management is important for AW-IPM programmes with an SIT component because, firstly, these programmes affect large segments of the community, as do other more generally accepted area-wide programmes such as police protection or other services, where virtually everyone within the concerned area is involved (Knipling 1980; Lindquist 2001; Klassen, this volume). This necessarily involves public financing, public accountability, legislation, enforcement, community participation, substantial infrastructure and organization, long-term support, and high levels of managerial skill. Secondly, as the SIT requires the successful handling of live insects, which have a very short "shelf life", as well as a number of other activities that need to be carried out in a defined time and space sequence, flexibility and high levels of technical responsibility to conduct and coordinate operations effectively and in a timely manner are essential.

Reyes et al. (1988) described the general organization and structure of an AW-IPM campaign involving the use of the SIT.

This chapter discusses various aspects of programme management and its impact on programme success or failure.

2. FEASIBILITY ASSESSMENT

A feasibility assessment is essential, e.g. DeBiasio (1988) on the codling moth *Cydia pomonella* (L.), and it includes financial issues such as funding and budgets (costs and benefits) (Rhode 1975; Mumford, this volume), expected benefits other than financial, the biological and practical feasibility of achieving the goal of pest suppression or eradication (Rhode 1970), details of the proposed organization and administration, and activities needed to conduct the programme. Also, the pest situation, current control methods, impact of the pest on agriculture, the environment and human health, and the complexity of the human, biological and physical environments must be assessed. An assessment of the political and fiscal stability of a country, government priorities, governance, public and programme security, social and economic values, and economic development all impact on how feasible it would be to conduct an AW-IPM programme that applies the SIT. J. Reyes Flores (unpublished data) is developing a model to consolidate all relevant factors to predict the feasibility of a proposed programme.

The baseline data needed for a feasibility assessment are: (1) data on population ecology, dynamics, and distribution of the insect pest in the proposed area using geo-referenced data, and an assessment of potential immigration of the insect into the area, (2) assessment of the pest problem (Tween 1993) — losses, both direct and indirect, caused by the pest, and the impact of the pest on human health, agriculture and the economy, (3) cost and effectiveness of current pest control methods, and their disadvantages, and (4) impact of the pest and of current control methods on the environment (Tween 1993; Vreysen et al. 1999; Nagel and Peveling, this volume; Vreysen, this volume). Newly available decision-support tools, such as geographic information systems (GIS), can greatly assist in planning these programmes (Cox and Vreysen, this volume).

3. PROGRAMME PLANNING

A programme can be planned properly only if the baseline data are available, and all required feasibility assessments have been carried out.

If an assessment shows that a programme is technically feasible, economically viable, and socio-politically acceptable, detailed plans can then be made and incorporated into a programme document. Such a document provides details of programme strategy, milestones, operational procedures (including standard operational procedures (SOPs)), budgets, business plan, and management policies and procedures. If eradication is the goal, the viability of maintaining pest free status, as well as the quarantine protocols required to prevent reinfestation, must be included (Krafsur et al. 1987, Reyes et al. 1988, Lindquist et al. 1992).

A legal agreement between the programme organization and commercial farmer associations, and local and regional authorities, may be needed (Lindquist 2000). This agreement would describe responsibilities of all parties, in terms of financial and in-kind contributions, monitoring activities, the required level of pre-release suppression, sterile insect quality, expected insect deliveries, identification and

handling protocols, expected degree of insect control, penalties for poor results, and insurance coverage.

Unfortunately, there is a tendency to begin implementation before planning is complete (Vreysen et al. 2000; Itô and Yamamura, this volume). A premature start can easily cause the programme to fail, or at least perform poorly, leading to discouragement, and possibly to abandonment of the programme. All components of the programme must be ready and harmonized, including a properly trained staff, before operations can begin. Sometimes programmes can begin on a pilot scale, to provide hands-on training for staff and to test all components, or start with areas in which success can be more easily achieved, and then move on to larger or more difficult areas. The lessons learned in the pilot programme are useful in making plans for the large-scale programme, and in any case flexibility (Norton 1986) is always needed as programmes proceed. In cases where a pest emergency occurs, such as an invasion or introduction of a new pest (Patton 1984), proper planning is usually not done, and ad hoc procedures are adopted with the attendant high risk of failure. However, in spite of the crisis of the New World screwworm *Cochliomyia hominivorax* (Coquerel) invasion into Libya, the appropriate time was still taken to develop a plan of action for an AW-IPM programme.

If programmes are aimed at eradication, especially of pests that attack export crops, plans to continue the appropriate activities indefinitely, e.g. pest monitoring, quarantine, and contingency plans and readiness to re-eradicate outbreaks if needed, should be included.

AW-IPM programmes that integrate the SIT, when compared with conventional insect control programmes, tend to be larger in size, more complex, comprehensive, flexible, dynamic, intense, and demanding, and are relatively long term. They require multidisciplinary teams of technically knowledgeable people with special skills to integrate different control methods, an organization involving whole communities and thus needing public and political support at many levels, and significant facilities and equipment. It is a real challenge to conduct such programmes, and both public and private sectors of society need to be educated and encouraged to play their essential roles in the programmes (Dyck, Regidor Fernández et al., this volume).

4. MANAGEMENT STRUCTURE

AW-IPM programmes that use the SIT along with other pest control methods are complex, and cannot be conducted by individual beneficiaries such as a farmer on a single farm. Instead, an organized group, in the area defined by the distribution of the pest population, must be formed (Tweddle 2002; Klassen, this volume). It is essential that coordination among all stakeholders is effective, and that tasks and procedures are well described and clear responsibilities shown.

Traditionally, SIT-related programmes have been managed "top-down", where the planning and directing is done by hired programme managers, usually government employees such as was the case in the melon fly *Bactrocera cucurbitae* (Coquillett) programme in Japan (Yamagishi et al. 1993), where national and prefectural technical specialists directed the activities. The eradication programme

for the New World screwworm in Libya (FAO 1992) was strongly supported by the national government and international donors, and managed by a team of international technical specialists and their Libyan counterparts. Strong support for a programme from its stakeholders is essential for its success (Hendrichs 1986).

A government-led programme has the advantage of political, social, and legal authority in the programme area, and therefore persons in the public sector appropriately may be programme staff members.

Political independence and power in the hands of a few, even though usually an advantage for an action programme where operations are time-critical, can nevertheless under certain conditions become a disadvantage. Empowerment of people can become a real detriment to success if the programme then becomes burdened by political concerns and vested interests. Those not accustomed to having access to large amounts of money and authority may be tempted to use it unwisely, jeopardizing the programme. Therefore, empowerment should not be granted unilaterally, but only after individuals and groups have demonstrated the ability to manage resources in the best interests of the programme.

The local community and direct clients can also influence the programme from the "bottom-up", such as the initiative shown by ranchers in the USA to get screwworm flies *C. hominivorax* eradicated (Baumhover 2001), and by fruit growers in Canada to study the feasibility of a sterile insect release (SIR) programme for the codling moth (Dyck et al. 1993). Community participation, at some level, is essential to obtain the cooperation of farmers (and sometimes the community at large) for pest suppression activities prior to the release of sterile insects, pest monitoring, quarantine, and publicity. Where pest suppression has to be carried out in residential areas, the active participation of homeowners is essential. However, AW-IPM programmes that apply the SIT almost always require both "top-down" and "bottom-up" elements in the organization. The area-wide nature of programmes, and the technical activities of sterile insect production and release, almost always need a centralized organization led by experienced and knowledgeable managers. For transboundary programmes involving more than one country (where the pest distribution range includes areas in two or more adjacent countries), a multinational or international organization is required to coordinate the activities (FAO 1992). Such an organization may introduce cost savings that are not feasible if there is only a national programme, and a regional approach to pest control often has technical benefits. Selection of a management structure for a programme must be made carefully since, once it has started, it will probably be impossible to change it.

Ideally, AW-IPM programmes include a mix of public and private management, but the balance depends on the local situation, and each case is different. Private companies are often cost-effective and dependable for routine tasks, e.g. maintenance and security of a rearing facility, purchase of materials, insecticide applications, aerial release of insects, and research contracts, but supervision of any out-sourced activity is essential to maintain the quality of the programme. However, the final cost to the community may be high if there is not enough competition among companies, and private firms take a large profit. In Mexico, an international institute contracts with the government to provide administrative services (with only

an 8% overhead charge) in the *Anastrepha* spp. rearing facility, resulting in an efficient work force and no labour union conflicts.

Another option is for a private firm to manage a programme, especially a permanent suppression programme, and where there is a continuous demand for sterile insects. A private company in The Netherlands produces and releases sterile onion maggots *Delia antiqua* (Meigen) as a pest control method for growers who request this, fully paid by the growers (Loosjes 2000). SIT Africa (Pty) Ltd in South Africa handles insect rearing, sterile fly distribution to release areas, and technical support for a Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) suppression programme. Currently, interest is increasing for private companies to supply sterile Mediterranean fruit flies in Israel, Madeira (Portugal), and Slovakia, and tsetse flies *Glossina* spp. in Slovakia. The risks of a business based on biology, and of matching correctly the demand for, and production of, sterile insects, are a significant challenge for private business. Production contracts must specify the required quality of the insects. Nevertheless, commercialization of sterile insect production, with the potential cost reductions (as already shown by successful companies producing biological control agents), is likely to become increasingly common (Quinlan et al. 2002, Quinlan and Enkerlin 2003). However, the area-wide nature of SIT field operations, with the consequent involvement of various stakeholders and the public, complicates privatization of these activities. To attract private investors, the industry supporting an AW-IPM programme must be robust and strong.

If insect rearing and field operations are managed by two different organizations, it is important that misunderstandings and poor coordination between them do not develop. A lack of coordination would be awkward at best, and disastrous at worst, for a programme. One organization with all programme components is the easiest to manage — the programme manager has authority over, and can coordinate, all components. However, when this is not the case — such as a programme that purchases sterile insects from another, possibly distant, organization (Quinlan and Enkerlin 2003; Cayol et al. 2004; Enkerlin and Quinlan 2004; Dowell et al., this volume) — ways must be found to ensure strong cooperation between the two organizations. If a programme is implemented by more than one agency in a joint undertaking, the roles of each agency must be clearly defined. To maximize effectiveness and efficiency, it is essential that the authority for day-to-day operations be held by the executing organization (Rhode 1969).

Corruption can be a significant problem for programme managers, and AW-IPM programmes with large budgets, such as those that use the SIT, are especially vulnerable to it. Examples of corruption are: (1) diversion of funds and materials for personal use or for other units of government, (2) personal use of equipment such as vehicles, and (3) personal or political favouritism or nepotism in selecting or rewarding staff, and in selecting or obtaining kickbacks from suppliers. Paying bribes, e.g. to customs personnel, cannot be done officially, but sometimes for the sake of programme efficiency it is possible to reward cooperators in a culturally acceptable way. Political disturbances and civil unrest are also a threat to programme operations (Rhode 1969).

5. OPERATIONAL FLEXIBILITY AND INDEPENDENCE

Due to the rather unique challenges of an AW-IPM programme involving the SIT, there will almost always be better results if the organization is politically and financially autonomous, and independent of the usual government regulations, bureaucracies, and politics, and sometimes corruption, that reduce efficiency and block progress (Hendrichs 1986). Insufficient operational independence, and the burden of stifling administrative restrictions, cause programme failure.

Operational independence, with a focused and goal-directed agenda, can produce stability and remarkable achievements. The melon fly programme in Japan was conducted through a special organization, the Okinawa Prefectural Fruit Fly Eradication Project Office. This office was independent, like a trust, and had considerable flexibility and freedom to conduct operations and follow procedures conducive to programme success. The prefecture also provided administrative support. The Mexico–United States Commission for the Eradication of the Screwworm, established in 1972 for the AW-IPM programme on screwworm flies *C. hominivorax* (Krafsur et al. 1987, Vargas-Terán 1991, Wyss 2000, Baumhover 2001), represents such an independent but legal body that has the authority but also the flexibility to "get the job done" effectively and efficiently. This commission is exempt from the usual bureaucratic rules, and decentralized from government with an independent management, with its own regulations, accountability, and financial audits (Tweddle 2002). Another example is Programa Moscamed which implements a trinational (Guatemala/Mexico/USA) Mediterranean fruit fly programme. The eradication programme for the New World screwworm in Libya (FAO 1991, 1992; Lindquist et al. 1992) was operated under a specially created organization, Screwworm Emergency Centre for North Africa (SECNA), which had the required independence and authority to achieve its objectives.

On-site programme managers have not only the responsibility to make a programme successful, but also require the authority to make management decisions in a timely manner so that the programme can achieve its goals. This should include authority to dismiss an employee for unproductive or disruptive performance (Rhode 1969).

6. PROGRAMME STAFF

An adequate number of qualified, competent, responsible, goal-oriented, motivated, dedicated, and hard-working staff members, having a high level of performance and morale, are essential to programme success (Lorraine and Meltsner 1987). Employees must have "can do" and "do it today, not tomorrow" attitudes. Staff recruitment should be done well in advance of the time when employees are needed.

Staff, especially the managers, must be full-time workers to encourage a high level of commitment and provide strong leadership, preventing other interests from diverting attention away from the programme (Hendrichs 1986). In the past, some programmes have suffered greatly because the leading professional staff could not spend the required time on the programme due to other duties or insufficient government salaries. It is important to provide incentives for staff to maximize job

commitment and continuity, and to improve their performance on the job. For example, higher than normal salaries can be paid to prevent staff taking on competing extra jobs to earn more money. High salaries also attract the best workers and discourage resignations to take more lucrative positions elsewhere. Staff members taking higher risks than usual, such as supervisors of insect release flights over mountainous terrain, should receive appropriate compensation. If the organization is unique or independent of government, promotion and the accumulation of pension benefits may not have much relevance to employees of the programme, and therefore a high salary is justifiable.

Job security and opportunities for promotion help to maintain job satisfaction. To make workers feel comfortable and able to concentrate on their tasks, safe practices in the work environment should always be a priority. It is vital that there be a high level of *esprit de corps*, using various forms of recognition and reward for good performance to encourage employees and make them feel proud to be a part of the programme. A biological programme has a high degree of dependence on the good performance of the programme's workers; sloppy work from one employee can negatively impact the whole programme.

Another incentive for staff can be specialized training, which also directly improves performance. It is important to train back-up employees so that the work usually done by a person who is absent can continue. However, if a programme results in pest eradication, there would appear to be little need in the future for the acquired knowledge and skills on that pest in that area. Nevertheless, experience shows that the knowledge acquired in a successful programme can be used by animal and plant health authorities in programmes against other pests or the same pest in other areas.

The appropriate expertise needed for all of the various operations must be available among the programme staff, not only biological but also engineering and managerial expertise. This is especially true of the management of insect production (Fisher 1984, Leppla 1984, Schwalbe and Forrester 1984). If the appropriate persons needed to operate a programme are not available, it is better not to start than to start and fail. Incompetent management leads to programme failure.

If there is a conflict of interest, such as between insect production and field work, or between insect production and quality control, the lines of authority must permit staff to report their results to managers who will not be biased, e.g. product quality control activities should be done by an organizational unit that is not responsible for sterile insect production (FAO/IAEA/USDA 2003).

Labour-management relations must be kept positive to maintain staff morale. Refusal to accede to union demands can lead to strikes or work sabotage, and capitulation results in the programme becoming a hostage and subject to blackmail. A labour strike, and the consequent failure to operate a biologically robust programme, can severely retard and even jeopardize the programme's success. Labour strikes at the New World screwworm production plant in Tuxtla Gutiérrez, Mexico, caused delays in the progress of the Jamaican screwworm eradication programme because it depended on this sole source of sterile screwworms (Box 1).

Especially if staff motivation is low, negative personal habits, attitudes, and values, and even local customs, can create significant problems. If not taken into

account and creative solutions found, holidays, religious practices, personal behaviour patterns and conflict among staff members can reduce programme efficiency, and lead to programme losses. Rearing and handling sterile insects is a 24-hours-per-day and 365-days-per-year job. In ways that do not offend individuals and local customs, the insects must somehow be given the first priority. After a clear explanation of the reason for timely actions, properly motivated workers will usually respond with the kind of behaviour that is good for the programme, especially if recognition and compensation for exceptional work are being given.

The need for timely action is not only true for the biological elements of a programme, but managerial actions also must be carried out at the appropriate time (Kakinohana et al. 1993). A delayed management decision, just like a delayed biological activity, could result in programme termination. In the Jamaica New World screwworm eradication programme (Grant et al. 2000), interruptions in critical programme components, such as the employment of temporary field inspectors, resulted in severe programme setbacks (Box 1, Fig. 1).

Box 1. New World Screwworm Eradication Programme in Jamaica — Lessons Learned

The programme began releasing sterile flies in 1999, but by mid-2004 little progress had been made. A special management configuration, which could operate independently of existing inflexible regulations of the government, was never established. Instead the programme was embedded in existing government structures, and was technically and financially supported by several "outside" stakeholders. The Government of Jamaica purchased the sterile flies from the Mexico-US Commission that operated the only screwworm mass-rearing facility in the world (located in Tuxtla Gutiérrez, Mexico).

The programme was implemented on the premise that the screwworm SIT technology was infallible. Consequently, emphasis was placed on operational procedures rather than on strategic considerations that took local conditions into account. Most of the problems encountered in the programme were not related to the SIT technology *per se,* but were due to a reluctance to address problems in a scientific way. Although there were many factors that contributed to the lack of programme progress, the following were the most significant:

- The importance of collecting baseline data on screwworm population ecology and dynamics prior to the initiation of sterile insect releases was not recognized. Sterile insects were dispersed using long-established protocols (which proved their usefulness in other countries under different environmental conditions) that did not take into account the distribution, and spatial and temporal fluctuations in density, of the local screwworm population. Consequently, the density of the screwworm population was greatly underestimated, and insufficient attention was given to adequate population suppression prior to and during the releases. The programme adhered to a dogmatic belief in the "supremacy" of the sterile flies, and ignored an important lesson from history, i.e. screwworm SIT cannot succeed without efficient field operations that continuously suppress the native fly population.

- Appropriate field data to monitor programme progress (Vreysen, this volume) were not collected. Evaluation of the programme was based solely on the number of reported positive screwworm cases, a very crude parameter influenced more by the willingness of farmers to collaborate and submit samples than by the effect of sterile flies. No attempts were made to systematically collect information on mating frequencies between sterile male and wild female flies. It was always assumed that the released sterile insects would perform adequately, and any potential inferior competitiveness was not considered. Many decisions were based on assumptions, the underlying causes of the lack of progress were never identified, the real problems were never rectified, and the programme was allowed to "drift along" for several years.

Box 1. Continued

- Privatization of the veterinary services created a conflict of interest for veterinarians working for the screwworm programme. The more time they spent treating animals against screwworms, the less time they had to treat animals for payment.

- AW-IPM programmes that integrate the SIT are inherently complex, and require flexibility to react swiftly to emergencies and to solve emerging problems. A flexible and proactive management style is required to ensure continuity in the implementation of all important programme components. Any interruption in a critical component will automatically result in severe setbacks. This is especially true in programmes against the New World screwworm fly (which has a very short life cycle — 21 days in optimal conditions). Fig. 1 shows that in 2003 optimal implementation conditions, i.e. continuity in all important components, were achieved only 52% of the time (during 27 of 52 weeks). Although some of the problems such as the interruption in the dispersal of sterile flies, or the accidental release of fertile flies, were beyond the control of the programme, the malfunctioning of the chilled fly unit (which reduced both fly quality and the number of sterile flies released), and the interruption in the employment of temporary field inspectors, could have been avoided.

- The lack of an independent centralized management structure, with the responsibility, authority and flexibility to implement all programme components, was a major constraint and at the core of many persistent problems:
  - o Programme staff members were usually civil servants, and especially the senior persons were assigned many other duties and responsibilities outside of the screwworm programme. The complex nature of AW-IPM programmes demands the full commitment and complete dedication of senior staff, and the many diversions caused by the other duties were detrimental to the programme.
  - o Due to the absence of locally available SIT-related expertise at the onset of the programme, expert advice had to be provided continuously by internationally recruited technical advisors. However, these scientists were authorized only to give advice and thus could not play a significant role in programme decision-making. Many management decisions were unduly influenced by political concerns instead of scientific principles and field data.
  - o Government regulations, that imposed severe restrictions on programme implementation, had to be followed, e.g. the programme could not employ temporary field staff according to the changing needs of the programme over time.
  - o Some critical components of the programme were beyond the control of the managers. For example, there was only one source of sterile screwworm flies, the screwworm production facility in Mexico. Delivery of sterile insects to Jamaica was interrupted twice due to labour unrest at the facility. The second interruption (in 2001) lasted for 38 days, a time equal to almost two screwworm generations, and as a result all progress made earlier was lost. Another catastrophe occurred in January 2003 when, due to a malfunctioning irradiator at the production facility (Merrell 2003), a batch of at least 400 000 fertile insects (potentially 200 000 fertile female flies) was delivered to Jamaica. The upsurge in screwworm cases after this incident is hardly surprising in view of the very large potential for female screwworm flies to proliferate (each fertile female can, in her lifetime, produce several egg masses, each with up to 400 eggs).

- The importance of culturally determined factors, such as the attitude of the public and farmers towards animals, was severely underestimated. The prevailing behaviour of abusing pets and livestock, creating small wounds (and oviposition sites for flies), generated ideal and persistent opportunities for the fly population to sustain itself. Obviously the public education activities were not enough to change this attitude. This problem could have been circumvented by not relying on farmer participation, but instead employing more permanent or temporary field staff to proactively screen animals and treat both infested and uninfested wounds with insecticide; government regulations and a tight budget prevented such actions.

- The programme was impaired by having too many stakeholders, each with a slightly different interest and agenda. Often the result was political and diplomatic dithering rather than firm and focussed decision-making. As a consequence, an attitude of "getting the job done", a prerequisite for programme success, was never developed.

Weeks in 2003	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52

Figure 1. Diagram showing, during one year, the weeks with suboptimal implementation of the releases and field activities in the New World screwworm programme in Jamaica.

Due to a shortage of funds, or for convenience, volunteers such as local farmers may be recruited to perform certain tasks, e.g. set up insect traps or distribute a lure-toxicant (Teruya 2000). The screwworm programme in Central America developed an honorary-inspector scheme that effectively complemented the activities of programme field workers. However, experience has shown that sometimes volunteers do not perform properly, and therefore close supervision is indispensable; the critical operations of a programme should not be assigned to volunteers.

7. PROGRAMME MANAGER

The management of AW-IPM programmes that integrate the SIT is multifaceted and intensive. Administrative tasks can be done by persons with a business orientation, but the senior managers have a much larger responsibility — to give leadership to the programme! Besides being able to manage personnel, infrastructure, finances, and the political side of the programme, and to make difficult decisions, managers must also have practical knowledge about relevant technical matters, and have experience in conducting area-wide and complex insect control programmes. Many of the day-to-day decisions in a programme require sensitivity to critical biological

issues, including environmental issues (Kinney 1993). Managers must be convinced that the technology being used will achieve the programme's objectives. Area-wide programmes require strong and persuasive leadership. Managers must be able to communicate effectively with the programme's stakeholders (Patton 1984; Dyck, Regidor Fernández et al., this volume).

Since there are relatively few persons in the world who have all of these required qualifications, hiring an experienced manager from outside the country may, at least initially, be appropriate (IAEA/FAO/Tanzania 1994). In fact, due to cultural norms, an "outsider" can sometimes achieve things more efficiently than a local person. Normally a programme manager would operate under the guidelines of a board drawn from the official supporting agencies.

If a government takes a leadership role in programme management, it can produce mixed results, since standards of good governance vary tremendously among countries. Even when appointed leaders are good managers of money and people, they may still be poor managers of the technical components of the programme; the result of this combination could be programme failure. Another risk of government-run programmes is that senior management positions may be seen as political rewards, independent of managerial and technical competence. Also, if a government changes, there is the potential for staff changes, and this lack of continuity could disrupt a programme (Rhode 1969).

The suggestions made by Lorraine and Chambers (1989) in the context of the 1980–1982 California Mediterranean Fruit Fly Programme are worth noting. In urban entomology, with today's increased participation of community activists and special-interest groups, it is a major challenge to deal with complex public policies and programme activities that are not derived from experimental sciences. In modern life, there is no longer a "best solution" but only bargained negotiated compromises that more or less balance competing interests. Coping with the public's desire to participate in decision-making, and even having public confrontations over technical issues, are rather new experiences for most managers of AW-IPM programmes.

8. FINANCIAL SUPPORT

AW-IPM programmes using the SIT may operate over large areas, and sometimes involve major facilities and equipment. The required financial resources may also be large. Even though a programme might be economical on a benefit/cost basis (Mumford, this volume), it is not always affordable, and obtaining operating funds can be the most important issue facing a programme. The type of financing used affects programme strategy and operations, the duration of programme support, and the reliability of this support.

Most programmes receive financial support from governments — national, regional or local, and in many cases this support is essential to the stability and success of the programme, e.g. melon fly in Japan (Kakinohana et al. 1993), New World screwworm in the USA, Mexico and Central America, oriental fruit fly *Bactrocera dorsalis* Hendel in Thailand, Mediterranean fruit fly in Latin America (Rhode 1975, Patton 1984), and codling moth in Canada. However, sometimes

government support is unreliable or not delivered in a timely manner, and in a biological programme this can easily cause delays, uncertainty, unnecessary repetition of work, and even programme failure (Hendrichs 1986). Convincing government officials (who may reside far from, and have very different concerns than, the people in the programme area) to support a programme is often a huge challenge.

A mix of public and private funds seems to work best in most programmes, and this is now the trend. For example, the successful *Anastrepha* spp. fruit fly programme in northern Mexico is funded one-third by grower associations, one-third by regional authorities, and one-third by the federal government. However, the larger the number of funding sources, the greater the complexity of financial management, but probably also the greater the funding stability.

Direct programme beneficiaries, such as farmers, growers, orchardists, and ranchers, are goal-oriented, and the pest directly affects their livelihoods. They have a personal stake in the programme's success. Such stakeholders are usually supportive of the programmes since they will reap many of the benefits of success, but often are also "cash poor", even if sometimes "asset rich", and tend to offer quite limited financial resources to a programme. Those farmers whose products for export must be residue-free, or even completely free of quarantine pests, are often very supportive. Even though in some programmes there is little or no contribution from the direct beneficiaries, many programmes obtain contributions "in cash" or "in kind" from stakeholders, e.g. fruit growers in Argentina and South Africa (Barnes et al. 2004) support the field operations of Mediterranean fruit fly programmes, cotton growers in California, USA, help support a pink bollworm *Pectinophora gossypiella* (Saunders) programme, cattlemen in the south-western United States made contributions to a New World screwworm programme (Wyss 2000, Baumhover 2001), and fruit growers in British Columbia, Canada, support a codling moth programme by paying a tax on the number of hectares of apple and pear orchards that they have (Dyck et al. 1993).

Support from programme beneficiaries is good from an operational point of view, but the financial aspects are often unstable. Contributions need to be reliable, and therefore probably compulsory. If the economic health of an industry is poor, the participants in that industry will be reluctant to support a programme. In addition, as in many AW-IPM programmes, not all beneficiaries tend to be supportive and educated in technical matters. They are also sometimes poorly organized, and reluctant or even opposed to cooperating in joint activities. In cases where individual farmers have sterile insects applied but not as a part of a coordinated area-wide approach, for example onion maggots in The Netherlands (Loosjes 2000), such support would benefit only the farmers who contracted to have the SIT applied. Neighbouring farmers, who are "free riders", obtain benefits from dispersing sterile flies without paying for them, while farmers that pay for the SIT suffer when mated fertile female flies enter their land from nearby farms that are not part of the programme (Klassen, this volume).

It is unusual for a community as a whole to financially support a pest control programme, but in the codling moth Sterile Insect Release (SIR) programme in Canada all property owners in the community, including non-farmers, pay an annual

tax to support it (Dyck et al. 1993; Bloem et al., this volume). However, this involvement of the community creates an opportunity for uninformed people to influence the programme, so the role of public relations becomes very important (Dyck, Regidor Fernández et al., this volume).

In developing countries, it is common for donor organizations and government-sponsored foreign-aid bilateral programmes to support national governments in area-wide pest control programmes, e.g. the New World screwworm programme in Libya (FAO 1992, Lindquist et al. 1992), and tsetse fly *Glossina austeni* Newstead programme in Zanzibar, Tanzania (IAEA/FAO/Tanzania 1994, Dyck et al. 1999). In Ethiopia, non-governmental organizations (NGOs) support the control of tsetse flies. It is also common in developing countries for international organizations to provide multilateral support to national governments (LaChance 1993), such as in the screwworm programme in Libya (FAO 1991, 1992; Lindquist et al. 1992), and the tsetse fly programme in Zanzibar (IAEA/FAO/Tanzania 1994, Dyck et al. 1999). Nevertheless, when donors provide support "in kind" instead of "in cash", sometimes the equipment provided is not appropriate to the practical operations and may therefore become unusable. Thus "in cash" support is more useful to a programme than "in kind" support.

9. MANAGING FINANCES

Often the biggest problem in managing finances is interrupted cash flow due to delays in receiving funds from financial supporters of a programme, be it from government or other sources. Budget cuts, caused by economic downturns or political decisions, especially if unexpected, have a strong negative impact on the progress of a programme (Patton 1984). Sometimes a shortage of money is caused simply by bureaucratic delays. If there is no money or money is delayed, usually the programme activities have to be restructured, curtailed, or even stopped, and this can be disastrous for a biological programme where "timing is everything" (Lorraine and Meltsner 1987). Insect rearing and release activities cannot be "put on hold" and resumed later when money becomes available. Failure to sustain activities can result in a total loss of previous achievements and the necessity to start the programme again (Box 1). On the other hand, if a programme suddenly receives an excess of funds, panic spending could lead to the inefficient use of money. One way to minimize the problem of local cash flow is for the funding organization to regularly advance money to the programme manager, who can anticipate expenses and budget these funds accurately.

A back-up plan must be included in the programme planning document so that, when a programme supporter fails to deliver promised money, funds can still be made available, and the programme can proceed on schedule. A possible mechanism to solve this problem would be for one financial supporter to agree, in advance, to provide contingency funds. In any case, a contingency fund is needed to cope with emergencies.

Reliability in funding and cash flow, and also the provision of large amounts of money at those times of year when biological operations require it, are essential for an AW-IPM programme involving the SIT. The seasonality of programme

implementation complicates financial planning. This emphasizes the need for a separate organization (section 5), located at the site of the programme, with its own financial accounting system and audits, which can control cash flow and prevent unauthorized use of money.

Especially in poor countries, payment of staff salaries and incentive bonuses on a regular basis is important for maintaining staff morale and a high level of performance, both of which are essential for effective field operations and the production of high-quality sterile insects for release.

Declining exchange rates between local and "hard" currencies can be a major problem. Under these conditions the conversion of funds has to be carefully managed, as a rapid decline makes it more difficult for a programme to purchase foreign-produced goods such as technical equipment or aircraft services.

10. LOGISTICAL AND PHYSICAL SUPPORT

Even though the quality of the human input into an SIT-related AW-IPM programme is a top priority, programme staff cannot do the work alone, and logistical and physical support is essential to achieve a programme's objectives. Materials and equipment must be of appropriate specifications and quality, cost effective, procured in a timely manner, and properly maintained. A well-executed procurement plan should take into account that biological processes, once started, must be completed, and that the lack of even one item could mean disaster. Stability in the supply of critical materials, and an adequate stock of essential items, are required. For example, in mass-rearing, diet ingredients for at least 3–6 months operation are required, and warehouses have to be sized accordingly (Parker, this volume).

Local rather than foreign procurement would appear, at first glance, to be the best policy, and can be advantageous in terms of availability, lower costs, and reduced transport expenditures. However, purchasing products from another country may be necessary to obtain a high-enough quality or items that simply are not available locally. Flexibility in procurement procedures is required to enable the correct materials to be purchased, and to permit quick action when biological events demand it. The lowest bid on an item is often not the correct criterion on which to base a purchase decision. Diet ingredients require especially stringent quality control procedures (Calkins and Parker, this volume; Parker, this volume).

The location of a mass-rearing facility has an impact on operational efficiency, and on the availability of materials and appropriate staff (Rhode 1969; Hendrichs 1986; Phillimore 2002; IAEA/FAO 2004; Dowell et al., this volume; Parker, this volume). If the programme is located in a difficult-to-access place, procurement must be done long before items are needed. This applies especially to an insect rearing facility, release materials and equipment, and field monitoring supplies. Vehicles, including release aircraft, must be adequate in number and maintained properly to provide reliable and safe service. The production and release of a perishable product like sterile insects need a strong support system to ensure that investments are not wasted by the absence of, or having low-quality, materials and equipment. Proper maintenance of equipment is essential so that mechanical failures

do not result in the production of fewer or low-quality insects. Equipment should not be more complex than necessary (Rhode 1969), but of good quality to minimize breakdowns that lead to inefficient operations. Back-up equipment and plans for all critical operations, and a stock of spare parts for essential equipment to minimize equipment downtime, are necessary. Accidents and emergencies do happen, and therefore redundancy is needed to avoid negatively affecting field operations or to prevent declines in quality insect production. For example, even though many small independently operated rearing rooms cost more to build and operate than one large room, the many small rooms provide much more production security than one large room (Tween 1987; Dyck et al. 1993; Phillimore 2002; Dowell et al., this volume; Parker, this volume). For the sake of maximizing efficiencies and productivity, competition among workers responsible for different rooms can be fostered.

Ideally, utilities must be reliable, but back-up systems (e.g. electricity generator) should always be available. To protect the environment and maintain respect for nearby communities, disposal of spent diet (diet should be biodegradable) through treatment of waste solids and water is an essential activity in mass-rearing facilities, requiring appropriate infrastructure (Wyss 2002; Nagel and Peveling, this volume).

The aerial release of sterile insects has sometimes been done from aircraft owned by the programme. However, it is now considered appropriate to contract out aerial release activities to private or government aircraft companies (Dowell et al., this volume). Nevertheless, to maximize programme effectiveness, programme staff must still supervise release operations. The high cost of aerial release, using fixed-wing or rotary-wing aircraft, necessitates a careful assessment of operational procedures to accommodate any concerns with economic, efficiency or technical issues (Teruya 2000).

Assuming that a regional rearing facility can produce enough sterile insects for the needs of the region, it is more efficient and economical to construct a regional facility than for each programme to have its own facility (Dowell et al., this volume). However, this is risky in view of unforeseen catastrophic or political events, and can lead to complete dependence on the supply of insects, especially if there is only one production facility in the world, as is currently the case for the New World screwworm. Such a monopoly situation had a negative impact on the Jamaica New World screwworm programme (Box 1). In the absence of competition, the operating procedures and policies of, and the cost charged by, the only source of sterile insects might not be in the interest of a particular programme.

11. PROGRAMME EVALUATIONS AND INDEPENDENT ASSESSMENTS

Programme managers, and even standing advisory committees and associated research scientists and institutions, need regularly to invite independent external experts and consultants to evaluate a programme and then give constructive advice and guidance. Both national and international consultants play valuable roles. External reviews of a programme reduce the likelihood of failure due to the "cannot fail syndrome", the unreasonable confidence that a process done correctly cannot fail to achieve the goal, and success does not need to be monitored (Dowell and Wange 1986). Experience gained in other programmes using the SIT can provide

invaluable information and ideas to resolve problems and help achieve the programme's goals as efficiently as possible. Advice is often available, but usually at a cost, from university professors and other technically trained persons that are not affiliated with the programme's organization. The advice should be impartial, unbiased, and given "at arms length". Sometimes it is appropriate to bring in full-time experts to advise the programme manager, especially on technical issues.

Even though there are numerous examples of good and helpful advice obtained from short-term consultants, there are also examples of bad advice that have damaged programmes (Box 2) (Klassen and Curtis, this volume). Some caution in selecting consultants is needed since persons with no stake in a programme might in fact be biased, unrealistic, or too theoretical. External reviewers are not always correct in their assessments or recommendations, but it is extremely difficult politically for a programme manager to reject them. It is also difficult for non-technical managers, and representatives of donor organizations who hire technical consultants, to know if a consultant is biased or not. Therefore external reviews are risky, but on balance in most cases they are very valuable.

Box 2. Mediterranean Fruit Fly Invasion of Central America

In Costa Rica, the Mediterranean fruit fly was first found in 1955, and by 1962 it had spread to southern Nicaragua. Field trials to contain it demonstrated that the technology (including the use of the SIT) was adequate, but that more funds, and a vision to achieve the goal, were required (IAEA/FAO 1970). Regarding the northwards invasion of the fly, the advice given in 1970 by a three-person review panel of technical specialists was to abandon any eradication efforts in Nicaragua. The panel did not accept that eradication should be the goal, questioned the cost of the programme, and instead recommended to "live with the pest" with a focus on enhancing biological control. Preconceived conservative notions against AW-IPM, and the economics and politics of the situation, evidently "ruled the day"; an opportunity to eradicate, or at least contain, the fly was lost. By 1977, abandoning all containment efforts resulted in the fly invading the next three neighbouring countries (Honduras, El Salvador, and Guatemala), and reaching southern Mexico. A costly trinational emergency programme had to be initiated to prevent the Mediterranean fruit fly from invading Mexico and the USA. Since then, the great amount of effort and money that has had to be spent each year (currently over USD 40 million), to prevent this invasion from continuing and to reverse the trend, is at least an order of magnitude larger than the budget (USD 1.5 million for 4 years) that the experts had rejected for the Nicaragua programme (IAEA/FAO 1970). The economic problems caused by the fly invasion are still present in Central America, with significant losses and conventional control costs, and representing the major limitation for countries in the subregion to develop a tropical fruit industry and access the nearby US export market. In comparison, Mexico recently celebrated 25 years of keeping the country free of the Mediterranean fruit fly, a result of which the fruit and vegetable exports have exploded to over USD 3000 million per year. Thus, in this case, the recommendation of a panel of outside technical experts turned out to be bad advice.

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12. PROGRAMME SUCCESS OR FAILURE

Even though often blamed for programme failure, problems with technical issues in fact usually only delay the successful achievement of a programme's objectives, and rarely lead to failure. However, several programmes have failed because of bad management, and inadequate funding and political support.

Many lessons about programme management have been learned from ongoing AW-IPM programmes that integrate the SIT. Some of these lessons are positive:

- Programmes with thorough baseline data collection and feasibility assessment for proper programme planning succeed.
- Programmes with an independent and efficient management structure are effective.
- Programmes with strong and reliable financial support, and strong political stakeholder support, succeed.
- Programmes with adequate and appropriate logistical and physical support succeed.
- Programmes are likely to succeed if they seek expert advice, especially technical advice. Even when pilot programmes encounter problems, eventual success can be achieved if adequate research support (probably provided from outside the operational AW-IPM programme), good cooperation, and international technical support and consultation are available.

However, other lessons are negative:
- Programmes with unrealistic objectives and expectations often fail.
- Programmes that are immature, based on inadequate knowledge, patterned after other programmes without recognizing unique local characteristics, started too soon or with too little advance planning, fail.
- Programmes that lack operational flexibility and independence, and carry the burden of stifling administrative restrictions, will probably fail.
- Programmes with managers having inadequate knowledge and experience, or who are not working full-time on the programme, will probably fail.
- Programmes with inadequate finances fail.

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CHAPTER 5.4.

PUBLIC RELATIONS AND POLITICAL SUPPORT IN AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES THAT INTEGRATE THE STERILE INSECT TECHNIQUE

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SUMMARY

The public relations component of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT) has a large impact on programme success. Full-time professionals should direct public relations activities and secure vital political support from governments and community organizations. Good communication among programme staff, and between programme staff and the public, is required to maintain participation and support, and to keep the work goal-oriented even when some programme activities are controversial. The media can be valuable and effective partners by informing the public about the real facts and activities of a programme, especially if this is done in a non-technical and straightforward way. Ongoing research support improves the programme technology, provides technical credibility on contentious issues, and solves operational problems. Programme failure can result from poor public relations and inadequate public support.

1. INTRODUCTION

Effective public relations for area-wide integrated pest management (AW-IPM) programmes that include the sterile insect technique (SIT) are critical to success. The overall communication of programme goals, objectives, and activities is often not given as much attention as are the more technical aspects of a programme, and inadequate public support can be a cause of failure. Good public relations are just as important for AW-IPM programmes as is good management. Public relations are important because these programmes affect whole communities (Lindquist 2000, 2001; Dyck, Reyes Flores et al., this volume; Klassen, this volume).

AW-IPM programmes are visible, as they often involve whole communities or even several contiguous communities. The participation, support, and possibly the financial contributions of many, if not all, members of these communities, even if disparate, are essential for success. Since urban areas are often included, it is necessary to inform this more sophisticated, "disinterested", and complex segment of the community about the programme's objectives and progress. Quarantine checkpoints established to maintain pest free areas affect travellers who may not live within the programme area (Hendrichs et al., this volume). Since it is environment-friendly, the SIT technology will usually be accepted by the public. However, sometimes problems from the field activities of AW-IPM programmes do arise, and these need to be resolved in a transparent manner (Reyes et al. 1988).

The public needs to be pro-actively and immediately informed about area-wide programmes, with emphasis on the benefits that a programme brings to the community and country (Lindquist 2000, 2001; Mau et al. 2003). Public relations activities require a plan and a budget just like other components of the programme; they are an integral part of it. Essential equipment and staff for these activities must be available at the beginning of a programme.

The details of the communication plan and messages to the audience will be unique to each programme. The type of media used, e.g. print media, radio, television, video, website (with various elements including a list of Frequently Asked Questions), public meetings, and one-on-one conversations with community

leaders, must be suited to the communication plan and the situation. Translation of the message into local languages may be necessary, and certainly the content, style and format must fit the intended audience (Schwarz 1983). A periodic evaluation of the public relations programme is a valuable tool to improve the communication strategy and activities.

2. PERSONNEL

Since technical specialists are often not good communicators with the public, a full-time professional with appropriate training and experience in communications, and also in all aspects of the programme, should be appointed to lead the public relations activities. Consideration should be given to contracting public relations companies to organize major media campaigns.

3. POLITICAL SUPPORT FROM THE PUBLIC

Educating the public about programme objectives and benefits is an indispensable pre-requisite to obtaining political support from the public. However, educating the public at large is difficult, and requires considerable effort and resources. Besides informing people about various aspects of AW-IPM and the SIT, using creative and effective methods are necessary to overcome the resistance that often develops when an area-wide activity is initiated (Klassen, this volume).

- The codling moth *Cydia pomonella* (L.) Sterile Insect Release (SIR) Program (SIR 2005) in British Columbia, Canada, produced two videos to explain in an attractive way the activities of the programme, especially those activities that affect most of the people in the region.

- Exhibits in public places and fairs are useful to describe a programme. A display about the SIR Program was set up in a museum (on the history of the fruit industry in the region), attracting local people and tourists.

- A toll-free telephone number should be available for persons to ask questions about the programme and to report on related activities.

- Potential urban-rural conflicts, such as in an agricultural programme involving insects attacking tree fruits, can often be alleviated through information exchange and one-on-one meetings with antagonists.

- Obtaining the support of labour organizations is highly desirable, and often essential, to assist in informing their members.

- Indigenous people should be informed about a programme and, where relevant to area-wide activities, their permission obtained to enter traditional or sacred areas.

- Programme donors, support groups, and schools may be able to provide good opportunities to educate the public. Students can take the information home and report to family members (who are more likely to listen to one of their own than to a stranger). Informing members of school clubs in Ethiopia stimulated cooperation in setting up tsetse fly traps. Students in Argentina and South Africa learned about AW-IPM programmes through official additions to the school

curriculum. Universities need to expand the teaching of the principles of AW-IPM (Lindquist 2001).

- In Madeira, Portugal, Caldeira (2001) emphasized the need to use both technical and non-technical materials, in a variety of formats and in various public settings, to inform urban dwellers, including school children, of the objectives and activities of the programme.
- An attractive and self-explanatory logo is needed to give a positive and easily identifiable image to the programme.

Obtaining political support for the programme, especially area-wide community support and cooperation, is perhaps the most difficult aspect of public relations (Patton 1984, Tween 1993, Pereira 2001, Allwood and Vueti 2003). Unfortunately, even when the majority of the public has been convinced through a public education campaign, there always are some individuals who are not supportive of a public good, which is beneficial for the community as a whole. In Canada, the group that was probably the most difficult to convince of the value of area-wide codling moth control was the urban property owners who had a few apple or pear trees in their backyards. Some of these persons did not want to cooperate with the programme, or cooperated reluctantly, and in an area-wide programme it is essential that all cooperate. In urban areas where there are no direct stakeholders or beneficiaries (e.g. programmes against pests that attack fruit), activities of AW-IPM programmes using the SIT may appear to be strange, intrusive, or even annoying (e.g. aerial release of sterile insects in bags or boxes, or formerly the application of aerial bait sprays over the Los Angeles basin in California, USA) (Lorraine and Chambers 1989; Mangan, this volume).

House-to-house visits are often a good way to ensure that residents are informed about the programme and the need for their cooperation. In the case of insect pests of animals, and where the programme may include urban animals such as pets and stray dogs, it is often hard to get urban residents to inspect and treat these animals.

Obtaining formal political support from the public probably requires endorsement through local referenda or the passing of legislation by public bodies (Dyck et al. 1993, El-Lissy and Grefenstette 2005). Prior to a public vote or formal response to the programme's plans, a thorough information campaign is needed to ensure that the public has a good understanding of the issues at stake, especially if public funds or taxes are involved.

It is exceptionally difficult to conduct a programme in areas of political or civil unrest. Under these conditions, programme staff members who live in the area should be the ones making contact with the local people; such staff members are probably more acceptable to the local population, and more able to find unusual and creative ways to address the specific concerns of the people, than other staff.

Local organizations, such as farmer associations and citizen groups, special-interest or donor groups, non-governmental organizations (NGOs), and industry-related groups, can be important supporters of a programme, and may even be helpful in combating social or civil unrest that impedes programme execution. If the livelihoods of local stakeholders are at stake (such as being able to export their produce), they can help convince governments to support a programme (Dowell et al., this volume). They often care deeply about the specific issues that support the

use of the SIT, such as environmental concerns. Nevertheless, local organizations may be small and therefore can be influenced by a few vocal or persuasive individuals; if they decide to oppose a programme, it will have significant negative impact.

It is usually an advantage to receive support from an international organization. Such a body lends stature to a programme. International support can stabilize a programme even if local support fluctuates or diminishes, but it cannot replace local commitment and "ownership". Furthermore, since such organizations are not greatly influenced by local concerns, over time their interests could change and support be withdrawn, even if local support for the programme is still strong.

4. POLITICAL SUPPORT FROM GOVERNMENTS

Obtaining the support of national, regional or local governments is dependent largely on the perceived importance of the commodity at stake, e.g. plant products for export (Buchinger 1993, Kinney 1993), health of livestock that are vital to the economy of the region, survival of subsistence farmers, human health threatened by insect vectors of disease, and recreational areas that require no or few environmental hazards such as insecticides that discourage tourism.

It is often difficult to obtain government support, and the legislation and regulations needed, to operate a programme. In the light of competing demands on financial resources, governments may regard pest control programmes, which usually benefit some segments of a community more than others, as a low priority. Positive examples of exceptional support from national and regional governments are the fruit fly programmes in Chile and Japan. These successful programmes resulted in the opportunity to export crops to previously closed markets (MAG/SAG 1995; SAG 1996; Teruya 2000; Enkerlin, this volume).

Legislation and regulations are usually required to support the implementation of complex area-wide programme activities. Programme staff and others, possibly perceived as plant or animal health "police", will need to enter private property, monitor the pest population, inspect and treat crops or livestock, and enforce quarantine activities. Legislation to collect funds from the community, and to obtain enforcement authority, may be needed (Dyck et al. 1993). A government shows that it is really committed to support an AW-IPM programme when it approves legislation and enforcement. For example, legislation that regulates the culture of alternate hosts of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) was recently passed in Spain to support an AW-IPM programme to control this major pest of citrus (W. R. Enkerlin, personal communication). Also, in California, USA, where fruit infested by this fly is sometimes smuggled into the state, legislation has been passed to try to prevent this, and to levy fines against those who are caught smuggling banned fruit (CDFA 2005).

Besides fines or other negative enforcement actions, positive incentives can also be provided to obtain the cooperation of people living within the area of an AW-IPM programme, e.g. low-cost trees for urban property owners to replace those that are susceptible to the target pest (SIR 2005), and free insecticide for farmers to treat cattle against other pests (Dyck et al. 2000).

Strong and long-term government support helps to stabilize, legitimize, and empower a programme so that it can operate until success is achieved. Usually only governments have the long-term view, and the all-embracing view of a whole community's welfare, needed to support the characteristically long-term AW-IPM programmes that apply the SIT. Endorsement of a programme by a government helps encourage the provision of the necessary resources, e.g. skilled personnel and facilities to rear and irradiate insects. Also, political support, programme endorsement, offer of technical assistance, or other kind of incentive from another country (that may represent a potential but important export market) will encourage programme success.

Nevertheless, political conditions change over time, and can change frequently (Patton 1984); the assignment by a government of new key personnel can radically decrease the support given to a programme. Therefore new people appointed to key positions in government need quickly to become informed about the programme. Some politicians are corrupt or may have other objectives, and thus long-term political support from a government is never a certainty. A programme needs protection from changes in government to provide the stability needed to attain its goals.

Political sensitivities (Patton 1984) can negatively affect a programme. Political uncertainties and civil unrest can impair the implementation of an SIT-related programme, e.g. for the Mediterranean fruit fly, the area-wide eradication programme in southern Mexico and Guatemala, or the transboundary suppression programme in contiguous areas of Israel, Jordan and the Territories Under the Jurisdiction of the Palestinian Authority (Enkerlin, this volume; Hendrichs et al., this volume).

Since politicians are usually not technical experts, there is a risk that they will misunderstand a programme, oversell it, use it for their own purposes, or push it in a wrong direction, and in the end damage or even destroy it. Care is needed to properly educate influential people.

A high level of support from governments usually means the involvement of high-level officials, both decision-makers in the executive branch and those who approve programme funds or appropriations in the legislative branch of government. It is useful to find a knowledgeable "champion" of the programme, who can steer politicians in the "right" direction. Obtaining authority and support from a senior government official is a big advantage, but this person must understand the programme well. Ownership of a programme is good, but unintended misuse of a programme is bad.

One of the important roles of government in successful pest eradication programmes is the official declaration that a particular species has indeed been eradicated in a certain area (Teruya 2000; Barclay et al., this volume; Vreysen, this volume).

5. INFORMATION COMMUNICATION

Not only do the biological and geographical characteristics of an AW-IPM programme impact the required features of an effective public relations programme, but so do the sociology of a community, the type of inhabitants, culture, traditions, economics, politics, and even the security concerns involving rebel groups and the military. Before a programme is initiated, a survey should be made to identify the important stakeholders, and also the segments of the community that will be affected by the programme, and what the primary concerns of each are likely to be. A strategy of public relations, and a plan of appropriate activities for each segment, can then be developed.

Communication within a programme through printed materials, electronic messages, radio and telephone communications, and meetings (Kakinohana et al. 1993) is essential to inform all interested parties of plans, progress, and problems. This is especially important if there is a language barrier between programme staff (Rhode 1969). Programa Moscamed, which involves three countries (Guatemala, Mexico, and USA), three mass-rearing facilities, and at least six field centres distributed over vast territories in southern Mexico and Guatemala, uses an internal web page to improve the information flow, and allow instantaneous access to an enormous amount of data information to all programme staff. Information helps to keep workers motivated, focused, coordinated, and productive. Opportunities should always be given to staff members to express their ideas on how the programme is progressing, offer suggestions for improvement, and participate in planning, assessment, and decision-making. A team that is informed encourages cohesive behaviour and mutual support.

A reliable communications system, between insect production facilities and field operations, is needed for operational efficiency and effectiveness (Rhode 1969, Dyck et al. 1999). The rapid sharing of data among staff members is essential for timely decision-making, and fostering good cooperation and coordination (Vreysen, this volume).

Communication activities within a programme should never detract from those directed towards people outside the programme. Nevertheless, field staff members at all levels have to be well informed about programme goals and activities; they interact directly with the community, and therefore play an important role in telling people about the programme.

Regular and timely communication with the programme's stakeholders or clients is essential to maintain participation, support, and accurate recording of field data. It is especially important that stakeholders realize, in terms they understand, that they will receive benefits from the programme. In some situations a website can be a good source of information for the clients (SIR 2005), and also communication through the mail and telephone and at public meetings is useful, but in most cases there must be direct communication at a personal level. Opportunities can be created in rural areas — informal meetings and using speakers on vehicles — to communicate with farmers and those who do not readily get messages through large-scale media services (Patton 1984).

It is important to be open and transparent about programme progress. Information should be withheld only when open discussion on a subject, such as detailed technical data, will confuse the stakeholders. Information provided must be clear, reliable, up-do-date, relatively simple, practical, and sensitive to the concerns of the clients. An opportunity for feedback should be provided. It must be recognized that perceptions among the various stakeholders will not be the same. The same issue may require more than one explanation, depending on the audience. Issues will relate to both technical and administrative matters. If prejudices or resentments are involved, not all issues can be answered satisfactorily. Staff must attempt to prevent stakeholders from having unrealistic expectations of what the SIT can achieve.

Financial supporters require regular reports on the financial status of a programme. However, in addition, donors (e.g. governments that have a political component) need to know that there is public support for it. Articles in the national press or items on television and radio provide indirect support to a programme.

6. PUBLIC MEDIA

AW-IPM programmes that apply the SIT need the public media! It is vital to build good relations with several media outlets, and with people in the media business, so that opportunities to communicate to the public become available, and the best way of reaching the right audience is found. The appropriate media outlets must be carefully discerned to maximize the desired communication to the target audiences. Programmes in Mendoza, Argentina, used television and radio broadcasts of sporting events to communicate short but key messages to the public. Press kits are a useful way of giving information to journalists. Sometimes communication in more than one language is required, but always in a format and style appropriate to the audience. Monitoring media coverage reveals which issues interest the public. The information provided to the media must be correct, authoritative, and also realistic about both the problem and the solution so as not to oversell a programme and create expectations that cannot be realized.

When the message is clear and straightforward, the SIT is promoted effectively through the public media. A humorous message, as in a cartoon used in Guatemala to show the basic concepts of the SIT (Fig. 1), is attractive, and gets the message across in an easy-to-understand manner.

However, relations with the commercial media are often complicated and full of pitfalls. The media tend to be aloof and sceptical about, impatient with, and uninterested in, pest control issues, and tend to oversimplify information, easily leading to incorrect statements. Explaining technical issues to the public, and even to clients, is difficult, and has to be learned. Technical experts have to realize that technical advice may not always be value-neutral (Lorraine and Chambers 1989). The public does not easily understand the concepts of AW-IPM and the SIT, may be quite sceptical (especially about the control of established pests), and special efforts have to be made to communicate the facts simply but effectively, using a variety of formats to ensure clarity. The public may require proof of the effectiveness of the technology. If not explained properly, the use of radiation to sterilize insects is easily

Figure 1. Cartoon illustrating the basic principles of SIT. (Figure from Programa Moscamed, Guatemala, reproduced with permission.)

misunderstood. Objections to releasing irradiated insects, which may be thought to be radioactive, must be handled carefully so that the facts are made clear and fears alleviated (Whitten and Mahon, this volume).

The response in Costa Rica to naturalists who objected to the eradication of the New World screwworm *Cochliomyia hominivorax* (Coquerel) was a public relations campaign called "The Ecological Fly", which emphasized the value of using the SIT to help preserve endangered species in the forests.

The public media can have a powerful effect on a programme, and it is important to inform the public about the facts. A World Health Organization (WHO)-supported programme in India, where research on using sterile mosquitoes for control of the vectors of dengue haemorrhagic fever was conducted, had to be terminated by Indian authorities after severe negative publicity, wherein the programme staff was falsely accused by journalists of working on biological warfare. A rebuttal in the international media, to openly defend the programme and clarify the scientific issues, should have been made at that time (Nature 1975), but

this was not done properly, and the true facts were not made public until a clear rebuttal was published later (WHO 1976).

Probably the hardest task for a programme manager, in regards to the public media, is handling criticism, both fair and unfair. Much criticism arises from incorrect information or a lack of information (Klassen, this volume). However, sometimes there are significant issues at stake. On the one hand, it is important to keep public support for a programme strong, and admitting to failure could weaken that support. On the other hand, avoiding a discussion on mistakes or problems could backfire since a discovered "cover up" would erode public support. A useful guideline in this regard is to discuss in simple terms the issues that are of fundamental importance to the programme, but omit those that are of transient importance. Temporary programme setbacks need to be explained within the perspective of the overall goals. In discussions it is not helpful to become too technical; people will be more supportive if they understand the basics than if they are confused or overwhelmed with detail. For example, if people object to regular flights (for sterile insect release) over their homes, the need for these flights should be explained in a simple way. It is important to be positive, sincere, and timely, and to prevent small issues from becoming large issues. It is also vital to describe how the programme impacts on the residents of the community, the benefits people will get from it, and how they can contribute to it, e.g. by controlling pests on their own property.

7. TECHNICAL SUPPORT

It is important to obtain the support of local, national, and international technical experts. This gives a programme technical credibility. Opposition from scientists, for whatever reason, tends to discredit a programme (Winston 1997; DFID 2002; Whitten and Mahon, this volume). Papers in technical journals, and presentations at scientific meetings, are essential for fostering an open and fair exchange of information.

Frequent communication between programme staff and the scientific community is essential to generate "programme ownership" and "goodwill", as well as to interest and involve researchers in improving technology (and thereafter possibly obtain the new technology for use in the programme). There are costs and operational limitations to adopting new technologies frequently, but programme managers must always be open to improve the techniques being used. Therefore a programme (especially a long-term programme) over time inevitably evolves in the technical and operational aspects (Buchinger 1993, Kinney 1993).

A technical unit conducting applied research on various aspects of a programme is a strong support for it, and helps to ensure its success (Lindquist et al. 1992, LaChance 1993, Tween 1993). The New World screwworm rearing facility in Mexico has an in-house research unit (Methods Development Unit) that is constantly testing improved methods of mass-rearing screwworms. The melon fly *Bactrocera cucurbitae* (Coquillett) programme in Japan received very strong support, through basic and applied research, from national institutions, local agricultural experiment stations, and various entomologists in the country; certainly this contributed to the

programme's success (Kakinohana et al. 1993, Yamagishi et al. 1993, Itô et al. 2003, Koyama et al. 2004).

8. PROGRAMME SUCCESS OR FAILURE

Even though adequate internal and external technical support is fundamental to progressively increasing programme efficiency, technical issues are rarely the main reason for failure. However, some AW-IPM programmes that use the SIT have failed or achieved limited success because of inadequate political support and poor public relations.

Many lessons about public relations have been learned, some positive and some negative:

- Political support at various levels is vital for the operation of an AW-IPM programme.
- Communication within a programme, and with the programme's stakeholders, the public, financial supporters, and commercial media, is essential for a programme to receive the support it needs.
- Agricultural leaders are role models in their communities, particularly influential in spreading the adoption of new technologies, and important for promoting the acceptance of AW-IPM programmes.
- Continuing input from technical experts is needed to keep programmes up-to-date with the latest research findings, and maintain technical credibility.
- Even though potentially costly and often not recognized by financial supporters, public relations are as important to a programme's success as is effective management. Failures in public relations definitely have a negative impact on AW-IPM programmes.
- Public relations activities that fail to incorporate local cultural values into the definition of the problem, the message to the public, and the implementation of its solution, probably will be ineffective.
- Hiding facts from the public can result in poor public relations. A lack of open communication, especially when a programme is criticized, can even lead to programme termination.

9. ACKNOWLEDGEMENT

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PART VI

APPLICATION OF THE
STERILE INSECT TECHNIQUE

CHAPTER 6.1.

STRATEGIC OPTIONS IN USING STERILE INSECTS FOR AREA-WIDE INTEGRATED PEST MANAGEMENT

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SUMMARY

The four strategic options, "suppression", "eradication", "containment" and "prevention", in which the sterile insect technique (SIT) can be deployed as part of area-wide integrated pest management (AW-IPM) interventions, are defined and described in relation to the contexts in which they are applied against exotic or naturally occurring major insect pests. Advantages and disadvantages of these strategic options are analysed, and examples of successful programmes provided. Considerations of pest status, biology and distribution affecting decision-making in relation to strategy selection are reviewed and discussed in terms of feasibility assessment, and programme planning and implementation. Unrealistic expectations are often associated with applying the SIT, resulting in high political costs to change a strategy during implementation. The choice of strategy needs to be assessed carefully, and considerable baseline data obtained to prepare for the selected strategy, before embarking on an AW-IPM programme with an SIT component.

1. INTRODUCTION

E. F. Knipling developed a theoretical model of the sterile insect technique (SIT) in the early 1940s (Klassen 2003; Klassen, this volume), but it was not until 1954 that the technique was successfully demonstrated with the elimination of the New World screwworm *Cochliomyia hominivorax* (Coquerel) from the island of Curaçao following sequential releases of sterile insects for 6 months (Baumhover et al. 1955). Since then, in line with Knipling's basic model, the SIT has on numerous occasions been confirmed in field programmes as an effective and very powerful method of insect pest management. In spite of this history of successful SIT applications, the existence of puzzling terminology, conflicting definitions, and inappropriate utilization of concepts/strategies continue to cause confusion.

Baumhover et al. (1955) referred to the strategy applied in the Curaçao experiment as screwworm "control". Both Knipling (1955) and Lindquist (1955) proposed "control" and "eradication" as possible SIT strategies (Lindquist et al. 1990). While Lindquist (1955) used the term "control" to refer to the general use of the SIT, Knipling (1969) defined for the first time "eradication" and "suppression" as the two major strategic options. In his 1979 book, Knipling proposed that the terms "suppression" and "management" could be used interchangeably. During the decade that the screwworm programme in the south-west of the USA was maintaining a "containment" buffer along the Mexican border, he explained that it was erroneously referred to as an "eradication" programme, and argued that, since long-range migrating flies reinvaded the area every year, the term eradication was

misleading in this context when compared with the Curaçao and Florida programmes.

Referring to programmes such as the one against the Mexican fruit fly *Anastrepha ludens* (Loew) in southern California, Knipling (1979) described "prevention" as another possible strategic use of the SIT:

> As more experience is gained in the use of sterile insects for insect suppression and with greater confidence in the value of this technique, releasing sterile insects routinely in certain areas may be more expedient to prevent establishment of major pests than eliminating them after they become established.

Since 1996, this principle has been applied in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) Preventive Release Program in the Los Angeles Basin, California, USA (Dowell et al. 2000) (section 4.1.).

The concept of applying the SIT as part of an area-wide integrated pest management (AW-IPM) programme against key exotic or naturally occurring (FAO 2005) insect pests is gradually gaining acceptance, largely due to a change in the mindset of decision-makers and stakeholders. This is driven mainly by demands of large retailers, in response to consumer requests, for cleaner food and a safer environment, the need for alternatives to the indiscriminative use of insecticides, and the increase in globalization and concomitant international agricultural trade, which requires enforcement, through national animal and plant protection organizations, of the Sanitary and Phytosanitary Standards (SPS) agreement under the World Trade Organization (WTO).

The programmes themselves tend to become grander in scale and scope, thereby increasing their commercial impact and simultaneously reducing their operational costs, mainly due to economies of scale and improvements in the technology of rearing and releasing large numbers of sterile insects (Hendrichs 2000). An example of a very large programme is the Moscamed Mediterranean fruit fly containment effort in Guatemala and southern Mexico, which operates on an annual budget of ca. USD 40 million (data from 2003), including the cost of producing over 2000 million sterile males per week (Tween 2004).

Within such contexts and under these pressures, the authorities deciding on AW-IPM campaigns are facing crucial choices regarding the inclusion of the SIT, and which strategy to apply. In fact, as noted by Klassen (2000), "the terms of reference and contingencies have a tremendous influence on the selection of strategies", which then are "the real decision-makers".

The objectives of this chapter are to review the terminologies, main characteristics, and strengths and weaknesses of the various strategic options where sterile insects can be used, and to propose definitions in accordance with international agreements. In addition, the chapter addresses important species and case-specific issues, e.g. biology of the target insect, buffers, release densities, topography, etc., that require careful reflection before selecting one of the four strategic options under which to integrate the SIT into AW-IPM programmes.

2. FOUR STRATEGIC OPTIONS: DEFINITIONS AND DESCRIPTIONS

The Food and Agriculture Organization of the United Nations (FAO), through its International Plant Protection Convention (IPPC), whose standards are accepted by signatory countries under the SPS agreement of the WTO, defines "control" of a given plant pest (FAO 2005) as encompassing:

> Suppression, containment or eradication of a pest population.

These three strategies would apply to most AW-IPM programmes with an SIT component, including those against insect pests of medical and veterinary importance. However, the most efficient and cost-effective "control" programme is the one that aims at preventing the entry of a pest (movement of a pest into an area where it is not yet present (FAO 2005)) rather than at dedicating resources to suppress, eradicate or contain an introduction (the entry of a pest resulting in its establishment (FAO 2005)) once it has occurred (Knipling 1979). On this basis, four strategic options, i.e. "suppression", "eradication", "containment", and "prevention", will be discussed.

2.1. Suppression

The FAO (2005) defines suppression as:

> The application of phytosanitary measures in an infested area to reduce pest populations.

Thus the main objective of a suppression programme is to maintain the pest population below an agreed and acceptable economic injury level and/or prevalence level. Until the early 1990s, the SIT was generally considered an appropriate technique for eradicating certain insect pests. This view largely resulted from the high visibility of the successful screwworm eradication programme in North and Central America (Baumhover 2002; Klassen and Curtis, this volume), and the assumption that the SIT was too expensive to compete with other control methods for routine pest management. In recent years, however, suppression of an insect pest is increasingly being viewed as a suitable strategy to apply the SIT as part of an AW-IPM approach for some pests of agricultural importance due to: (1) crucial improvements made in the rearing techniques for some key insect species, which have significantly improved the cost-effectiveness of the SIT (Caceres et al. 2004; Parker, this volume), (2) increased restrictions imposed on the use of insecticides (Matteson 1995), (3) increased intermingling of commercial production areas and human settlements which complicates the routine use of insecticides, (4) increased customer demand for "organic" products (Economist 2001), and (5) difficulties in establishing effective quarantine measures to maintain an area pest free (Hendrichs et al. 1994; Cayol et al. 2002).

One major advantage of applying a suppression strategy is the significantly lower investment needed to monitor the pest population as compared with the intensive monitoring required in an eradication campaign (Table 1). Moreover, the set-up and

rigorous implementation of effective quarantines (which demand legislation and considerable investment) to maintain a pest free area (FAO 1999) are not needed or demand less attention and resources when a suppression strategy is applied (Table 2). Another strength of the suppression strategy is the focus on environmental benefits compared with conventional control methods (Table 1). These trends have culminated in the implementation of some suppression programmes using the SIT as an environment-friendly replacement for the use of insecticides (no longer disrupting the biological control of secondary pests) (Hendrichs et al. 1994, Enkerlin et al. 2003). This strategy has gained acceptance mainly for pest insects of phytosanitary importance, since a certain level of "crop damage" to agricultural commodities is usually acceptable. However, this concept is much less applicable to insect pests of veterinary, and particularly medical, importance.

It should be emphasized that implementing a suppression strategy in an area does not preclude exporting agricultural commodities from that area to countries that require a pest free status. Export markets that accept only pest free commodities can be accommodated within the context of a "systems approach" (USDA 1997), whereby an effective preharvest suppression programme with an SIT component can be integrated with other efficient pest risk-mitigation measures (for example, postharvest treatments) to guarantee pest free agricultural products. This strategic approach is applied successfully, e.g. Arava Valley in Israel, where the use of greenhouses, as an additional risk-mitigation measure, has allowed the export of Mediterranean fruit fly-free vegetable commodities from this area to the USA (Cayol et al. 2004). Whereas eradication aims at eliminating the last individual of a population in the target area, a suppression strategy can tolerate a certain residual pest population, and lead to the establishment of an "area of low pest prevalence" (FAO 2004, 2005). Suppression can be achieved much more quickly than eradication, is less complex, demanding, and management intensive, and therefore less expensive in the initial years (Enkerlin and Mumford 1997; Mumford, this volume). However, a suppression strategy requires continuing releases of sterile insects to maintain the low population level (Table 1).

Permanent application of a suppression strategy, including continuing releases of sterile insects, could be considered disadvantageous when compared with the sustainable elimination of a pest from an area. However, this permanent need for sterile insects could stimulate and promote investment in, and the commercialization of, the mass production of sterile insects (Enkerlin and Quinlan 2003) (Table 2). A similar demand has already resulted in a rapidly growing augmentative biological control industry (ANPB 2005, IMBA 2005).

Table 1. Strengths and weaknesses of the four control strategies applied in AW-IPM
programmes integrating the SIT, depending on type of area

Control Strategy				
Suppression (infested area)	**Eradication** (infested area)	**Containment** (part infested and part pest free area)	**Prevention** (pest free area)	**Eradication** (introduction in pest free area)
Strengths				
Decreased pesticide use	Insecticide use eliminated eventually	Protection of neighbouring pest free areas	Proactive rather than reactive approach (risk mitigation)	Establishment of pest precluded (if action taken immediately)
Lower investment in monitoring	Access to "specific pest" free export markets[1]	Environmental and economic benefits for protected area	In case of introduction, effective sterile to wild insect ratios (low wild population density)	Much cheaper in medium and long term than having to "live with" new pest forever
No need for quarantine measures	Activities and costs limited in time	Pest free area can be expanded gradually		Infrastructure and experience gained can be applied against other invasive pests
	Strengthened quarantine infrastructure against other pests	Threat of further pest expansion helps to obtain public and political support	No trade disruption in case of outbreak in release area	
	Strong public and political support for disease vectors		Infrastructure for SIT is in place in case of other outbreaks in the region	Success in avoiding pest establishment promotes preparedness plans for other invasive pests
Weaknesses				
Pre-export treatment	Rigorous quarantine set-up	Cooperation between infested and non-infested areas	Investment while the targeted pest is not present (political aspect)	Species-specific preparedness (plan of action, SIT technology and sterile insect supply, etc.)
Permanent control activities	Intensive public relations campaign	Intensive public relations campaign	Not all areas at high risk of introduction can be protected	Early detection and quarantine to isolate findings
More active participation of grower organizations	Long-term investment in permanent monitoring networks	Disruption of trade and free movement of commodities between infested and non-infested areas	Difficult to quantify cost/benefits of programme and thus susceptible to budget cuts when financial situation becomes difficult	Long-term investment in permanent monitoring networks
	High short-term investment			

[1] Only for agricultural pests, excluding insect pests of medical importance

Table 2. Characteristics of the major control strategies applied in AW-IPM programmes integrating the SIT, depending on type of area

Adjunct Issues	Control Strategy				
	Suppression (infested area)	**Eradication** (infested area)	**Containment** (part infested and part pest free area)	**Prevention** (pest free area)	**Eradication** (introduction in pest free area)
Quarantine	No	Yes	Yes	No	Yes
Investment in monitoring	Low	High	Varies along gradient (Fig. 1)	Medium	High
Trade advantage	Low (but other risk-mitigating measures can complement suppression to guarantee pest free products)	High	Medium	High	High
Demand for sterile insects	Continuous	Short term[1]	Medium term (but can be continuous)	Continuous	Short term
Potential for commerciali-zation of SIT	High	Low[1]	Medium	High	Low

[1] Nevertheless, where the area is very large, requiring a division into many blocks or phases, there could be a long-term demand for sterile insects and increased potential for commercialization

For some key fruit fly and moth pests of major agricultural crops, using sterile insects as part of a suppression strategy has become cost-competitive with conventional or other population reduction methods (Table 3), e.g. Mediterranean fruit fly in Israel and Jordan (Cayol et al. 2004) and in South Africa (Barnes et al. 2004), oriental fruit fly in Thailand (Enkerlin et al. 2003), and codling moth in British Columbia, Canada (Bloem and Bloem 2000), and is under development for a few other species of Diptera and Lepidoptera. These programmes are driven mainly by the need to decrease the use of insecticides to comply with minimum residue levels required for export and/or local markets, or to offer an alternate method of control for pests that have become resistant to the majority of available insecticides, such as the false codling moth and diamondback moth.

Table 3. Examples of AW-IPM programmes applying the SIT according to major control strategies in relation to type of area

Control Strategy				
Suppression (infested area)	**Eradication** (infested area)	**Containment** (part infested and part pest free area)	**Prevention** (pest free area)	**Eradication** (introduction in pest free area)
Carob moth[1]: Tunisia	New World screwworm[2]: North and Central America (1958–2002)	New World screwworm: Panama (2003–present)		New World screwworm: Libya
Codling moth[3]: British Columbia, Canada	Mexican fruit fly[4] and West Indian fruit fly[5]: North-west Mexico		Mexican fruit fly: Baja California and Rio Grande Valley (Mexico-USA border)	Codling moth: Brazil
Diamondback moth[6]: Mauritius	Pink bollworm[7]: Southern USA and northern Mexico (2001–present) (NCCA 2001)	Pink bollworm: San Joaquin Valley in California USA (1969–2000)		Cactus moth[8]: South-eastern USA
Mediterranean fruit fly[9]: Israel/Jordan, Madeira, South Africa, Spain, Tunisia	Mediterranean fruit fly: Argentina (Mendoza, Patagonia) (1992–present), Chile (1992–1995), Mexico (1978–1982)	Mediterranean fruit fly: Guatemala-Mexico (1983–present), Peru-Chile (1996–present)	Mediterranean fruit fly: Los Angeles Basin in California USA (1996-present), Tampa-Miami in Florida USA (1998-present)	Mediterranean fruit fly: Los Angeles Basin in California USA (1980s-1996), Southern Australia
Oriental fruit fly[10]: Thailand	Melon fly[11]: Japan (1982–1994)		Melon fly: Japan (islands near Taiwan) (1995–present)	Painted apple moth[12]: New Zealand
Onion maggot[13]: The Netherlands	Sweetpotato weevil[14] and West Indian sweetpotato weevil[15]: Japan	Queensland fruit fly[16]: South-eastern Australia		Queensland fruit fly: Western Australia
Greenhouse whitefly[17], sweetpotato whitefly[18], and serpentine leafminer[19]: Europe, USA (greenhouses)	Tsetse fly[20]: Unguja Island (Zanzibar)			Old World screwworm[21]: Australia[22]
False codling moth[23]: South Africa				

Table 3. Continued (footnotes)

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[1] *Ectomyelois ceratoniae* (Zeller)
[2] *Cochliomyia hominivorax* (Coquerel)
[3] *Cydia pomonella* (L.)
[4] *Anastrepha ludens* (Loew)
[5] *Anastrepha obliqua* (Macquart)
[6] *Plutella xylostella* (L.)
[7] *Pectinophora gossypiella* (Saunders)
[8] *Cactoblastis cactorum* (Berg)
[9] *Ceratitis capitata* (Wiedemann)
[10] *Bactrocera dorsalis* Hendel
[11] *Bactrocera cucurbitae* (Coquillett)
[12] *Teia anartoides* Walker
[13] *Delia antiqua* (Meigen)
[14] *Cylas formicarius* (F.)
[15] *Euscepes postfasciatus* (Fairmaire)
[16] *Bactrocera tryoni* (Froggatt)
[17] *Trialeurodes vaporariorum* (Westwood)
[18] *Bemisia tabaci* (Gennadius)
[19] *Liriomyza trifolii* (Burgess)
[20] *Glossina austeni* Newstead
[21] *Chrysomya bezziana* (Villeneuve)
[22] Although free of Old World screwworm, Australia maintains a contingency plan against this pest (AFF 2002)
[23] *Cryptophlebia leucotreta* (Meyrick)

2.2. Eradication

Eradication is defined (FAO 2005) as:

> Application of phytosanitary measures to eliminate a pest from an area.

This definition, which is accepted and used by the agricultural community, clearly implies the elimination of a local population of a pest. Nevertheless, for public health (used by the World Health Organization (WHO) mostly for human diseases), the term eradication is restricted to global extinction of a pest at the species level (WHO 2001). Classical examples are the eradication of small pox or the current worldwide efforts to eradicate polio.

An eradication strategy also leads to a reduction in the use of insecticides that is often larger and more long-term (once eradication is eventually achieved) when compared with a suppression strategy, although pre-release population reduction and "hot-spot" treatments may temporarily require increased localized use of insecticides. In addition, eradication allows the establishment of internationally recognized "pest free areas" (Malavasi et al. 1994; FAO 1999, 2005) which can permit access to otherwise closed export markets. These trade advantages are often the major motivation for eradication programmes against insect pests of agricultural importance. The eradication of the Mediterranean fruit fly in Chile (SAG 1996) opened trade opportunities annually worth several hundred million USD, and the

eradication of the Mexican fruit fly and the West Indian fruit fly in north-western Mexico allows fruit trade with the USA without the need for costly postharvest treatments (Reyes 2000; Enkerlin, this volume).

In the past, most AW-IPM programmes integrating the SIT aimed at eventual eradication of the target population, and sterile insects were often released only during the last phase of the programme (section 3). The eradication strategy is applied mainly in the following two situations (Hendrichs 1996):

- Eliminating an established pest population, e.g. the tsetse fly *Glossina austeni* in Unguja Island (Zanzibar) (Vreysen et al. 2000)
- Eliminating outbreaks of an exotic invasive species before full establishment can occur, e.g. the painted apple moth in New Zealand (Suckling 2003) (additional examples in Table 3)

The second situation is likely to increase, with more pest introductions due to globalization, and the growing awareness by governments of the need for monitoring networks for early detection to facilitate eradication. Once the target pest has been eliminated from a given area, it is imperative to maintain this area pest free. This will require (with the exception of the second situation described above, or geographically isolated populations) efficient, permanent, and stringent quarantine procedures to preclude reinvasion.

For eradication, two very important concerns (which have significant economic implications) have to be addressed: (1) the period of time in which releases of sterile insects should continue after the last wild insect has been detected (Vreysen, this volume), and (2) the duration of continued monitoring after releases have stopped, to be able to declare with sufficient confidence the status of eradication (Barclay et al., this volume). Various mathematical models have been developed that, given the biology of the pest species, and the efficiency and density of the deployment of monitoring tools, determine the time required to ascertain, within certain confidence limits, the probability that the target population has been eliminated. These models, which are important decision-making tools, have in practice unfortunately seldom been used.

The decision to establish a pest free area is often questioned in view of the permanent threat of reinvasion, which would result in a loss of the investment. The likelihood of reinvasion of a pest insect is related to its biology, its geographic distribution, the efficiency of the quarantine infrastructure, and the location and size of the pest free area. Therefore the selection of an eradication strategy will also be influenced by these parameters. The reinvasion potential of a species in a given area is also inherently linked to the economics of the various strategic options, which will therefore significantly influence the decision to select a suppression or eradication strategy. "Sequential eradication" in a target area can still be economically beneficial, notwithstanding periodic reinvasion, if the average period between reinvasions is long enough so that the economic and environmental benefits of each pest free period between reinvasions exceed the costs of having to "live with" the pest and the effects of continuous control.

J. Mumford (personal communication) proposed a concept of "serial eradication", which entails the elimination of a pest population in a certain target

area without heavy investment to prove the "status of eradication". If the area is reinvaded, the eradication effort is simply repeated. The critical issue here is "not demonstrating the pest free status", as this could save considerable sums of money. In situations where there is a high probability of reinvasion, the pest free status would often be in doubt, e.g. the day after it was proved, a new introduction might occur. However, with a "near zero" or "low prevalence" population, which requires less funding to assess the status as compared with an absolutely zero population, growers could relax local treatments and monitoring, and thus manage both quality and residues effectively and inexpensively. To obtain the trade advantages, the concept of serial eradication would entail a "systems approach" (USDA 1997), which includes a combination of low-cost monitoring, postharvest treatments, and occasional intensive area-wide control actions.

The decision to adopt a strategy of permanent suppression or periodic eradication is, however, based not only on economics and trade. The affordability of "living with" a pest population, even when suppressed, also needs to be considered. Eradication of major vectors of human or livestock diseases is often the preferred option, since a low density of the pest population does not necessarily lead to low levels of disease transmission (Otieno et al. 1990, Feldmann and Hendrichs 2001).

2.3. Containment

Containment is defined (FAO 2005) as:

> Application of phytosanitary measures in and around an infested area to prevent spread of a pest.

Containment programmes are adopted to avoid the spread of invading exotic pests that have become established, or to consolidate progress made in an ongoing eradication programme. An example of the first case is the Queensland fruit fly Tri-State Fruit Fly programme, which has operated since 1988 in eastern Australia to protect an area that contains much of the horticultural production areas of southern New South Wales, northern Victoria, and eastern South Australia (Jessup et al. 2004). Other examples are given in Table 3. Some of these programmes are stationary and thus become permanent containment efforts, whereas others successfully advance or gradually retreat and eventually collapse.

A typical containment situation is illustrated spatially or geographically in Fig. 1, showing a gradient in the density of the pest population from an infested area (NAPPO 2004) on the left to a pest free area on the right. In between there is a relatively steep population decline across the various operational areas where the pest is being contained. Various wild population reduction tools have to be integrated in areas where pest levels are too high for sufficient numbers of sterile insects to be released, whereas the SIT is increasingly implemented over lower population levels, mainly low pest prevalence areas with remaining pest remnants or incursions (an isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future (FAO 2005)). Sterile insects are particularly effective in adjacent areas that already are largely pest free,

but that are subject to regular pest entries (FAO 2005). Ideally, sterile insects are also released as insurance in a buffer zone, over parts of the contiguous pest free areas, in view that the pest may occasionally be moved by the transport of infested host material. The degree of population reduction, sterile insect release, and monitoring efforts along this gradient are also indicated in Fig. 1, reflecting requirements to maximize their effectiveness. For example, to be able to detect as early as possible any entry or incursion, monitoring activities have to be the highest in the buffer zones and adjacent area (FAO 2005).

Figure 1. Schematic representation of containment (and also rolling-carpet approach to eradication in large blocks, section 7.1.), illustrating geographically the gradient in pest population density, operational phases, and degree of activities in the different types of areas.

2.4. Prevention

The prevention strategy is defined here as the "application of phytosanitary measures in and/or around a pest free area to avoid the introduction of a pest" (however, it applies also to the preventive application of sanitary measures against animal pests). Prevention has been applied where the invasion pressure is very high, and quarantine activities are not sufficient to maintain the area pest free (Table 3). An example is the permanent release of sterile melon flies over the Japanese islands closest to Taiwan (Kuba et al. 1996). In situations where the invasion risk is not very high, sequential or serial eradication approaches are probably more viable economically (section 2.2.).

For agricultural trade, the strategic options of containment and prevention are designed to maintain market accessibility, although the prevention option is most desirable in terms of cost, as it is always cheaper to prevent a pest problem than to deal with it later. (Enkerlin (this volume) provides a more detailed explanation of the costs of containment and prevention.)

3. TEMPORAL SCENARIO FOR AREA-WIDE IPM PROGRAMMES INTEGRATING SIT

Irrespective of the strategic goal, AW-IPM campaigns that include the SIT will likely have similar basic elements or distinct temporal phases:

- Pre-intervention phase. The collection of baseline data on the distribution, dispersal, and population dynamics of the target species (Itô and Yamamura, this volume; Vreysen, this volume), the development of basic human and physical infrastructure (including the mass-rearing and sterilization facility, packing and emergence centres, and quarantine infrastructure), and the launching of a public relations campaign (Dyck, Regidor Fernández et al., this volume).

- Population reduction phase. Control measures are applied, with varying intensity depending on the strategy and seasonal variation in pest population levels, to reduce the density of the target population prior to the release of sterile insects. Various population reduction methods are available, depending on the target insect, which typically involve the application of non-residual insecticides, "lure and kill" devices, male annihilation, fruit stripping, wound treatment, cultural controls, etc. (Mangan, this volume). However, these are not always required, e.g. when the pest emerges in low numbers after a winter, or when the population undergoes a natural decline as a result of climatic conditions.

- Release phase. The sequential release of sterile insects over the target area to reduce the target population to a pre-determined level (suppression strategy), to drive the target population to extinction (eradication strategy), or to avoid pest establishment (containment and prevention strategies). This phase can in some instances overlap with the reduction phase, e.g. insecticide treatment of wounds for population reduction in screwworm eradication campaigns is carried out simultaneously with the release of sterile insects.

- Maintenance phase. To sustain the low prevalence status (suppression strategy) or the pest free status (containment and prevention strategies) through the permanent implementation of release activities, or

- Verification phase. To confirm and preserve the pest free status (containment and eradication strategies) through permanent implementation of monitoring and quarantine activities (Barclay et al., this volume).

4. STRATEGIC CONSIDERATIONS IN RELATION TO PEST STATUS AND TARGET MARKETS

The selection of a control strategy is significantly influenced by two main factors of economic importance: the status of the pest in the target area, and the target market for the produced crop or livestock commodities.

4.1. Area and Pest Status

Pest insect status depends not only on its biology and whether the insect is naturally occurring or exotic (FAO 2005), but also on the specific situation and legislation of the importing and exporting contracting parties; thus, a pest can be non-regulated or regulated (both quarantine and regulated non-quarantine pests (FAO 2005)). The pest status, together with the infested or pest free status of a given area (Tables 1, 2 and 3), are the primary factors (Klassen's "decision-makers") that will influence the type of strategy(ies) that can be developed and implemented:

- An area is considered "pest free" when the insect pest is not present in that area (FAO 2005). If there are frequent entries (FAO 2005) of a major exotic pest, and the area is endangered (area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss, FAO 2005), the most economical strategy to protect the area from any potential introductions will often be preventive releases of sterile insects, a successful example being the Preventive Release Programme in California (Dowell et al. 2000; Enkerlin, this volume). About 300 million sterile male Mediterranean fruit flies are released per week in the Los Angeles Basin, a "high-risk" area for introductions of this pest because of favourable ecological factors, the large amount of trade, large number of fruits imported by tourists, substantial volume of postal shipments, and high traffic in contraband fresh commodities.

- A pest free area, contiguous to an infested area, can be protected by a containment strategy, e.g. the release of sterile pink bollworms to prevent the spread of this pest from infested areas into the cotton production areas of the San Joaquin Valley in California, USA (Henneberry 1994). The threat of entry and introduction can be reduced further by supporting actions of "offshore pest risk-mitigation" in places where the pest originates (i.e. neighbouring countries or regions), e.g. release of sterile Mediterranean fruit flies along the border between Chile and Peru to avoid reinvasion of this pest from Peru into Chile (Enkerlin et al. 2003). Another example is the USA's support of the Moscamed programme along the Guatemala/Mexico border.

- An area is considered here as infested (NAPPO 2004) when the pest is naturally occurring (FAO 2005) or exotic pest establishment (perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2005)) has taken place and the pest has spread (expansion of the geographical distribution of a pest within an area (FAO 2005)). A suppression or eradication strategy can be applied under these circumstances, e.g. a suppression programme against the Mediterranean fruit fly in Valencia, Spain (Primo 2003), and eradication of the

same pest in temperate fruit-production areas in Mendoza and Patagonia, Argentina (De Longo 2000).

- When establishment of an exotic insect pest has taken place very recently, and the outbreak (a recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area (FAO 2005)) has not yet spread significantly out of the outbreak area, the preferred strategy is eradication, e.g. elimination of the New World screwworm in Libya (Lindquist et al. 1992; Krafsur and Lindquist 1996; Vargas-Terán et al., this volume). Containment is the second-best strategic option, but only when immediate eradication has failed.

Situations of multiple presence of major pest species in one area necessarily complicate all these strategic considerations, although there are a number of suppression or eradication programmes where more than species have been simultaneously addressed. Examples are the eradication of both the Mexican and the West Indian fruit flies from north-west Mexico (Reyes et al. 2000), or the elimination of three tsetse populations (*Glossina p. gambiensis*, *G. tachinoides* and *G. m. submorsitans*) from 3500 km^2 in Burkina Faso (Politzar and Cuisance 1984, Clair et al. 1990).

4.2. Market Requirements

The type of market targeted for the agricultural commodity produced plays a major role in selecting a control strategy. There are four major potential markets that can be accessed (Table 4):

- Domestic markets are normally the least demanding, but also the least lucrative, and a suppression strategy using conventional methods is normally sufficient. Only when there are important local markets having special requirements in terms of pesticide residues is the use of more environment-friendly pest control methods warranted.

- Non-discriminating export markets often also have no special requirements, and thus are similar to domestic markets. Increasingly, as a result of globalization, these markets tend to decrease in importance, whereas export markets demanding low pesticide and/or pest commodities are growing.

- Low residue export markets are typically in developed countries that have demanding supermarket chains (in terms of maximum allowed residue levels) and a growing demand for organic products. They either already have the pest or have climates in which the pest cannot become established. A suppression strategy involving application of more environment-friendly methods is suitable to gain access to these markets.

- Pest free export markets are the most demanding, with zero tolerance for the presence of the pest in the export commodities. Accessing these markets requires an eradication strategy to establish certified pest free areas. Alternatively, a suppression strategy can be followed to establish a low prevalence area, but this strategy demands in addition a systems approach involving integration with complementary pest risk-mitigation measures such as, in some instances,

postharvest treatments to guarantee pest free commodities. Alternatively, in situations where the export areas are already partially or fully pest free, the logical approaches to protect or maintain these markets are containment or prevention strategies.

Market access is, of course, not relevant for areas infested with major vectors of animal or human health diseases. Here, an eradication strategy is the norm since even low pest populations can result in significant disease transmission.

Table 4. Access to potential markets for available control strategies

Control Strategy	Potential Markets			
	Domestic markets	Non-discriminating export markets	Low residue export markets	Pest free export markets
Suppression (infested area)				
Conventional control	Yes	Yes	No	No
Low pesticide including SIT	Yes	Yes	Yes	No
Low pest prevalence and systems approach	Yes	Yes	Yes	Yes[1]
Eradication (pest free area developed)	Yes[2]	Yes[2]	Yes[2]	Yes
Containment (pest free area protected)	Yes[2]	Yes[2]	Yes[2]	Yes
Prevention (pest free area maintained)	Yes[2]	Yes[2]	Yes[2]	Yes

[1] Only when combined with complementary measures, such as post-harvest treatments, except for marginal hosts of the pest

[2] Development, protection or maintenance of pest free areas, involving quarantines and intense monitoring, is usually not cost-effective for these markets unless they target mainly pest free export markets

5. STRATEGIC CONSIDERATIONS IN RELATION TO PEST BIOLOGY AND ECOLOGY

There are several factors related to the biology and ecology of the pest that influence the selection of a control strategy, and affect both its planning and implementation, e.g. pest mobility, pest densities, average lifespan, potential of direct and indirect damage, and ecological aspects such as topography, vegetation, and climate, as well as the capacity to monitor pest presence.

5.1. Pest Dispersal

One of the most critical aspects is the degree of isolation of the target population and, associated with it, the dispersal ability of the target insect. Knowledge about dispersal ability is essential to assess the likely invasion pressure, which remains an important consideration in selecting and developing a strategy. Highly dispersive (sterile) insects are advantageous for programmes that release sterile insects, as this trait will increase their chances of finding and mating with virgin wild female insects. However, at the same time, the greater the dispersal of the wild insect, the more difficult it will be to protect pest-cleared areas (Lance and McInnis, this volume).

Mark-release-recapture studies, using both wild and sterile insects, can be used to study dispersal, but this approach, although valid, can be expensive and cumbersome (Itô and Yamamura, this volume). An alternative is the analysis of the gene frequencies of target and neighbouring populations as an indirect approach to dispersal (Krafsur, this volume). Dispersal ability is a decisive component in strategy selection, in assessing the minimum size of an intervention block where the SIT can be effectively applied (section 8; Klassen, this volume), and in determining the need for temporary and permanent buffer zones (section 9).

5.2. Release Density

Itô and Yamamura (this volume) provide detailed mathematical guidance on estimating absolute densities of insect populations under different situations and for pests with different biology. An estimate of the absolute population density of the pest in the target area is key to determining the need and extent of population reduction before the application of the SIT, and to make accurate estimates of the required number of sterile insects needed. The latter will obviously influence the total cost of the programme, and will represent a major consideration when selecting a strategy (Mumford, this volume).

The cost of the SIT component of AW-IPM programmes has traditionally been calculated using data from the production of sterile insects, and the cost associated with handling and release operations. Even though very large variations in the production and release costs of different insect pests exist, i.e. from only USD 0.25 to produce 1000 Mediterranean fruit flies to USD 85 to produce the same number of tsetse flies (Table 5), the ultimate cost of an SIT operation will also be determined by the number of sterile male insects required per unit of surface, and the time

needed to obtain adequate sterile to wild male overflooding ratios. A comparison of costs per surface and time unit for different pest species is presented in Table 5. For example, following application of population reduction measures, only 15 sterile male *Glossina palpalis gambiensis* Vanderplank (riverine species of tsetse) are required per km^2 per week, whereas for the Mediterranean fruit fly, ca. 200 000 sterile males per km^2 per week are needed. Consequently, although the production and release costs of Mediterranean fruit fly are very low and for tsetse very high, the costs per km^2 per week of the SIT operation are much higher for Mediterranean fruit flies (USD 80) than for riverine species of tsetse (USD 2.7). Therefore, considering the cost of the sterile fly requirements per surface and time unit provides a much more accurate indicator of the economic implications of SIT operations.

5.3. Pest Aggregation

Aside from the absolute population density, the degree of population aggregation or dispersion is important. Sterile insects are usually released by aircraft, and are thus distributed fairly homogeneously over the target area, irrespective of whether the target pest is distributed evenly or clumped. Pest insects with a clumped distribution require higher release rates (Barclay, this volume) as compared with a homogeneous

Table 5. Comparison of costs of AW-IPM programmes that release sterile insects (data for codling moth calculated from information provided by R. Fugger and L. Tomlin, Sterile Insect Release Program, Canada and Bloem et al. 1998)

Pest species	Cost of producing sterile male insects (USD per 1000 insects)	Cost of handling and releasing sterile males (USD per 1000 insects)	Total cost (production and release) (USD per 1000 insects)	Release rate (number of sterile males per km^2 per week)	Cost of sterile male production and release (USD per km^2 per year)
Codling moth[1]	1.9	0.65	2.55	100 000	5167
Mediterranean fruit fly	0.25	0.15	0.4	200 000	4160
Glossina austeni (savannah tsetse species)	85.0	33.0	118.0	80	491
New World screwworm	1.0	0.4	1.4	2500	182
Glossina p. gambiensis (riverine tsetse species)	85.0	96.0	181.0	15	141

[1] Data for codling moth based on releases for only 20 weeks per year

distribution, to obtain the required sterile to wild male ratios (Vreysen, this volume), and thus pest aggregation also affects strategy selection and its cost. Only if the released insects can find the same aggregation sites and aggregate in a similar manner as wild insects, so that adequate sterile to wild male overflooding ratios are obtained in those sites, is there no need to increase release rates to compensate for such clumping.

5.4. Sterile Male Longevity

The density of the sterile male population in the field, which fluctuates in relation to the release frequency and the sterile male mortality rate, should not decrease below that needed to maintain the critical ratio (Fig. 2, upper graph) (Barclay, this volume; Kean et al. 2005). Therefore, the frequency and number of sterile males released has to be carefully assessed in relation to the average longevity or survival of the sterile males, to effectively avoid periods when insufficient sterile males are present in the field (Fig. 2, lower graph).

As generations normally overlap in multivoltine species, releases have to be continuous, with survival determining whether they have to occur on a weekly (New World screwworm), twice a week (Mediterranean fruit fly, tsetse), or even daily basis (pink bollworm). The importance of assessing the survival of sterile male insects in the natural habitat must be emphasized here (Vreysen, this volume), as their actual survival in open field conditions is often drastically lower than in protected field-cage situations, where sterile males have easy access to food and are protected from predation (Hendrichs et al. 1993). In addition, mass-rearing conditions often inadvertently select for short-lived individuals (Cayol 2000). A shorter sterile male lifespan, although not directly representative of competitiveness, often requires higher release frequencies, and thus can significantly increase programme costs compared with longer-lived sterile insects.

5.5. Topography and Other Conditions of Target Area

The topography of the target area, combined with the density of roads, has major implications for programme implementation and the selection of an intervention strategy. A flat terrain and a good road network will facilitate most field activities (including ground release in some cases), whereas mountainous areas, dense vegetation, and the absence of roads will complicate implementation. In most of the larger programmes, releases and some of the population reduction activities use aircraft (usually with fixed wing), and the topography and presence/absence of a road network are less critical. Monitoring, however, is mostly ground-based, and extreme terrain conditions make eradication campaigns (which have a more intensive monitoring component) much more complex and costly than programmes following a suppression strategy (which have less intensive monitoring activities). Conversely, the absence of a good road network is advantageous for the establishment of efficient quarantine procedures in support of an eradication strategy. Likewise, topography
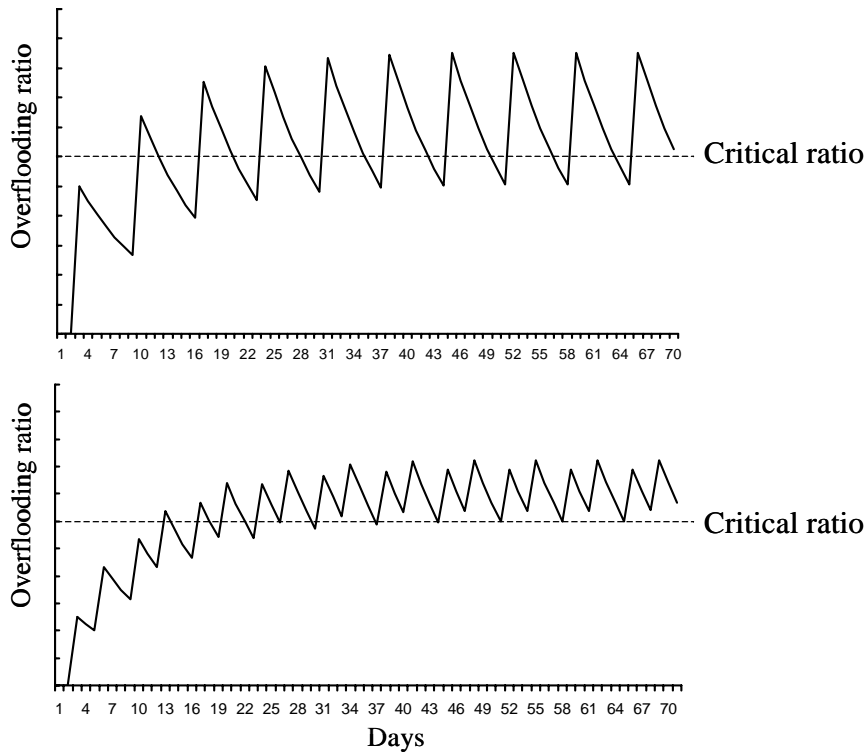
*Figure 2. Effect of sterile insect longevity (assume daily mortality rate of sterile males is 0.1)
on sterile to wild overflooding ratio. Upper: Due to only weekly releases, sterile insect
population routinely decreases significantly below the critical overflooding ratio; Lower:
twice-a-week releases overcome this problem.*

influences the requirements of sterile insects or bait sprays, e.g. mountainous areas
have a larger surface area per square kilometre as compared with two-dimensional
conditions, demanding higher sterile insect release rates. Furthermore, helicopters,
which are more expensive to operate than fixed-wing aircraft, are often needed in
difficult terrain for safety reasons and to properly treat narrow valleys.

5.6. Capacity of Monitoring and Delimiting Pest Presence

The distribution of the target insect has to be accurately known; direct and indirect
monitoring methods are usually available to assess this for the different target
species. Each monitoring tool, however, has its limitations, and none is perfect.
Knowledge about the efficiency and limitations of the monitoring methods is
essential to assess the accuracy of, and the ability to demarcate, the total distribution
of the target population (Vreysen, this volume).

The different phases of programme implementation require specific adjustments to the monitoring system according to the pest density (Fig. 1). The intensity of monitoring tools, or of indirect surveys, will gradually change from the initial relatively low monitoring effort in the baseline data collection phase to increased activity in the initial population reduction, low-prevalence, and eradication phases (highest need for detection capability), and will decrease again during the maintenance/verification phases (IAEA 2003).

For each phase, the density of monitoring tools will also vary according to their efficiency. For eradication, very efficient monitoring tools are particularly important once populations have decreased to very low densities; the same is true for population remnants or entries and incipient incursions (Fig. 1). In pest free areas, monitoring efficiency is important also for early detection of, and delimiting, the population of a new introduction. Inefficient monitoring tools to assess the status of eradication, or for delimiting purposes, will increase the cost of programmes — high deployment densities are required to compensate for the lower efficiency, and longer deployment times to obtain the required confidence limits (Barclay et al., this volume).

6. STRATEGIC CONSIDERATIONS IN RELATION TO PEST DISTRIBUTION

The characteristics of the spatial distribution pattern (dispersion) of the target population will be a crucial factor when selecting a strategy and developing an implementation plan. An insect population can be confined to an island, or be distributed in fragments or continuously on a continent.

6.1. Island Distribution

Targeting a pest population confined to an island is the ideal and simplest situation for which to develop an AW-IPM programme, e.g. New World screwworm on various islands in the Caribbean, *Anopheles arabiensis* Patton on Reunion, sweetpotato weevils on islands of the Okinawa archipelago (Kohama et al. 2003), etc. Islands can be relatively small, making area-wide intervention easier, and, if the size is not too large, the insect population can be tackled at one time. Typically, insect populations on islands are isolated, with the ocean being an ideal natural barrier. Sustaining such areas pest free after eradication is usually only a minor concern provided adequate quarantine procedures are developed and implemented. However, migration over considerable distances has been reported for species such as the melon fly (Koyama et al. 2004), where movement between Taiwan and islands in southern Japan has been recorded routinely.

6.2. Fragmented Distribution

Insect populations on continents are fortunately not always distributed continuously, but often appear to be fragmented and confined to "population islands". Examples

are the carob moth in date palm oases in the Mediterranean Basin and the Near East, the mosquito *An. arabiensis* confined to isolated mosquito habitat along the Nile River in Sudan, and various tsetse species in valley systems in the south-western part of Ethiopia. Although the distribution of insect populations is sometimes perceived as vast and continuous, topographical features (e.g. mountains, lakes, cities), and factors such as host availability, climate, and vegetation, often result in the division of populations into smaller population units. This subdivision occurs more easily at the edge of the distribution, where the insects are confined to those areas with favourable conditions. Field surveys assisted by a GIS-based analysis (Cox and Vreysen, this volume), supported by population genetics studies (Krafsur, this volume), are needed to determine the degree of reproductive isolation between such smaller population units .

Detailed data on the distribution of the target population are often not available when needed, and satellite-derived parameters are used increasingly to develop models to predict the probability of presence or even abundance of a certain pest in a given target area (Cox and Vreysen, this volume). These prediction models can greatly facilitate the demarcation of the pest's distribution, and provide the foundation for developing an adequate intervention strategy. For example the fragmented nature of the distributions of *Glossina austeni* and *Glossina brevipalpis* Newstead in South Africa was confirmed through field monitoring and fine-tuned by developing such a distribution prediction model (Fig. 3).

Insect populations are rarely stable in space and time, and temporal changes in their dispersal potential can significantly affect the isolated nature of seemingly distant population pockets. Seasonal changes in vegetation cover can be particularly dramatic in subtropical and tropical environments, with important consequences for insect dispersal, e.g. populations can be confined to "vegetation islands" during the dry season, when conditions for survival are harsh, but during the rainy season dispersal potential increases, spatial distribution expands, and larger distribution areas are created.

6.3. Continuous Distribution

The most complex scenario is when insects are distributed continuously over very large areas. For both suppression and eradication strategies, a vast distribution of the pest will make it technically, logistically and economically impossible to tackle the entire infested area at one time. Consequently, the AW-IPM programme has to be developed and implemented in different phases, and the target area has to be subdivided into "intervention blocks". The appropriate block size will depend on the sterile insect production capacity and the biology of the particular species (section 8), but as much as possible it has to preclude migration of mated fertile females into the core area. Determining the exact size and precise demarcation of the blocks is a very challenging task, as it could determine success or failure. Topographical and ecological features (e.g. major cities, mountain ranges, rivers, non-host areas, and open areas of unsuitable habitat), which constitute natural barriers for the target insect, should separate to the maximum possible extent two adjacent intervention
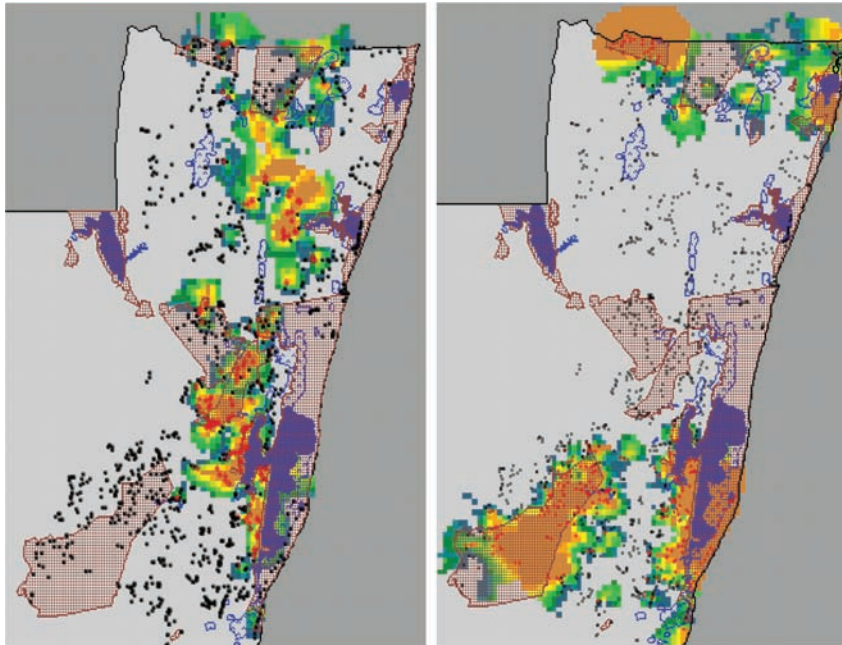
Figure 3. Presence-absence prediction model using the geostatistical kriging method, indicating the probability of presence of the tsetse species Glossina austeni *(left) and* Glossina brevipalpis *(right) in KwaZulu Natal in South Africa. (Shaded polygons: conservation areas; Blue areas: lakes and dams; Blue contours: major marshes; Blue – Green – Yellow – Orange – Brown: low to high probability of occurrence; Black spots: negative trap catches; Grey in the east: Indian Ocean; Grey in the north: Mozambique.) (Map adapted from AVIA-GIS 2002.)*

blocks. As a principle, to reduce the probability of migration, the common border between adjacent blocks should be as limited as possible, and the size and number of temporary buffer zones between the blocks should also be limited.

7. STRATEGIES FOR CONTINUOUS DISTRIBUTION

For target populations that are distributed continuously, the "rolling-carpet principle" or the "wave principle" can be used to plan and implement AW-IPM programmes. Intervention according to the rolling-carpet principle entails a unidirectional front (Figs. 1, 4), whereas this front is bidirectional or multidirectional when using the wave principle (Fig. 5). The different blocks have to be demarcated and selected in such a way as to minimize the invasion pressure from various directions. Multiple fronts complicate the programme, and necessitate the establishment of temporary buffer zones, which obviously will reduce the probability of success. In both approaches, it is of prime importance that the starting point of operations be selected

carefully. Once the programme has started, it must be continued, and cannot be interrupted until a pest free area (eradication strategy), or the maintenance phase to sustain an area of low pest prevalence (suppression strategy), has been obtained in all blocks.

7.1. Rolling-Carpet Principle
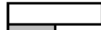
The rolling-carpet principle is dynamic. The basic operational phases described in section 3 (pre-intervention, population reduction, releases of sterile insects, and maintenance/verification of low prevalence/pest free areas) are carried out simultaneously in a phased manner (Fig. 4). Obviously this approach is more cost-efficient than a static approach, in which each of the four different phases would be implemented in a given block before proceeding to the next block.

The diagram in the lower part of Fig. 4 shows the implementation of the rolling-carpet principle in space, i.e. the programme is initiated with the collection of baseline data and capacity building in block 1 (phase 1), and these activities are progressively shifted to adjacent blocks. Simultaneously, reduction of the population, release of sterile insects, and maintenance/verification of low prevalence/pest free areas will be carried out in those blocks where the previous activities have been completed. The phased approach will entail a gradual increase in operational activities (and operational complexity) during the first four phases, with expanding needs for funding, personnel, and logistics.

As the duration of each operational phase can be different, the different phases of the rolling-carpet principle should be considered not only in space but also in time (upper part of Fig. 4), i.e. baseline data collection (phase 1) usually has to take into account temporal changes in the density, distribution, and structure of insect populations, and often takes at least one full year to complete, whereas reduction (phase 2) of the target population is often implemented in shorter periods.

To achieve the desired population reduction, sterile insects must be released for a period of several generation times (Knipling 1979). The duration of sterile insect releases to obtain a pest free area (eradication strategy), or to establish an area of low pest prevalence (suppression strategy), will depend on the reproductive rate and the generation time of the target species. For species such as tsetse flies, which have a long lifespan and long generation times, the release phase can take up to 18 months. Theoretically, this will be less for insects that have shorter generation times, like mosquitoes or fruit flies, but their high rates of reproduction can be a complicating factor.

Legend   No activities

Assumptions: Duration of the baseline data collection = 1 year
               Duration of population reduction = 0.5 year
               Duration of releases = 1.5 years
               Verification phase (eradication) = 2 years
               Maintenance phase (suppression) = indefinite

Overlap of releases in adjacent blocks

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
Block 1								
Block 2								
Block 3								
Block 4								

No overlap of releases in adjacent blocks

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
Block 1								
Block 2								
Block 3								
Block 4								

	Pre-control	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6	Phase 7
Block 1								
Block 2								
Block 3								
Block 4								
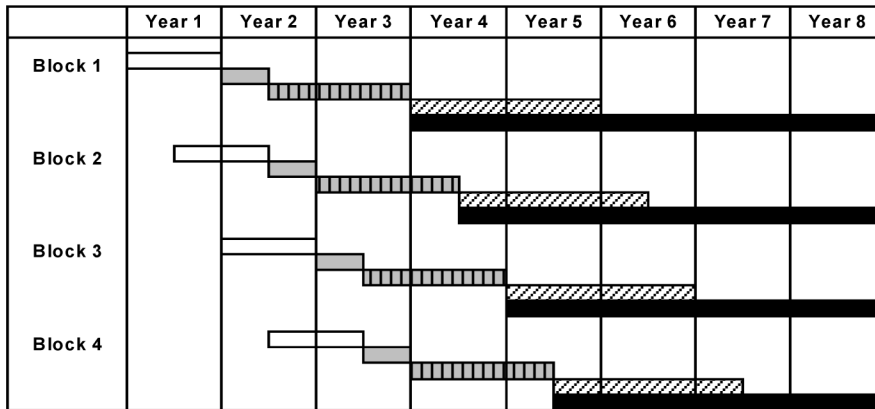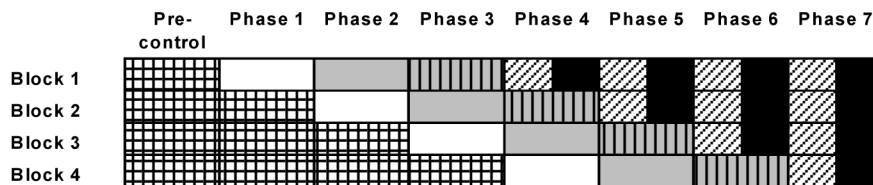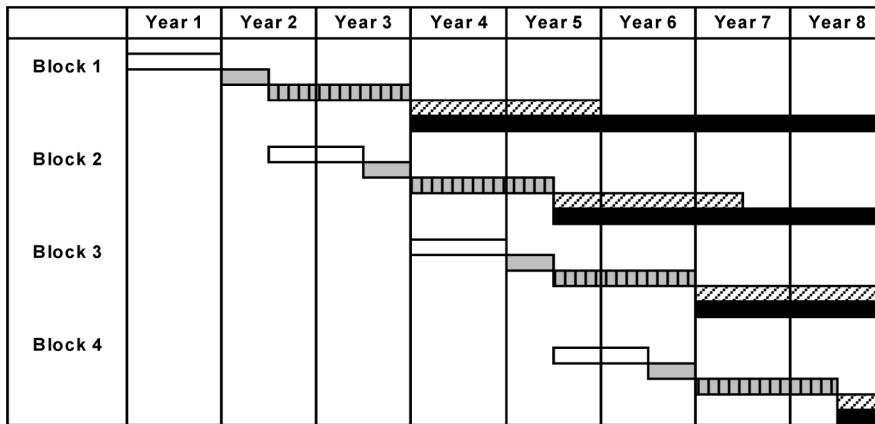
Figure 4. Temporal (upper, with or without overlap of releases in adjacent blocks) and spatial (lower) diagrams of the rolling-carpet principle applied in four intervention blocks using eradication and suppression against a pest population distributed continuously.
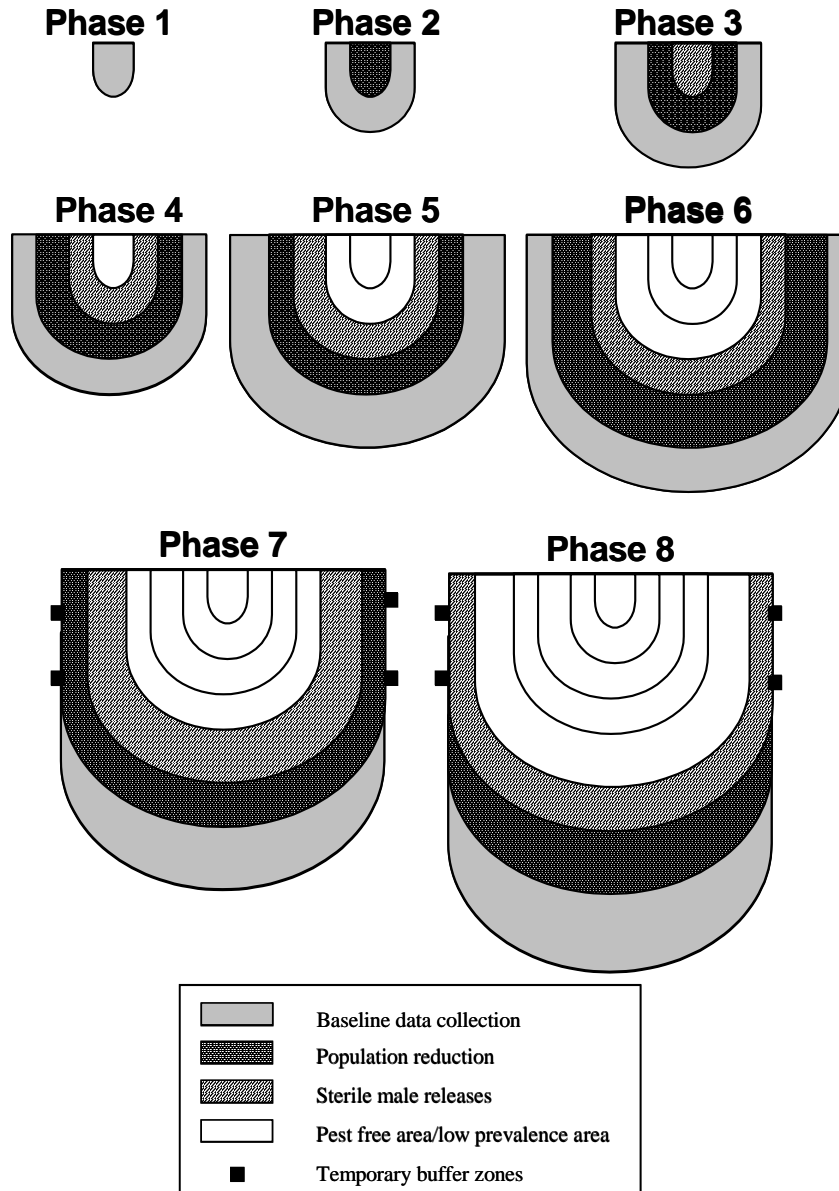
Figure 5. Diagram of different phases of an AW-IPM programme using the SIT according to the wave principle against a pest population with a continuous distribution. In this theoretical example, the intervention starts at the edge of the pest distribution and develops along a multidirectional front in the first 6 phases, until full production capacity of sterile males is reached. Beginning in phase 7, the intervention continues along a one-directional front, and requires the establishment of temporary buffer zones.

The exact timing and initiation of each activity will be crucial for efficient implementation of the programme, e.g. a too-early start of the population reduction phase could result in a reduction of the target population before sterile insects are available for release. Therefore, the availability of sufficient sterile males should be taken as the "baseline", and all other activities should be scheduled in relation to the releases. These, in turn, depend largely on the production capacity of the rearing facility. The overlap of releases in certain blocks (upper part of Fig. 4) has important implications for the cost of the programme, the time to reach programme goals, and the need to establish protective buffer zones (section 9). For example, in the hypothetical case with an overlap of releases for 12 months in two adjacent blocks (and even an overlap of 6 months in three blocks), the maintenance/verification period in the last low prevalence or pest free block can be initiated in the third quarter of year 5, whereas if there is no overlap of releases in adjacent blocks, this is delayed until the third quarter of year 8. In addition to the cost and timing of implementation, the first example offers a much more effective operation when compared with the second example, since the insect population in the block adjacent to the release area is always under active population reduction to minimize the dispersal of gravid females into the release area (Fig. 1). On the other hand, in the second example the population reduction activities in block 2 are only initiated 12 months after the initiation of releases in block 1, requiring the establishment of temporary buffer zones to prevent the migration of females from the still "untreated" block 2. It is equally important that the contiguous part of the area that is already pest free be treated preventively with sterile insects as a biological buffer zone (Fig. 1), an ultimate insurance that individual entries or incipient incursions cannot become permanently established in the free area.

7.2. Wave Principle

The wave principle entails an expanding operational block size with each phase (Fig. 5), and this must be considered when designing a production facility. For example, if an intervention were initiated in a 10 x 10 km (100 km^2) area, and in each phase expanded by 10 km to the east, west, and south, the total size of the intervention zone would expand from 100 km^2 in phase 1 to 6600 km^2 in phase 6 (Fig. 6).

In an eradication strategy, the release area (shaded area in Fig. 6) would expand from 100 km^2 in phase 1 to 500 and 2100 km^2 in phases 2 and 6, respectively. If the release density of sterile males were 200 000 per km^2, the sterile male requirements in each phase would increase linearly by 80 million insects, i.e. from 20 million sterile insects in phase 1 to 420 million in phase 6.

In a suppression strategy, however, the sterile insect requirements would be different. If sterile males were released at a density of 200 000 per km^2 during the release phase (shaded area), and at a density of 50 000 per km^2 during the maintenance phase of low prevalence areas (white area), the sterile insect requirements would increase more dramatically with each phase when compared with an eradication strategy, and are best described by the following equation:
$y = 2E + 0.7x^{1.9119}$ (Fig. 6).
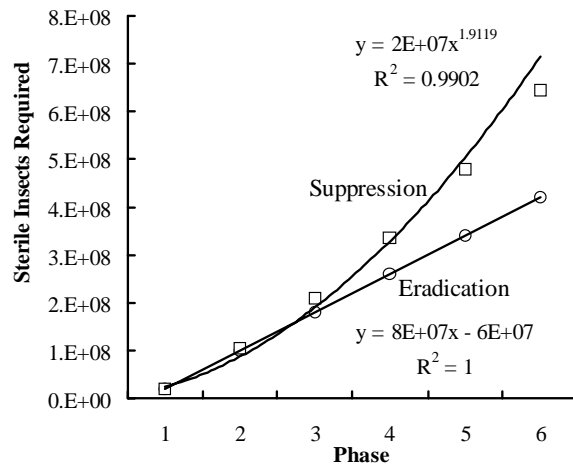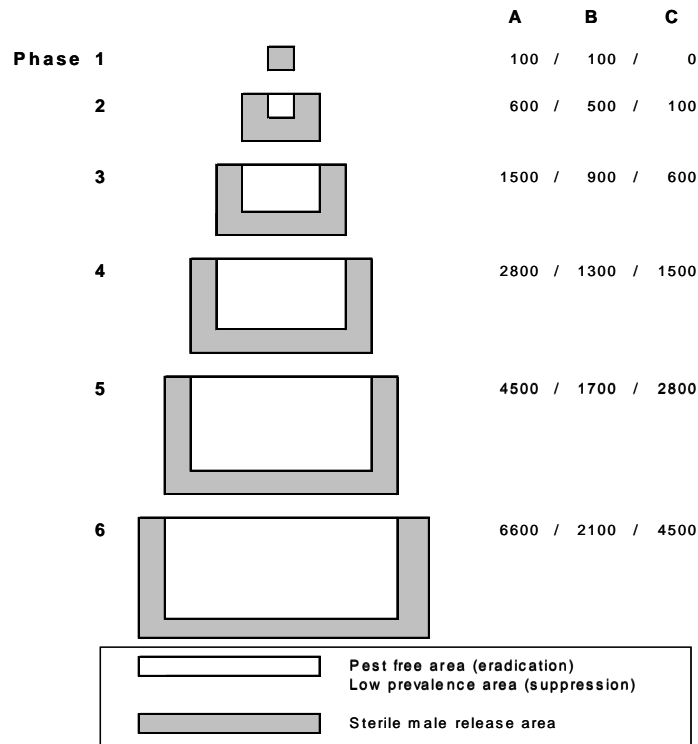
*Figure 6. Diagram of the wave principle (upper drawing) and sterile male requirements (lower graph), with an expanding intervention front of 10 km south, east, and west, and each phase using eradication and suppression. Numbers indicate: (**A**) total surface area in km^2 / (**B**) area in km^2 where sterile males are released / (**C**) pest free area in km^2 (eradication) or low pest prevalence area (suppression). (More details in the text.)*

The number of sterile males needed for each phase is also very different in a strategy using the wave principle compared with that using the rolling-carpet principle. In the rolling-carpet principle, subsequent intervention blocks are approximately the same size (Fig. 4). In the wave principle approach, the requirements of sterile males and other resources will increase with each phase; there will be a limit beyond which the operations cannot be sustained. At a given point, the programme will need to advance in one direction (south), and temporary or permanent buffer zones have to be established in the west and east to protect the pest free zones (Fig. 5).

A mobile, modular insectary approach, e.g. containers as production modules, would in some situations have the advantage that, if the target area were expanding, modules could be added fairly easily. Furthermore, the entire insectary could be moved with the expanding eradication or low pest prevalence front. This would also reduce the transport time and cost between a stationary factory and the release area (in the case of an eradication strategy, the distance between the target zone and the factory increases with each phase). A mobile insectary could be relocated occasionally to areas where the pest is still present, and, in the case of eradication, expensive insect-proofing systems would not be needed. However, the relative merits and economics of insect-proof systems versus relocation costs of the modules need to be considered. An example is the New World screwworm mass-rearing facility in Tuxtla Gutiérrez, Chiapas, Mexico. As the programme succeeded and then advanced southward into Central America, the factory remained in an area now free of screwworms, requiring expensive and cumbersome biosecurity measures. These included the continuing release around the facility of several million sterile insects per week to deal with any fertile flies that might have escaped accidentally from the facility.

8. SIZE OF INTERVENTION BLOCKS FOR AW-IPM PROGRAMMES

At what spatial scale is an area-wide approach no longer technically and economically feasible? Is there a minimum size of the target area below which effective implementation of AW-IPM programmes using the SIT becomes technically impossible and economically unjustifiable? These questions are of major importance since the SIT, similar to mating disruption (Cardé 2005), does not kill the pest and hence is particularly susceptible to immigrating gravid female insects, which are completely unaffected by the sterile males.

Blocks are composed basically of two distinct areas, i.e. the core and the edge areas. The core area contains the commodity that is being protected (e.g. fruit crop, cattle, etc.), whereas the edge area, although included in the treatment zone, is defined by the edge effect: the level of infiltration by gravid wild females from the surroundings into the area under treatment (Fig. 7). In some situations the edge area completely surrounds the core area, often requiring the establishment of a protective buffer zone (section 9) to minimize this edge effect.

The size of both core and edge areas is determined mainly by the combined effects of the dispersal potential of the pest species and the degree of isolation
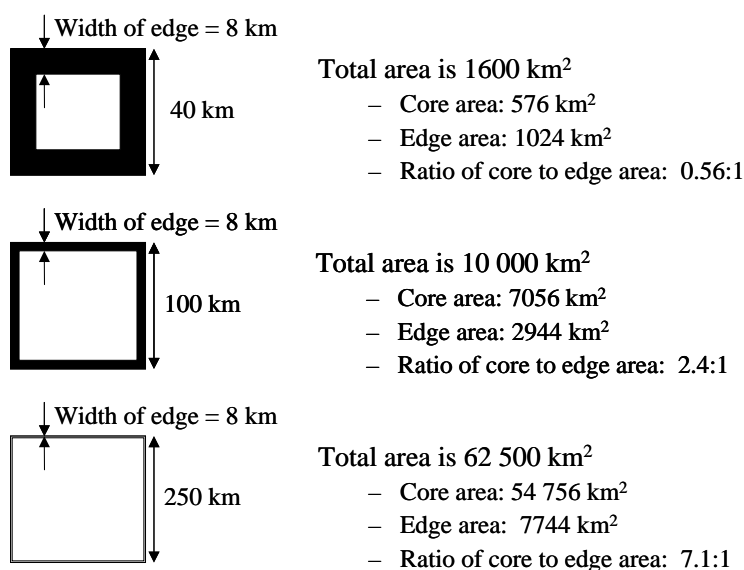
Figure 7. Relationship of core area to edge area at three different scales of block size. Assumptions: edge effect based on pest dispersal of 8 km, and square core areas for either pest free (containment and eradication) or low pest prevalence (suppression) area.

required for the target area, which depends on the strategic objective of the programme. Other biotic and abiotic factors, such as presence of natural barriers, ecological and topographic characteristics of the area, acceptable economic threshold, economic feasibility, and managerial-logistic capacity, are likewise of relevance.

If the selected target area were completely isolated, e.g. an island, the edge effect would be absent and therefore no need to establish protective buffer zones. Consequently, the total size of the intervention block can be quite small. However, when a continuous pest population surrounds or is adjacent to the selected core area, the size of each block must be much larger to absorb the edge effect without affecting progress in the core (Fig. 7). For pests having a high dispersal rate, individual blocks will have to be larger.

The size of the blocks will also affect the strategic approach and the economics of each approach. If the goal is to eradicate the pest and to maintain an area pest free, larger blocks will most likely be required to reduce the relationship of edge to core area (Fig. 7). However, if pest suppression is the strategic goal, a certain damage threshold is usually acceptable, and relatively smaller blocks and smaller protective buffers are acceptable (Larcher-Carvalho and Mumford 2004). This confirms earlier observations that, by nature, an eradication strategy is a more extensive and intensive strategy when compared with suppression (sections 2.1. and 2.2.).

The correlation between the size of the core and edge areas is related to this, i.e. the larger the core area, the smaller the edge area in relation to the whole block surface. Also the relative effort needed to maintain protective buffer zones becomes proportionally smaller with larger block areas. The following example illustrates this point. Assuming that the core and edge areas are squares, and that reinvasion occurs in an edge of 8-km width (Fig. 7), the percentage of the total surface area represented by the edge area decreases from 64 to 29 to 12% for a core area of 1600, 10 000, and 62 500 km^2, respectively. For the small block of 1600 km^2, the effort going into the edge area is almost equal to that going into the core area (Fig. 7). Such a situation could only be justified if the value of the commodity being protected outweighs the costs incurred to maintain protective activities in the relatively large edge area.

The scale of application will also influence the economics, especially when the programme includes the initial capital investment of a mass-rearing and sterilization facility, as well as holding and emergence facilities. Particularly in those cases, the programme needs to be applied on a scale large enough to become economically competitive with alternative control options (for which production costs operate at real economies of scale, e.g. insecticides). Therefore, larger blocks incur a lower cost per surface unit or per unit of the commodity that is being protected. This relates directly to the size of the mass-rearing facility; experience has shown that the cost of a unit of produced insects is inversely related to the level of production (Enkerlin 2003). Higher fixed costs are associated with factories designed to produce relatively small numbers of insects. The size of blocks varies — from tens of square kilometres, in the case of the oriental fruit fly pilot suppression programme in Thailand (Sutantawong et al. 2004), to thousands of square kilometres in the case of large programmes such as the New World screwworm eradication campaign, where each treatment block encompassed at least one and a half of the Central American countries (Wyss 2002). At present, no guidelines are available that could be used to recommend quantitatively the minimum size of blocks for the range of different conditions where sterile insects can be applied. A mathematical model should be developed that considers the main variables involved to produce estimates of minimum-size intervention blocks for different pests and programme objectives.

9. BUFFER ZONES

To address the edge effect as described above, there is often a need to establish buffer zones (FAO 1999, 2005) to maintain containment or eradication, or to protect low prevalence commercial production areas. This may necessitate the application of conventional control methods or the release of sterile insects or both, and may be either temporary or more permanent.

9.1. Temporary Buffer Zones

Eradication programmes that proceed in phases or blocks, irrespective if a rolling-carpet or wave principle is adopted, might require buffer zones between the

intervention blocks to temporarily protect achievements made in each phase of the advancing programme. These temporary buffer zones have two main objectives:

- To prevent a massive influx of the pest from areas where it is still uncontrolled to blocks where sterile insects are being released. Population reduction activities, normally implemented in blocks adjacent to the release blocks, often serve as temporary buffer zones (Fig. 4).

- To consolidate progress in areas where the pest has already been eliminated. Preventive sterile insect releases and other control activities are implemented in these areas adjacent to the population reduction and low prevalence areas (Fig. 1). Hence, these buffer zones attempt to ensure that any migrating gravid females, or those that are transported across a permeable quarantine, cannot re-establish new populations.

Temporary buffer zones were established during the progressive eradication campaign of the New World screwworm in Mexico and Central America (Wyss 2002). The programme was implemented according to the rolling-carpet principle, and always included (at the back end of the moving eradication front) a large screwworm-free buffer area in which sterile insects were released as an insurance in case screwworm-infected cattle was moved into the pest free area.

Such buffer zones are only established temporarily in the dynamic rolling-carpet or wave-principle approaches, and are progressively moved from block to block with the advancement of the eradication campaign (Fig. 4). Occasionally, special temporary buffers are needed when the increasing size of the intervention front of an expanding programme exceeds the available resources, e.g. at a given stage, the multidirectional moving front of a wave-principle strategy will be shifted to a front that proceeds temporarily in only one or two directions (Fig. 5). The areas where such buffer zones have to be established should be identified during the collection of baseline data.

9.2. Permanent Buffer Zones

Permanent buffer zones are often established for containment (Fig. 1). These buffers should have a width sufficient to intercept any immigrating insect and deal with progeny of any gravid female that enters the area. In tsetse flies, for example, which are relatively poor fliers and have no free-living immature stage, the reinvasion potential is much lower than that of New World screwworms (Lance and McInnis, this volume). However, the potential for reinvasion of screwworm flies is much lower than that of polyphagous fruit flies, which often have hundreds of small hosts that can contain larvae, and are easily transported by travellers or postal shipments. Therefore, in addition to buffer zones, rigorous quarantine measures/procedures have to be established to intercept any insect that is transported passively with animal or plant commodities, e.g. fruit fly larvae in fruit, screwworm larvae in livestock, pets, and humans, codling moth pupae in packing boxes, and tsetse flies resting on vehicles.

A good example is the buffer zone that has been established permanently in eastern Panama to prevent New World screwworms from dispersing from Colombia

to the screwworm-free areas of Central and North America (Fig. 8). Three aircraft release nearly 40 million sterile insects per week over a 30 000-km^2 area bordering with Columbia (covering 56 flight hours along about 20 000 km) (APHIS/USDA 2001). The large size of the Darien buffer zone is required because of the high mobility of screwworms; individual flies can cover distances up to 290 km (Hightower et al. 1964). Another example is the sterile Mediterranean fruit fly buffer zone that has been maintained for more than 20 years between Mexico and Guatemala as part of the Moscamed containment programme (Villaseñor et al. 2000). The requirements and optimal dimensions for efficient buffers are often underestimated and, when combined with insufficient resources, frequently result in the establishment of localized inefficient buffers that "leak".

Permanent buffer zones are also needed around low pest prevalence commercial production areas where suppression, rather than containment or eradication, is the strategic goal. The objective is to reduce the impact of gravid females moving into such areas — by applying conventional methods of pest control or extending the sterile insect releases beyond the core commercial production areas. Once low pest prevalence has been achieved, releases could be decreased in the core area, while the buffer would continue with higher release rates and other population reduction activities (Larcher-Carvalho and Mumford 2004). Since suppression is the strategic objective in these situations, these permanent buffer areas do not require the establishment of rigorous quarantine procedures, and can be much more modest than buffer zones for containment or eradication programmes. Nevertheless, the width of these buffers also needs to be determined, taking into account pest mobility, host density, tolerable damage threshold, and population pressure from the surrounding
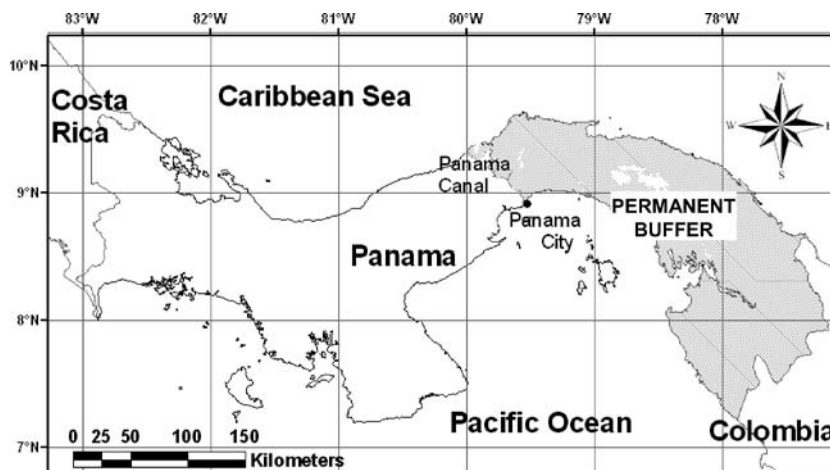
Figure 8. The permanent buffer zone in the Darien Gap in Panama, consisting of the weekly release of sterile New World screwworm flies. (Map from R. B. Matlock, adapted.)

areas. Examples include: (1) the integration of released sterile codling moths and mating disruption, between British Columbia, Canada, and Washington State, USA (Calkins et al. 2000), and (2) the buffer area (treatment of "hot spots" and releases of sterile Mediterranean fruit flies) covering wild-host areas and commercial orchards at the entrance to the Hex River Valley in South Africa (Barnes 2004).

10. CONCLUSIONS

Insect pest control using an AW-IPM approach with an SIT component is more complex and management-intensive than applying simpler control tactics, and hence requires more initial baseline data collection, feasibility assessments, careful planning, and stakeholder involvement to ensure effective implementation. The SIT is a very powerful technique that, unfortunately, too often has been promoted as a "silver bullet", resulting in unrealistic expectations that usually cannot be fulfilled. Programmes that attempt to eradicate a target insect are particularly ambitious and challenging, but in reality are often poorly prepared, frequently underfunded, and lacking in regulatory support to achieve the objective. In some cases, such programmes are operating without even the indispensable establishment of quarantine measures to protect a pest free area. In some of these situations, changing the strategy from eradication to containment or suppression is a more realistic option, but changes during ongoing implementation are politically costly, even if eventually successful. More modest initial goals, starting with a suppression objective to reduce insecticide use, or a containment effort to stop the spread of a pest, are often more realistic. If successful, such strategies can, in some favourable situations, eventually be upgraded to an eradication strategy.

Before embarking on an AW-IPM programme with an SIT component, the choice of strategy must be carefully assessed and preparations made accordingly, biological considerations of the specific pest taken into account, considerable baseline data collected, and in-depth feasibility assessments carried out.

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CHAPTER 6.2.

MISCONCEPTIONS AND CONSTRAINTS

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SUMMARY

In theory, the sterile insect technique (SIT) is applicable to a wide variety of invertebrate pests. However, in practice, the approach has been successfully applied to only a few major pests. Chapters in this volume address possible reasons for this discrepancy, e.g. Klassen, Lance and McInnis, and Robinson and Hendrichs. The shortfall between theory and practice is partly due to the persistence of some common

misconceptions, but it is mainly due to one constraint, or a combination of constraints, that are biological, financial, social or political in nature. This chapter's goal is to dispel some major misconceptions, and view the constraints as challenges to overcome, seeing them as opportunities to exploit. Some of the common misconceptions include: (1) released insects retain residual radiation, (2) females must be monogamous, (3) released males must be fully sterile, (4) eradication is the only goal, (5) the SIT is too sophisticated for developing countries, and (6) the SIT is not a component of an area-wide integrated pest management (AW-IPM) strategy. The more obvious constraints are the perceived high costs of the SIT, and the low competitiveness of released sterile males. The perceived high up-front costs of the SIT, their visibility, and the lack of private investment (compared with alternative suppression measures) emerge as serious constraints. Failure to appreciate the true nature of genetic approaches, such as the SIT, may pose a significant constraint to the wider adoption of the SIT and other genetically-based tactics, e.g. transgenic genetically modified organisms (GMOs). Lack of support for the necessary underpinning strategic research also appears to be an important constraint. Hence the case for extensive strategic research in ecology, population dynamics, genetics, and insect behaviour and nutrition is a compelling one. Raising the competitiveness of released sterile males remains the major research objective of the SIT.

1. INTRODUCTION: CHALLENGE OF A NOVEL TACTIC FOR AREA-WIDE INTEGRATED PEST MANAGEMENT

The 20th century witnessed at least four major advances in methods of suppressing pestiferous invertebrates. The two most ubiquitous of these, synthetic pesticides and biological control, did not require any prior conceptual revolution in scientific thinking for their development and adoption. Both approaches witnessed significant technical innovations, but, conceptually, each was essentially "more of the same". In contrast, the other two important approaches to pest management, autocidal/genetic control (including the sterile insect technique (SIT)) and, more recently, genetically modified organisms (GMOs), have emerged from a totally new worldview as to how each generation passes on the "instructions of life". No language could even come close to explaining these genetic approaches before the 20th century and the influence of Darwin and Mendel. They were simply beyond anticipation (Whitten 1985).

The existence of DNA, and just how these giant molecules might carry the heredity blueprints from one generation to the next as cryptic coded information, was completely outside the thinking of 19th century scientists. Advances in the discipline of genetics — the study of change during an individual's development, and variation between populations over space and time — became the hallmarks of 20th century biology. Not surprisingly, genetics and evolutionary concepts now impinge on many aspects of pest management. They shape our understanding of pesticide resistance and its management (McKenzie 1996), help us determine the specificity and safety of biocontrol agents (Whitten and Hoy 2000), and facilitate the design of novel genetic tactics for pest suppression such as the SIT (Knipling 1955, 1979) and chromosomal rearrangements (Curtis 1985, Whitten 1985). They have also enabled researchers to identify toxin-producing, and pesticide- and disease-resistant, genes, along with the means to transfer such information to unrelated species, whether crop plants or insects (Hoy 2003).

Unlike biological control or pesticides, both household concepts, an important corollary of the genetics revolution has been a need for the broader community to understand the nature of the hereditary material, and how we can manipulate it, to

appreciate the risks and benefits of genetics-based approaches to pest control. Even then, some understanding of ecology and economics is required to put the basket of tactics into a realistic context. In comparison with other pest control tactics, it is difficult for stakeholders, without a basic level of knowledge, to make informed decisions about whether to support or oppose the SIT and transgenic GMOs. Herein lies the biggest constraint to the successful application of the SIT and other genetic means of pest suppression.

It is important that scientists and their institutions enter into meaningful dialogue with the ultimate beneficiaries of genetic approaches to pest control, particularly farmers and communities affected by insect pests and insect-borne diseases. This is especially true in developing countries where the funding and momentum for an area-wide integrated pest management (AW-IPM) programme that integrates the SIT are usually not generated locally (Box 1).

Box 1. Unsuccessful Application of SIT and Genetic Control of Mosquito-Borne Disease Vectors in India

The value of achieving a joint understanding and ownership of genetic control tactics can be illustrated by reference to the unsuccessful attempts of the World Health Organization (WHO) and the Indian Council of Medical Research (ICMR) to develop novel genetic control tactics for three mosquito vectors of disease (malaria, yellow fever and filariasis) in rural India during the 1970s. This ambitious programme evaluated three approaches to genetic control, namely the SIT using either irradiation or chemosterilants, and chromosomal rearrangements (Davidson 1974, Rao 1974). Initially, some common ground for this pioneering project was created when the general approach was described to rural communities as family planning for mosquitoes — a concept widely promoted for human population control by the Indira Ghandi Government. Unfortunately, the mosquito programme floundered when journalists, exploiting ignorance and prejudice, persuaded Indian politicians that the project was designed to serve foreign interests and not the national well-being. The programme was abruptly abandoned in 1975, to the chagrin and embarrassment of the WHO and ICMR, to the bewilderment of many, and, undoubtedly, to the lasting detriment for rural communities scourged by mosquito-borne diseases (Nature 1975; WHO 1976; Dyck, Regidor Fernández et al., this volume). There may be some parallels between the attempted genetic control of mosquito species in India and the eradication of a suite of tsetse *Glossina* populations in Africa using the SIT. These include the risks of using foreign aid to implement sophisticated technologies, not adequately understood by the intended beneficiaries, with the expectation of eliminating a suite of disease vectors on a large area-wide basis covering different political entities and diverse ecologies.

A recent study on the efficacy, economics and social impact of genetically engineered *Bt* cotton in China illustrates well the importance of technology users having a reasonable understanding of the available pest control tactics (Pemsl et al. 2003). A similar conclusion can be drawn about the evolution of resistance and its management, whether we are talking about pesticides, GMOs, or the SIT. Not surprisingly, in very few countries is the concept of resistance management understood and effectively applied (McKenzie 1996). Without doubt, a major challenge for proponents of genetic-based interventions is to secure from participating countries and their communities (especially in rural areas) the prior informed consent and genuine ownership of the proposed programme (Dyck, Reyes Flores et al., this volume). Otherwise, misconceptions will arise, and serious constraints can emerge unnecessarily.

Finally, a brief case study of tsetse suppression/eradication in Africa illustrates how misconception and constraint can interplay to influence decision-makers on the feasibility of pest control options surrounding the SIT. Ironically, tsetse fly suppression/eradication, using sterile hybrids (Vanderplank 1947), is often cited as the first convincing field demonstration of autocidal control of an insect pest (Krafsur 1998; Klassen and Curtis, this volume; Robinson, this volume). Yet, subsequent attempts at area-wide eradication of tsetse species, first with chemicals, and then integrating the SIT, have achieved some success (Vreysen et al. 2000), but have also attracted controversy (Linear 1985, Rogers and Randolph 2002). For example, it is claimed that the pesticide campaign of the Food and Agriculture Organization of the United Nations (FAO), costing about USD 1000 million by 1985, increased rather than reduced the distribution of tsetse (Linear 1985). Important ecological aspects of the debate, such as the role of tsetse in "protecting" vast tracts of natural habitat from occupation by human populations with environmentally damaging cattle ranching (Nagel and Peveling, this volume), are not directly germane to this discussion. However other criticisms leveled by Linear, and partly revived more recently (Rogers and Randolph 2002), at SIT-based tsetse programmes, are pertinent (Box 2). Again, the vexing issue of international funding to finance such initiatives emerges.

Controversies about the net pestiferous status of species such as tsetse flies, and the commitment of resources for control measures, can only be resolved by broad community debate. Such projects, according to Rogers and Randolph (2002), should be demand-driven. In itself, ambiguity about the pestiferous/beneficial nature of a target species is a separate issue to any particular control measure, whether it be the SIT, pesticides, biological control or hybrid sterility. Clearly, it is important for agencies like the FAO and the International Atomic Energy Agency (IAEA) to create and promote a range of pest control options for guaranteeing food security and the health of humans and animals. However, the WHO/ICMR experience in India needs to be borne in mind (Box 1).

2. SOME COMMON MISCONCEPTIONS

2.1. Are Irradiated Insects Radioactive?

During the early development of the SIT, chemosterilants were assessed but abandoned, mainly because of concern over the release of non-specific chemosterilants into the environment (Bakri et al., this volume; Klassen, this volume; Lance and McInnis, this volume; Robinson, this volume). All current and proposed programmes that release sterile insects use cobalt-60 (^{60}Co), caesium-137 (^{137}Cs) or X-rays as the irradiation source. Is there a similar risk that irradiated insects contain harmful residual radiation?

A study of the irradiation of foodstuffs, where irradiation levels are much higher, dispels this fear. The conclusion of all the studies on irradiated foods indicates that there are no levels of residual radiation to give cause for concern. The issue of greater concern was on the production of biologically active compounds that could be harmful. Again, this concern has been dismissed as insignificant.

Box 2. Can Tsetse Species be Suppressed/Eradicated by SIT?

The debate on the potential of the SIT to suppress/eradicate tsetse species indicates a mix of misconceptions, and possibly some significant constraints. These include:

- The SIT is highly technological (a misconception driven by inadequate understanding, but to the extent that this is true, it is largely irrelevant since production and sterilization are done centrally).

- At least 31 species and subspecies of *Glossina* are disease vectors, with various mating barriers between taxa. (In fact, most tsetse taxa are not economically important, and are restricted to rain forests, having little contact with humans or domestic animals. In Kenya, for example, elimination of populations of *G. pallidipes* Austen would eliminate the entire agricultural problem. In Ethiopia, two species are important — *G. pallidipes* and *G. morsitans* ssp.).

- The complexity and difficulty of the problem — with 10 million km^2, more than 30 countries infested, and muliple taxa — pose immense logistical, financial and coordination problems, especially if eradication is the objective. In fact, only selected populations in priority areas at the edge of tsetse belts are currently being targeted (Feldmann et al., this volume).

- The possibility exists that a neighbouring non-target tsetse species would invade an empty niche following the application of the SIT. This could apply to *G. pallidipes* and *G. morsitans*, which are sometimes sympatric.

- Expensive pre-release field studies are required to determine species presence, distribution and abundance (part misconception, and probably an overstated constraint).

- Target tsetse populations are often remote from human population centres (a logistical challenge?).

- Multiple-mating of females, thereby possibly reducing the effective impact of sterile males, is largely a misconception, since only a small percentage of females mate more than once.

- Sterile tsetse males can act as disease vectors, and the argument of "net benefits" may not suffice (in fact, steps are routinely taken to prevent males from acting as disease vectors (Feldmann et al., this volume; Nagel and Peveling, this volume).

- Pre-release tsetse population reduction measures, e.g. pesticides and trapping, are required (integration with other pest management approaches could be seen as a strength).

- The SIT is prohibitively slow and costly, with low economic returns from ensuing livestock production. Large up-front costs presupposing eradication represent a constraint. In addition, while the economic return from animal traction and livestock production may or may not be "low", it is difficult to place a value on human health.

- Failure to eradicate could represent a zero return on a substantial investment, although temporary suppression is also the goal of other tactics.

- Technological weaknesses and poor economics create difficulties in obtaining sponsorship (a constraint).

Is irradiated food radioactive? The process of irradiating food is regulated and designed to ensure that that is not the case. The radiation sources used to irradiate food and agricultural products (including insects) are restricted to: gamma rays from the radionuclides ^{60}Co and ^{137}Cs, and accelerator-generated radiation either as 10 million electron volt (MeV) electrons or 5 MeV X-rays (FAO 2003). Also it is important to realize that food (non-irradiated), and any natural substance, contain natural radioactivity that can be measurable. Therefore it is important to put in perspective the issue of possible induced radioactivity in food and natural radioactivity in food. In a document published by the IAEA (2002a), all relevant data generated over the last 40–50 years, and various nuclear mechanisms that can produce radioactivity, were analysed, and it was concluded that:

> . . . the increase in radiation background dose from consumption of food irradiated to an average dose below 60 kGy with gamma rays from cobalt-60 or cesium-137, with 10 MeV electrons, or with X-rays produced by electron beams, with energy below 5 MeV, is insignificant. It is best characterized as zero.

The above facts about food irradiation can be extended to insect sterilization procedures, since the same sources of irradiation are used (^{60}Co, ^{137}Cs, electrons or X-rays). Furthermore, insect sterilization for sterile insect releases requires a much lower radiation dose than for food irradiation. In food irradiation, the dose varies from about 100 Gy to 60 kGy, depending on the end objectives (IAEA 2002a, b). In insect sterilization, the dose is in the range of only 100–300 Gy, depending on the insect and irradiation conditions (Elias and Cohen 1977; Terry and McColl 1992; Hallman 2000; IDIDAS 2004; Bakri et al. 2005; Bakri et al., this volume). Thus, the induction of radioactivity in insects irradiated for programmes that release sterile insects can also be "best characterized as zero".

Given the low levels of irradiation for insects, the small biomass of the released insects and their wide dispersal, there are no plausible grounds for concern about residual radioactivity, or radiation-induced toxins, in released insects. Conversely, one might argue that the SIT is more environment-friendly than some alternative suppression tactics such as synthetic pesticides or augmentative or classical biological control. Pesticide contamination of foodstuffs, exposure of workers, and environmental damage are often very real issues, particularly in developing countries (EJF 2002, 2003). The absence of any collateral damage following directly from the release of radiation-sterilized insects into the environment is indeed one of the strengths of the SIT, compared with chemical control, biocontrol or even GMOs. As with GMOs, concern about the process, and not the product itself, is sometimes the basis for apprehension. It would be very unfortunate if this misconception about the SIT ever became the reason for an otherwise viable AW-IPM programme not being implemented.

2.2. In What Sense Are "Sterile Insects" Sterile? Is This Misnomer or Misconception?

In terms of physiology and behaviour, male insects "sterilized" by gamma radiation are not sterile (Klassen, this volume; Robinson, this volume). Indeed, they are expected to generate viable and functional sperm capable of fertilization, to produce a full complement of seminal fluids, and also to enjoy undiminished libido. The population-suppressing impact of released sterile males is realized via radiation-induced dominant lethals that are lethal in the next or subsequent generations. By way of contrast, irradiated females may produce fewer or no eggs, and their behaviour may well differ in other critical ways from normal non-irradiated females. Thus "conventional" sterility is often induced in females, but that is of no consequence in most programmes that release sterile insects.

In species where females multiple-mate and store sperm in spermathecae, it is important that irradiated sperm from sterile males are competitive with non-irradiated sperm in fertilizing eggs. In species where female receptivity is normally terminated after the first mating, again it is important that sterile males induce this

behaviour. In species where it is predominantly sperm from the last mating that fertilize egg batches, it is important that sterile males are equally capable of maintaining this outcome.

The above considerations have become important since recent advances in biotechnology and molecular biology have suggested alternative means of sterilizing males. For example, the use of gene-silencing techniques (Nowak 2003) to "switch off" a gene essential for sperm development could well induce true sterility. However, unless the male accessory gland product that "switches off" female receptivity is transferred during mating, these techniques would only serve to create a situation where the released males had little or no impact on the target population (Robinson and Hendrichs, this volume).

By way of contrast, Gould and Schliekelman (2004) suggested a range of novel molecular approaches that rely on lethality in the progeny of released males. Such approaches are unlikely to encounter problems in generating the envisaged impacts. However, as stressed by Gould and Schliekelman, it is important that molecular biologists remain in dialogue with field entomologists so that practical outcomes are optimized. These authors noted that closer collaboration existed during the early period of SIT and autocidal programmes than often exists today between laboratory-based researchers and field ecologists, especially with the latter now an "endangered species" in many countries. Burt (2003) discussed some recent advances in molecular biology, with far-reaching and possibly profound implications for genetic engineering or suppression of natural populations. These revolutionary ideas address the potential of removing the need to mass-rear or irradiate insects before releasing engineered individuals into target populations.

2.3. Is It Essential That Females Mate Only Once?

Conceptually and operationally, it is simpler if females of the target species mate only once. Initially, E. F. Knipling thought that monogamy was desirable, but later realized that it was not of central importance (Knipling 1955, 1979; Klassen, this volume). Monogamy can be a significant constraint where migration introduces inseminated females from outside the sterile-male-release area (Barclay, this volume).

In species where females are monogamous, it is essential that sterilized males evoke the same response as normal males during mating to terminate further receptivity by the inseminated female. The competitiveness of the released male is then measured by the difference between the two ratios — sterilized males to normal males versus field females that have been inseminated by the two classes of males. Multiple matings complicate the situation, but do not disqualify a pest from being a candidate for the SIT. In the latter case, the issue centers around sperm viability and competitiveness in fertilizing eggs, and the competitiveness of sterile males for second or subsequent matings. However, for tsetse flies *Glossina* spp. (Curtis 1968), females mated first to an irradiated male, and then to a non-irradiated male, were less than 50% fertile, just as the reverse order of mating produced greater than 50% fertility. Curtis also addressed another special problem with a viviparous female containing a mix of dominant lethal and normal sperm — an embryonic death might

affect the time of the next ovulation. It turned out that this is only a minor problem, and, in the event of an early embryonic death emptying the uterus, the time before the next ovulation is advanced only from the normal 9 to 7 days.

2.4. Should Released Insects Be Fully Sterile?

There is little doubt that released females should be fully sterile — not so much for ecological or operational reasons, but for good public relations and to avoid potential litigation or compensation claims. Fortunately, for the majority of species of Diptera where the SIT is deployed, females require a lower dose than males for complete sterilization, although for a few groups of insects this is not true (Bakri et al. 2005). However, where suppression and not eradication is the objective, males need not be as sterile, especially if the higher dose required for complete sterility significantly reduces competitiveness (Toledo et al 2004, Bakri et al., this volume; Carpenter et al., this volume; Lance and McInnis, this volume; Robinson, this volume). The objective of the SIT, like any genetic control stratagem, is to impart a genetic load to the target population. If that can be imparted more efficiently through the release of fewer but more competitive males, then that must be the favoured strategy.

2.5. SIT Requires Isolated Populations or Area-Wide Approach

The SIT, and genetic control methods, are generally sensitive to the effects of immigration, particularly the incursion of females already mated to wild-type males, e.g. studies on compound chromosome strains of *Drosophila* sp. (McKenzie 1976, 1977). In this situation, monogamy can be disadvantageous since an immigrant inseminated female, immune to the abundance of sterile males, can produce fertile offspring (Barclay, this volume). Apparently the immigration of inseminated females was the cause of only limited success in a large-scale trial on *Culex quinquefasciatus* Say (then known as *C. fatigans* Wiedemann) conducted by the WHO/ICMR in villages in India during the 1970s (Curtis 1976). Immigration was also a negative factor during trials against *Anopheles albimanus* Wiedemann in El Salvador. In one trial an isolated population was eliminated (Lofgren et al. 1974), but in a larger trial immigration reduced the efficacy (Dame et al. 1981), and suppression was achieved only after increasing the release rate of sterile males (Benedict and Robinson 2003).

The negative impact of immigration on the efficacy of the SIT can be countered by applying the technique on an area-wide basis (Klassen, this volume) or "rolling carpet approach" (Hendrichs et al., this volume). For a newly established pest population, such as a recent introduction of an exotic pest, the target area could be less extensive than for an endemic and widespread pest. However, where eradication is the objective, as is likely for a recent introduction, the releases would need to extend beyond the known distribution, taking into account habitat suitability and the pest's dispersal capacity.

There would be very few situations where it would be appropriate for a single beneficiary, e.g. owner of a single orchard or farm, to use the SIT independently

(Klassen, this volume). Area-wide approaches require organization, coordination, and importantly, financial backing. To provide those resources, the imposition of a levy on stakeholders/producers may be considered (Dyck, Reyes Flores et al., this volume). However, in many situations, some individuals would prefer not to contribute to area-wide control, either because of a perceived loss of independence or a preference for alternative measures (Krafsur 1998). Often the actual or potential beneficiaries of the SIT are farmers, a group that traditionally is independent-minded; and it is inevitable that some would robustly resist making mandatory payments.

It is self-evident that populations of organisms on "islands", either physical (surrounded by water or mountains) or ecological (a patchy distribution of suitable habitat), are isolated to varying degrees from gene flow (Krafsur, this volume). The "island" concept might include urban mosquito populations (where the target species is absent in the surrounding countryside) as a form of ecological island population; its eradication would benefit many people (Curtis 1976). Such situations present attractive targets where eradication is the objective for the application of the SIT. Isolation ensures that the method is at its most economical. Also, if eradication is achieved, suitable quarantine measures are easier to implement to ensure that the benefits are long-term. However, while long-distance dispersal is a disadvantage in any programme releasing sterile insects, imposing "barrier zones" can, in suppression programmes, accommodate certain levels of immigration (Hendrichs et al., this volume). If barrier zones are "reasonable", and the costs and benefits remain favourable, physical barriers to gene flow have not proved to be absolutely necessary.

In terms of an area-wide approach to release strategies (Hendrichs et al., this volume) and impact, an interesting contrast can be made between biological control and the SIT. Box 3 illustrates the enormous regenerative powers of some insect species when colonizing a vacant niche.

2.6. Is Eradication Necessary When Using SIT?

The term "eradication" may be interpreted in a variety of ways. An extreme view would necessitate the removal of every last individual of a species on the earth. Such range-wide species extinction is a natural, indeed inevitable, consequence of the evolutionary process (Wilson 1992). Mankind has been responsible for many accidental, but few deliberate, "eradications".

A more reasonable and acceptable view of eradication at the population level was presented by Newsom (1978), and is adequate for our purposes:

> Eradication is the destruction of every individual of a species from an area surrounded by naturally occurring or man-made barriers sufficiently effective to prevent reinvasion of the area except through the intervention of man.

Newsom (1978) argued (with some cause given the situation that prevailed at the time) that eradication of the New World screwworm *Cochliomyia hominivorax* (Coquerel) in the USA using the SIT had been an "abysmal failure". However, there should be little argument now, using Newsom's own definition, that the New World

screwworm has indeed been eradicated from the USA, Mexico, and Central America (Wyss 2002).

Box 3. Comparison Between SIT and Classical Biological Control for Area-Wide Control of Pest

Both pest management tactics require an area-wide impact to be practical, but operationally, the procedures to achieve this outcome can be quite different. For example, in August 1996 the South African tephritid bitou bush seed fly *Mesoclanis polana* (Munro) was released at two sites, some 300 km apart, along the eastern seaboard of Australia to control seed production by the invasive weed, bitou bush *Chrysanthemoides monilifera rotundata* (L.) (Edwards et al. 1999). In total, 124 adults of mixed sexes were liberated (67 at one release site, 57 at another). By October 1998, the species had occupied virtually the entire range of bitou bush, a distance of over 1200 km (Edwards et al. 1999), establishing vast populations in the process. Thus, within 2 years, these two miniscule founding populations displayed exceptional rates of dispersal, located and colonized scattered and quite isolated pockets of bitou bush, and in the process traversed vast stretches, even cities like Brisbane, that contain no host plants. In May 2004, a survey of six sites, along 700 km of the New South Wales coastline, revealed that 98% of all flowerheads were attacked by *M. polana*, with a mean of 12 eggs laid in each (P. B. Edwards, personal communication). It would be a sobering thought to contemplate the effort and efficacy of rearing and releasing sufficient sterile males of *M. polana* along the eastern seaboard of Australia to reverse the phenomenal outcome achieved by the bitou bush seed fly following its release. Yet we have no reason to doubt a similar dispersal and reproductive capacity of a pestiferous tephritid such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). One is a beneficial insect, the other a pest, but only because of the economic status bestowed by their hosts.

Where repeated immigration of fertile females occurs, either natural or man-made, eradication may be unachievable. The problem of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in California is such a situation (Krafsur 1998). The release of sterile flies certainly may bring about temporary eradication of this pest, but it is difficult to sustain the area fly-free in the face of repeated man-assisted incursions. In such situations, it is perhaps preferable to view the SIT as a preventive, rather than eradication, tool to maintain the fly-free status. Economically sound prevention, rather than eradication, has become the objective (Dowell et al. 2000; Hendrichs et al., this volume).

A final example of accommodating the objectives of an AW-IPM programme integrating the SIT, by recognizing immigration, is the pink bollworm *Pectinophora gossypiella* (Saunders). In cotton-growing areas of the San Joaquin Valley, California, infestations of the pink bollworm are kept below economic-impact levels through the implementation of an AW-IPM programme in which the SIT is a key component. The SIT is also expected to play a role in an ambitious plan to eradicate the pink bollworm in the USA and northern Mexico (Elstein 2002). In this programme, the proposed eradication will employ the SIT, and to reduce pest numbers, in combination with growing transgenic cotton, the use of pheromones for mating disruption. In addition, attempts will be made to limit the number of generations each year by reducing the period when suitable alternative hosts are available.

The eradication of a population of a pest species from all but trivial areas is a challenging objective. Even deciding whether or not to attempt eradication is not a simple matter. Myers et al. (2002) stated that:

> . . . evaluation of the ecological and economic costs and benefits of removing an exotic species is difficult, and often the benefits are exaggerated and the costs are understated.

Rogers and Randolph (2002) emphasized a sensitivity to the desired end result:

> Failure of any eradication attempt is much more serious than failure of control [suppression] because failure to eradicate has no fallback position.

While the SIT does become more effective as the target population dwindles and the ratio of sterile to fertile males increases, this positive attribute of the SIT should not condemn it to be exclusively an eradication option. Krafsur (1998) noted:

> . . . because SIT can lead to eradication, anything less may come to be judged a failure.

In this sense, the necessity of eradication is a misconception.

Frequently, suppression, not eradication, is the objective of using the SIT, necessitated perhaps by unacceptable levels of immigration. In this common situation, the integration of the SIT into AW-IPM programmes, such as those for the pink bollworm, codling moth *Cydia pomonella* (L.) and Mediterranean fruit fly, demonstrates that the SIT can be an important component of AW-IPM.

2.7. SIT as Part of Area-Wide IPM Strategy?

The SIT is not a stand-alone technology, and thus it has to be integrated in various degrees with other control tactics (Bloem et al., this volume; Mangan, this volume). Even in the North American New World screwworm regional eradication programme, there has been some integration with other suppression measures, such as dressing infested wounds with larvicides, mobilizing ranchers to report cases of infestation, as well as quarantine activities (Klassen and Curtis, this volume; Vargas-Terán et al., this volume). In contrast, all the various fruit fly programmes that release sterile flies range from area-wide suppression to eradication; the SIT for fruit flies is integrated with baits, lures, and cultural practices (Enkerlin, this volume; Hendrichs et al., this volume; Klassen and Curtis, this volume; Mangan, this volume).

One impressive example of integrating the SIT into AW-IPM, where the goal is sustained suppression, is the Sterile Insect Release (SIR) codling moth programme in British Colombia, Canada; it uses a "knowledge-based" approach (Bloem et al., this volume). SIR for the codling moth requires the cooperation of commercial fruit growers and home owners with backyard trees. The programme is funded essentially by three tiers of government (75%), and a levy on fruit growers (25%) (SIR 2004). The SIR depends on an integration of the following tactics (baits, UV zappers, and *Bt* sprays were not deemed to be effective):

- Various tree-husbandry practices and field hygiene
- Multiple chemical sprays coinciding with critical life-cycle stages
- Twice-weekly sterile moth releases
- Sex pheromone traps used for monitoring (about one trap per hectare)
- Mating disruption with sex pheromone (about 200 dispensers per hectare)

Although eradication was the initial objective, no quarantines were implemented, and thus this is no longer deemed practical as a short-term solution. Instead, a sustainable programme has evolved (SIR 2004) which has successfully implemented the revised objective to:

> . . . substantially reduce the amount of toxic pesticides currently used to control [suppress] codling moth, and that means a more competitive fruit industry and healthier local environment for everyone.

Two important take-home messages emerge from the Canadian codling moth SIR Program: informed stakeholders are not hampered by misconceptions, and constraints are viewed as challenges to be met and overcome.

3. SOME CONSTRAINTS ON SIT

3.1. *Changing Contextual Framework for SIT*

In spite of the successes of other significant AW-IPM programmes integrating the SIT, such as the codling moth and various fruit fly species (Bloem et al., this volume; Enkerlin, this volume), the successful eradication of the New World screwworm in North and Central America (Vargas-Terán et al., this volume) remains a primary model, in terms of its size, economic importance and durability, for most SIT endeavours. Nevertheless several changed economic and social circumstances, especially over the past 30 years, suggest that even this highly successful programme would encounter difficulties if it were launched today. Changing social, economic, and political circumstances have probably reduced the relevance of the programme as a role model for similar programmes elsewhere in the world. In recent times, perhaps of greater relevance as successful SIT models are the on-going Latin American Mediterranean fruit fly AW-IPM programmes.

The successful North American screwworm programme has been characterized as having an opportunistic "can do" approach. It adapted a "mothballed" hangar at a well-sited but unused air force base in southern Texas. This facility did not demand the construction and safety standards required of a similar facility today. Today, in most countries, occupational health and safety issues, and general worker conditions, are more stringent, and remuneration is more substantial, than in the 1960s. Public liability, insurance premiums, and tendencies to litigate have increased enormously in the past 40 years. Such factors mean that the initial outlay costs for constructing and operating a mass-rearing facility are now much higher. Nevertheless, a facility to rear the New World screwworm is being built in Panama, and several fruit fly rearing facilities are being constructed.

The decline in primary-producer contribution to the gross domestic product (GDP) of many developed nations (now often less than 3%, according to the World Bank) has reduced the political influence of farmers (Europe, Japan, and the USA remain exceptions to this trend). This decline, together with a greater emphasis on the "user-pays principle", aggravates the situation. If pest eradication is the principal objective, breakdowns due to the increased global movement of people, livestock and materials, and threats of bio-terrorism, all serve to reduce the prospects of a

sound economic return on the not-insignificant initial outlay. Also, as mentioned earlier, large up-front costs, and the lack of a fall back position in the event of failure to achieve eradication, are serious disincentives to invest in the SIT for eradication.

A general decline in financial support for relevant research and development (R and D), compared with that available to the US screwworm programme during the 1960s and earlier (Krafsur 1998), linked to some of the factors listed above, will often mean that less is known about future target populations. There will be less control over the quality and competitiveness of mass-reared and released insects (Calkins and Parker, this volume; Lance and McInnis, this volume; Parker, this volume), and a poorer ability to monitor progress or make appropriate adjustments (Krafsur 1998; Gould and Schliekelman 2004; Vreysen, this volume).

The construction of multi-purpose mass-rearing facilities could lessen the impact of some of the above negativities, and provide significant economies (Fisher 2002). However, in turn, such centralized facilities create new problems, such as increased complexity in management and greater technical challenges relating to the competitiveness of released males. The logistics of material supplies for mass-rearing, and transport of sterile insects to release sites, are also likely to represent greater challenges. Certainly many of the above considerations would impact on risk-adverse decision-makers in Australia, New Zealand and Canada, and probably the European Union, regarding the wisdom of conducting area-wide eradication programmes based on the SIT (Vargas-Terán et al., this volume).

Realistically, if an area-wide campaign were to cover diverse countries in a region like South-East Asia or Africa, the political, logistical, and ecological challenges would be significant. Such considerations have meant that, in Australia (for area-wide eradication of an existing pest such as the Australian sheep blow fly *Lucilia cuprina* (Wiedemann), or pre-emptive construction of a mass-rearing capacity to cope with an intrusion of the Old World screwworm *Chrysomya bezziana* (Villeneuve) (Vargas-Terán et al., this volume)), the SIT is effectively not on the agenda, in spite of policies to the contrary. Nevertheless the SIT continues to be used as a rational approach to several fruit fly related problems in Australia (Fisher 1996, Meats et al. 2003).

Compared with most other pest management tactics, the SIT is uniquely affected by another constraint — limited independent and robust experimental field data to evaluate the short- and long-term impact of released sterile insects on population dynamics. Information is inadequate on key issues such as competitiveness (Vreysen, this volume), dispersal rates, and density dependence (Itô and Yamamura, this volume); however, data obtained on the Mediterranean fruit fly in Guatemala provide insight into pest population suppression and competitiveness in large-scale field programmes (Rendón et al. 2000, 2004). As Krafsur (1998) noted, many experimental SIT trials have striven to isolate and reduce a target population. A combination of suppression measures is often applied to achieve this outcome (Mangan, this volume; Nagel and Peveling, this volume). Thus, frequently, according to Krafsur (1998):

> . . . the effects of sterile male challenge are confounded by other treatments.

In this sense, AW-IPM programmes that integrate the SIT, even highly successful ones like the New World screwworm programme in North America, are usually not designed to test hypotheses, but to achieve specific operational outcomes. While this strategy is understandable, it has a serious downside — sometimes little is learned from SIT successes (and failures) that could be applied to other pests.

In spite of the limitations imposed by changes in attitude and resources in the world, the SIT flourishes for species for which the technology is well established, such as the New World screwworm and fruit flies. Some encouraging new developments include a revival of interest in the application of the SIT for *Anopheles* spp. mosquitoes (Touré et al. 2004), and recently a species of tsetse, *Glossina austeni* Newstead, has been added to the list of targets against which the SIT has been successfully applied (Vreysen et al. 2000). Also significant is the exploration of opportunities for commercially viable sterile insect production facilities (Quinlan et al. 2002) that hopefully will serve as examples and, in time, provide alternatives to government-funded and government-controlled programmes.

3.2. Is SIT Too Expensive, or Does It Fail "User-Pays" Principle?

When control options in AW-IPM programmes are being assessed, whether to suppress or eradicate an existing pest or deal with an incursion, the question of cost is often foremost. The cost of SIT strategies generally will include the following: expense for some other form of control to reduce an initial high target population, cost of purchasing the sterile insects, and cost of release programmes and monitoring. In many programmes, the cost of providing sterile insects comprises a high proportion of the costs. However, model inputs show that other costs may outweigh the cost of sterile insects (Mumford, this volume). For example, the proof of eradication required by a trade partner may drive up trapping costs significantly.

In cases where a reliable and economic supply of sterile insects does not exist, the "deal breaker" need not be just the total cost of the sterile insects, but the substantial up front investment needed to construct and equip a new production facility. The start-up time for establishing a colony and reaching sufficiently high production is also a factor. A high initial cost is not always an issue with other control options. For example, for pesticides, the costs of identifying a new product, and taking it through the registration and marketing processes, are much more substantial, but there are important differences. At each step in the pesticide-development pathway, evidence is available to assess the merits of further investment; it is an incremental process. Also, the prospect of obtaining a sound return on the investment has been sufficient, at least during the second half of the twentieth century, to ensure that private enterprise would invest heavily in pesticide technology. However, in recent years, the cost of identifying, registering, and marketing pesticides has escalated; multinationals are increasingly less enthusiastic about identifying new chemistries. Equally, it could be argued that the development costs for a biocontrol agent are substantial, but, like pesticides, the process is incremental, and if progress is not promising, withdrawal is possible along the way.

In some instances, the SIT has been used in suppression or seasonal control programmes in a manner similar to pesticides, e.g. codling moth management in

Canada, and onion maggot *Delia antiqua* (Meigen) control in The Netherlands. The onion maggot control programme is the only example of an ongoing long-term (over 20 years) suppression programme that has used sterile insects from a private source. This programme faced the challenge of those who would turn to the SIT only when other options had failed, and therefore the population of the pest was too high to achieve good results using the SIT (Loosjes 2000). Also, due to rotation of crops and some "free-riders", there was no accumulated benefit to the users of the SIT, only a season's benefit. Furthermore, early support from the Dutch government, in recognition of the enterprise as an environmental business, was terminated due to a change in policy. The loss of this funding eliminated any recognition of public benefits from the reduced use of pesticides, and also caused some users to wonder if the pull-out of the government reflected on the overall product or concept. In spite of these setbacks, the private supply of sterile onion maggots has been maintained, and the programme continues to be successful.

Several large-scale eradication programmes have been cost effective. Without doubt, successful programmes such as the North and Central American screwworm programmes have proved to be enormous economic successes (Vargas-Terán et al., this volume). These successes have served to ensure a serious evaluation of other potential programmes. However, the problem of cost is more complex than just raising the substantial resources to construct and operate a mass-rearing facility, and conduct the release and monitoring activities. The risk of the venture failing, with no return on investment, can daunt all but the hardiest of optimists. One must keep in mind, in any case, that since the SIT does not entail any intellectual property rights (IPR) at this stage, there is little incentive for private enterprise to participate, other than as a "hired hand". Profits must come from the sale of services, as there is little scope for sale of assets of such a company compared with other types of businesses (Quinlan et al. 2002). In the case of long-term suppression programmes that continuously require sterile insects, the financial risk to private investors and governments would be lower than in eradication programmes (Quinlan et al. 2002; Bloem et al., this volume; Enkerlin, this volume; Hendrichs et al., this volume; Klassen, this volume).

It can be concluded, in terms of cost-effectiveness, that the SIT is not too expensive where the beneficiaries better understand the technology and participate in the programme. It is equally important to convince those who will capture the benefits to contribute resources in an increasingly "user-pays world". Possibly the most vexatious financial issue is ensuring that those who capture the benefits pay equitably. In suppression programmes, the beneficiaries often contribute to programme costs, e.g. the codling moth SIR Program in Canada, where growers, property owners, and three levels of government share the costs of implementation (Bloem et al., this volume), and the Mediterranean fruit fly programmes in Latin America and South Africa (Dyck, Reyes Flores et al., this volume; Enkerlin, this volume). The actual public benefits have not been well-measured to date, with some exceptions such as the evaluation in Madeira of indirect impacts on reduction of pesticide use, preservation of cultural heritage, and support of tourism (IAEA 2005). Unintentional subsidies by government for the production of sterile insects used in third-party programmes will certainly discourage private investment in the sterile

insect production sector (Quinlan et al. 2002). This could lead to further use of government funds for the SIT, even in those cases where users are willing to pay their fair share of programme costs.

Much more problematic is the task of identifying the appropriate financial resources, and securing the necessary budget, for programmes that have wider benefits or where the beneficiaries could not afford the cost. For eradication programmes, often the only realistic source is probably the public purse — governments in developed countries, and international aid agencies in less-developed countries (Dyck, Reyes Flores et al., this volume). Even when the identity of beneficiaries is clear, if the benefit is not sufficiently appreciated, users are unlikely to pay. For example, in Australia, various attempts to develop a genetic control programme for the Australian sheep blow fly (Foster et al. 1993; Krafsur 1998; Klassen, this volume), either on the mainland or the island of Tasmania, were abandoned, either because the cost was deemed to be too high (Waterhouse 1962), or because it was difficult to obtain the necessary financial backing (King et al. 1992).

In conclusion, AW-IPM programmes that release sterile insects are often susceptible to the "user-pays principle", but many cases fall outside this approach. It will be just as important to establish the "public good" in privately funded programmes, and obtain partial support for that benefit in the future, as in the past it has been to identify and engage private beneficiaries in government-led programmes.

3.3. Adverse Population, Ecological, and Behavioural Parameters

The suppression effects of flooding a natural insect population with sterile males depend on a suite of factors. Difficulty in adequately satisfying some of these could amount to a serious constraint in successfully applying the SIT to a particular pest. However, articulating such issues can sometimes transform a constraint into a challenge, and ultimately into an opportunity. Constraints and complementary opportunities can include:

- Large size of the natural target population versus methods of suppressing numbers by other means (e.g. pesticides, cultural interventions), or simply exploiting seasonal fluctuations (Mangan, this volume)
- Migration from non-target populations versus choice of target area to maximize effective immigration barriers (Itô and Yamamura, this volume)
- Low competitiveness of released insects (due to initial laboratory colonization, artificial diets, radiation damage, artificial rearing conditions over many generations, especially for adults and mature larval stages which may normally enjoy solitary conditions in the field) versus emphasis on quality control (diet and more natural abiotic rearing conditions), and regular injection of new genetic field material into mass-reared colonies (Bakri et al., this volume; Calkins and Parker, this volume; Lance and McInnis, this volume; Parker, this volume)
- Adverse impact of released females (sting damage from fruit flies or biting behaviour of blood-feeding pests) (Nagel and Peveling, this volume) versus

genetic means of removing females, preferably early in the life cycle (Franz, this volume)

- Frequency-dependent factors affecting competitiveness (i.e. ratio of released to field males) versus release ratios that minimize this effect (Vreysen, this volume)
- Increased ability of field females to discriminate against released males versus exploration of mass-rearing conditions and regular strain replacement to minimize risk of discrimination (Lance and McInnis, this volume)

It is one thing to identify constraints and the complementary opportunities, but to turn these to our advantage requires long-term research. As noted by Krafsur (1998) and Gould and Schliekelman (2004), conditions for the necessary underpinning strategic research, both in terms of resources, expertise, and collaboration, were more ideal during the 1960s and 1970s. Resources and creative ideas are abundant in molecular genetics (Burt 2003), but unless these are linked to field-oriented research in ecology and behaviour, the advantages are unlikely to be captured (Gould and Schliekelman 2004; Itô and Yamamura, this volume).

3.4. Mating Barriers — Serious Constraint on Programmes that Release Sterile Insects?

For the SIT to work, sterile males must be competitive with the field males of the target population(s) of a pest in seeking mates. If we are dealing with a group of distinct species, or even subspecies with limited interbreeding, it is clear that each taxon will have to be tackled separately for progress to be made. This is especially true if the species in the complex are ecologically equivalent, and where a neighbouring taxon can be expected to occupy the vacated niche (Box 2). If the pestiferous species, perhaps vectoring the same disease, is already coexisting, then there is little to be gained by targeting one taxon with the SIT. In such cases, the specificity of the SIT could be a disadvantage.

The above constraint applies equally to cryptic species, where the situation could be more insidious if the mating barriers are undetected because the relevant biological knowledge is simply unavailable. Even supposedly well-known entities such as major malaria vectors, e.g. *Anopheles gambiae s.l.* Giles (White 1974), were found to harbour morphologically indistinguishable, but genetically distinct, species with important consequences for insecticidal suppression programmes (Paterson 1963). Conversely, raising taxa that are not genetically isolated to species status could unnecessarily discourage SIT practitioners. To avoid both types of problem, it would be prudent to conduct the definitive test — determine if sterile males compete effectively with field males in the target populations (Calkins and Parker, this volume). Vanderplank (1947) confirmed that this was the case in his pioneering work on autocidal control of tsetse (Klassen and Curtis, this volume). Below several situations are described where this critical behavioural test was conducted, demonstrating that the SIT should, and did, work.

Based on morphological, cytological, and allozyme differences, Richardson et al. (1982) postulated that the New World screwworm in Mexico and the USA consisted of a number of sympatric, and generally non-interbreeding, populations. The existence of these "gamodemes" was hotly debated (LaChance et al. 1982), and the

subsequent eradication of the pest from these regions argues strongly against the Richardson scenario. While the presence of such entities cannot be disproved, the question has been rendered irrelevant, now that all screwworm populations north of the Panama Canal have been eliminated (Wyss 2002). However, what is relevant is that the Richardson analysis could have been used to stop the successful expansion of the screwworm programme, and thereby denying farmers in North and Central America the enormous economic benefits that the SIT actually provided.

Equally important has been the application of the direct-mating test in northern Africa, following the introduction of the New World screwworm into Libya in the late 1980s (El-Azazy 1989). Taylor et al. (1991) examined the "reproductive compatibility" of Libyan flies and the mass-reared strain. They also examined genetic variability (chromosome morphology, homolog pairing and m-DNA restriction-site analyses) of populations from Libya, the mass-reared strain, and extant Central American, Mexican, and Caribbean populations. Their analyses indicated that mating barriers should not prevent the eradication of the Libyan populations using "Mexican" sterile flies. Indeed, the successful programme proved to be one of the landmark achievements of the SIT (FAO 1992, Lindquist et al. 1992, Krafsur and Lindquist 1996).

A second example of research being conducted to better ensure mating and genetic compatibility (Calkins and Parker, this volume), and thus success of the SIT, is provided by the Old World screwworm. Given that the species is found over a large geographical range, possible incursions of this fly might be refractory to the SIT when using a colony established from a distant population. In response, samples of screwworms from southern Africa, the Middle East, and South-East Asia were subjected to various analyses — allozyme (Strong and Mahon 1991), cytological (Bedo et al. 1994), cuticular hydrocarbons (Brown et al. 1998), and laboratory hybridization tests (J. P. Spradbery, unpublished data; Mahon 2002a). No indication of discontinuities, that might indicate the presence of sibling species, was found. Subsequently, analyses of mitochondrial DNA (Hall et al. 2001) resulted in a similar conclusion. However, with the increased resolution of the technology employed, these authors detected the existence of two mitochondrial "races", one in sub-Saharan Africa and the other extending from the Persian Gulf to South-East Asia. Thus, if the SIT were to be implemented in Australia, it would be prudent to establish a colony from either the introduced flies or the source population.

The absence of mating barriers could reasonably be assumed where the evidence suggests recent movements of an insect species around the globe, including the Mediterranean fruit fly, codling moth, and Australian sheep blow fly.

3.5. Evolution of Resistance to SIT

Insects have shown a remarkable propensity to evolve resistance to chemical challenges used by man in efforts to suppress them. Some species, such as the house fly *Musca domestica* L., certain mosquitoes and whiteflies, are recidivists in that they have evolved resistance to a wide range of insecticides. With this background, it is not surprising that the question is sometimes asked, "Can insects evolve resistance to the SIT?"

Certainly a species could "escape" if it was able to adopt asexuality. Perhaps only those species that had a pre-existing mix of sexual and asexual modes of reproduction could respond rapidly enough to make use of this mechanism. A sexual species would more likely be driven to extinction before the drastic genetic changes necessary for this process could occur.

A possible caveat to the belief, that an asexual response from an SIT-targeted population represents only a minor threat, is if the cytoplasmic symbiont *Wolbachia* sp. is already present in the species, and plays a role in the determination of sex and the sex ratio. *Wolbachia* is a rickettsia-like bacterium that is found in up to 76% of arthropod species (O'Neill et al. 1997). The presence of these organisms produces a wide range of effects, including conversion of genetic males into functional females, male-killing, cytoplasmic incompatibility, thelytoky, and also may have effects on viability. Since the effects of an infection with these cytoplasmically-inherited organisms can be so comprehensive, e.g. thelytoky in hymenopteran species (parthenogenesis where the population consists only of females), the implementation of a programme releasing sterile insects could cause the conversion of an apparently sexual species into an asexual one through the elimination of the uninfected, i.e. the sexual, portion of the population. The dramatic effects of *Wolbachia* vary between groups of species, with perhaps conversion to thelytoky being an extreme effect. Thus, while consideration should be given to the possibility of a programme being thwarted by the effects of *Wolbachia* or similar symbionts, existing programmes have not encountered such problems. More often, consideration is given to the possibility of exploiting some of the characteristics of *Wolbachia* as a means of "driving" desirable genotypes into populations (Turelli and Hoffman 1999; Robinson, this volume).

While an asexual response is considered unlikely, and there are certainly no examples of it, the form of resistance to the SIT more commonly considered (at least to be a possibility) is that the target species could evolve mechanisms to avoid mass-reared sterile males. The argument for such a possibility has been put forward most strongly by Boesiger (1972), who considered it almost inevitable that, while early releases of sterile males might be highly efficacious, the survivors would be enriched for genotypes capable of avoiding sterile males, and thus selection for this ability.

Through genetic divergence, or physiological changes associated with mass-rearing, there should be ample "markers" for field insects to discriminate against mass-reared insects, but the question remains, "Is it likely?" The likely outcome is that the population will become extinct before resistance has evolved. Indeed, AW-IPM programmes integrating the SIT have been active for decades against both the Mediterranean fruit fly and the New World screwworm. To date there is no evidence that resistance has developed, and the SIT remains effective for both species (Krafsur 1998). Similarly, the SIT has been deployed against other species as well, and again there is no evidence to suggest the evolution of resistance to the SIT (but note comments by Lance and McInnis, this volume) (Robinson and Hendrichs, this volume).

A possible exception is a study on the melon fly *Bactrocera cucurbitae* (Coquillett). Hibino and Iwahashi (1991) examined the mating behaviour of the

melon fly using males from a long-term colony, and females from two islands in the Ryukyu Archipelago of Japan. Females from Okinawa Island (where the SIT had previously been applied) discriminated against mass-reared males, whereas females from Ishigaki Island did not. This finding was contrary to an earlier study performed before implementing the SIT, wherein Koyama et al. (1986) found that the mass-reared strain and those from Okinawa mated at random. Hibino and Iwahashi (1991) interpreted this as evidence that Okinawa females had "evolved sexual isolating barriers" to the mass-reared males (note comments by Itô and Yamamura, this volume).

Clearly care should be taken to document any changes in mating compatibility during the course of a programme releasing sterile insects. However, perhaps it is more appropriate simply to avoid the problem by maintaining the genotypic variation within the mass-reared colony as similar as possible to that of the target population. This can be achieved by "refreshing" the genetic variability within the mass-reared strain, such as the regular replacement of the mass-reared New World screwworm strain with field-derived colonies (Mangan 1992), or the regular infusion of field genetic material into the culture (Parker, this volume).

Recent advances in our ability to "cryopreserve" the embryos of insect species of economic importance, including species where the SIT is either ongoing or could potentially be employed (Leopold et al. 2001, Wang et al. 2000), should facilitate this process. Mahon and Leopold (2002) reviewed the potential uses of this technique within programmes that integrate the SIT, one of which would be to prevent the "deterioration" of colonies through genetic changes brought about by long-term selection in mass-rearing. The suggested procedure is to cryopreserve batches of embryos as soon as a newly established colony becomes sufficiently laboratory adapted to be suitable for mass-rearing. After a few generations, the mass-reared colony could be discarded and a fresh batch of embryos revived to re-establish the colony (Parker, this volume).

3.6. Eradication May Have Deleterious/Unanticipated Consequences

Eradication, through whatever means, could conceivably have unexpected consequences. If the species is exotic to the area, and the incursion is small, the non-target impacts should be limited. However, at the other end of the scale, eradication of a widespread indigenous species, such as a tsetse or screwworm species, may lead to undesirable impacts that need to be considered. Myers et al. (2000) provided examples of "secondary" consequences of eradication, following the eradication of exotics from islands, such as the removal of vertebrates, rats, and rabbits. These authors point out that reversing the changes to native communities following the eradication of exotics is not a trivial undertaking, and requires a sophisticated ecological understanding. In general it is anticipated that the negative externalities of the SIT are likely to be less than those of most alternative pest suppression tactics, especially pesticides (Nagel and Peveling, this volume).

3.7. Poor Competitiveness of Released Insects Can Be Major Constraint

In this volume, Calkins and Parker, Lance and McInnis, and Vreysen address aspects of "competitiveness". Competitiveness is an important feature of released insects. When it occurs, poor competitiveness contributes to an increased cost of the SIT, and cost is perhaps the major constraint to a more general application of the technique. Superficially, many of the costs of any successful AW-IPM programme that releases sterile insects may appear to be fixed, but the size of the required mass-rearing facility, and the costs of the diet and insect release operations, are all dependent on the competitiveness of the sterile males. Reduced competitiveness inflates the cost if, as is the norm, mass-reared and sterilized insects perform poorly in the field in terms of longevity, flight behaviour or ability to compete for mates. Consequently the programme must commit to produce and release more insects than would be required if released insects were equal to field (wild) insects in their mating propensity and capability.

Lance and McInnis (this volume) suggest that the competitiveness (as defined by Haisch 1970 and Fried 1971) of Mediterranean fruit fly sterile males may in very extreme cases be less than 1%. This implies that more than 100 sterile males are required to equate to the mating behaviour of a single wild (fertile) male. Clearly, a slight improvement in competitiveness would have a dramatic effect on the costs and benefits of the SIT. The low tolerance to fruit damaged by Mediterranean fruit flies, and the relatively low cost of insect production for the SIT, provide positive benefit/cost ratios for this species. However, for other species, this may not be the case. For example, Australian authorities are re-evaluating appropriate preparedness strategies to respond to a potential future incursion of the Old World screwworm (Vargas-Terán et al., this volume). In a recent SIT trial conducted in Malaysia (Mahon 2002b), the competitiveness of mass-reared and irradiated Old World screwworms was approximately 4%. A re-analysis of a similar trial in Papua New Guinea performed by Spradbery et al. (1989) yielded a similar value (R. J. Mahon, unpublished data). If competitiveness is calculated in this way, the merits of an eradication strategy could be questioned, but much depends on the programme costs and benefits in relation to alternative control tactics. Further work must be done to improve competitiveness, thereby ensuring that the costs and benefits of the SIT for this species remain positive (Calkins and Parker, this volume). Unfortunately, increasing the number of sterile insects released (and hence the release:wild ratio) may not be the simple remedy for low competitiveness, especially if there is a negative relationship between the ratio of released:wild males and mating success, as suggested in field trials of sterile tsetse *Glossina palpalis gambiensis* Vanderplank (Rogers and Randolph 1985) and translocation-bearing males of the Australian sheep blow fly (Mahon 1996).

4. CONCLUSIONS

The SIT has proved to be a robust and "green and clean" technology, yet the range of species to which it is presently being applied is limited. The outstanding successes of the SIT with the New World screwworm in North and Central America, and in Libya (Vargas-Terán et al., this volume), and with the Mediterranean fruit fly and other species of fruit flies in a number of regions of the world (for suppression, eradication, exclusion or prevention) (Enkerlin, this volume; Hendrichs et al., this volume), raise questions about why the SIT has fallen short of the high expectations for the technology.

Some misconceptions are partly to blame but, in general, these seem to be more like irritants than real impediments. Two strengths of the SIT are its specificity, and the absence of negative externalities that haunt other pest management tactics such as synthetic pesticides and, to a lesser extent, classical and augmentative biological control. Concern about residual irradiation, or the production of toxic metabolites, is discussed primarily to dismiss this issue as lacking foundation. Other common misconceptions identified include: the need for monogamous females, and for released males, to be fully sterile; maintaining that eradication is the only possible goal; the SIT is too sophisticated for developing countries; and failure to recognize the SIT as a valuable component of area-wide IPM strategies. In each case, we concede that these warrant analysis, but conclude that each is not a necessary feature of a successful programme that releases sterile insects.

The identified constraints pose more serious threats to extending the SIT to other insect pests. In particular, we recognize that the SIT and other genetic methods of pest control are knowledge-based technologies. Failure of the public to understand the nature of genetic material, and how interventions to disrupt the orderly inheritance or expression of this genetic information can be achieved, can introduce fear of the unknown. Financial considerations rank high on the list of constraints. The need for large up-front expenditure, risk aversion (failure to get any return on a failed eradication campaign), and rigid application of the beneficiary-pays principle, have proved to be serious disincentives for some potential SIT eradication projects that otherwise appeared to be feasible technically.

In the technical arena, the low competitiveness of released males is a serious constraint. Even the accurate measurement of competitiveness, and its implications for suppressing populations, are shrouded in uncertainty. Lack of support for the necessary underpinning strategic research also appears to be an important constraint. Hence, the case for extensive strategic research in ecology, population dynamics, density-dependent regulation, genetics, insect behaviour, and insect nutrition is a compelling one. Raising the competitiveness of released males remains the major research objective for the SIT. Exciting new developments in molecular biology promise to open doors to entirely novel genetic tactics. Even if these do not depend on the release of large numbers of mass-reared insects, with all the associated challenges, stronger links between laboratory and field workers will need to be forged to identify targets, and implement and evaluate these approaches.

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PART VII

IMPACT OF AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES THAT INTEGRATE THE STERILE INSECT TECHNIQUE

CHAPTER 7.1.

IMPACT OF SCREWWORM ERADICATION PROGRAMMES USING THE STERILE INSECT TECHNIQUE

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SUMMARY

The use of the sterile insect technique (SIT) in New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programmes has been successfully demonstrated. As a result of a 45-year area-wide campaign, suppression and eradication have been achieved in the USA, Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama north of the Canal, some Caribbean Islands, and the outbreak in Libya, North Africa. The humans, livestock, and wildlife in these countries are now free of this dangerous pest. It has been estimated that the annual producer benefits are: USA – USD 796 million, Mexico – USD 292 million, and Central America – USD 77.9 million. In Libya, the estimated benefit/cost ratio was 5:1 in the infested zone, and 10:1 in the whole country. If the New World screwworm were eradicated in South America, it has been estimated that each year USD 3592 million could be saved. Small field trials have confirmed that the SIT would be effective for the area-wide control of the Old World screwworm *Chrysomya bezziana* (Villeneuve).

1. INTRODUCTION

The application of the sterile insect technique (SIT), as part of an area-wide integrated pest management (AW-IPM) approach for the suppression and eradication of the New World screwworm *Cochliomyia hominivorax* (Coquerel), has been comprehensively demonstrated (Klassen and Curtis, this volume). The similar biology of the New World screwworm and the Old World screwworm *Chrysomya bezziana* (Villeneuve) indicates that the SIT should also be effective against the Old World screwworm. Field trials in Papua New Guinea provided strong indications that the SIT would be effective in suppressing the Old World screwworm (Spradbery 1990, Spradbery et al. 1989), but were not conclusive, although more recent studies in Malaysia have provided validation of the SIT for this screwworm species (R. J. Mahon, unpublished data).

Today, animal production is a high priority in world agriculture. There is an increasing demand for meat, dairy and egg production — major sources of animal protein for the world's growing population. To satisfy this demand, a diversity of livestock production systems is found in the different continents, including keeping cattle, buffaloes, sheep, goats, hogs, and poultry on traditional smallholder farms, and in extensive grazing or in more intensive systems, depending on the local circumstances.

In developing countries, animal production makes a major contribution to local and national food supplies. This production provides food security, cash income to a large number of rural people, and benefits to the whole economy. Commercial livestock-keeping increases total farm produce and income, provides year-round employment, and reduces the investment risk of raising livestock. Income from livestock products provides funds to purchase additional means to improve crops, or for other farm investment. Livestock production enhances the economic viability and sustainability of the farming system (FAO 1992).

Predictions made by the International Food Policy Research Institute (IFPRI), International Livestock Research Institute (ILRI), and Food and Agriculture Organization of the United Nations (FAO), suggest that, between 1993 and 2020, total world meat consumption will double from 180 to 300 million tonnes, and milk production will increase from 400 to 650 million tonnes (Delgado et al. 1999). To satisfy this growing demand, the world must find mechanisms to develop greater

production efficiencies without damaging the already stressed environment. Part of this productivity increase can be realized through improvements in animal health.

Animal diseases affect the livestock sector directly through mortality, reduced fertility, and loss of weight, and together with other factors have chronic debilitating effects on livestock and their production. This results in inefficient utilization of scarce resources. Both ectoparasitic and endoparasitic diseases are recognized as major factors limiting production. The cumulative effect of parasitic diseases is perhaps a greater cause of economic losses than that of any other disease.

Myiasis is caused by an infestation of a living vertebrate's tissue or fluids by larvae (maggots) of flies (Diptera). Even minor infestations cause annoyance to animals, disrupting normal habits including feeding and resting. In some situations there is loss of milk, meat or wool production, or in the value of hides.

There are at least 20 species of flies responsible for myiasis, feeding specifically on living animal tissues to complete the larval stage of the life cycle (James 1947). Two of the most important obligatory parasitic myiasis flies are the New World screwworm and the Old World screwworm.

In the Western Hemisphere's tropical and subtropical regions, the New World screwworm is one of the most damaging insect parasites of livestock. It alone represents economic losses each year of hundreds of millions of dollars (USD) (section 2.3.). Losses result not only from direct reduction in productivity due to sickness and death, but also from the labour and insecticide costs incurred by continuously having to inspect and treat wounds. In endemic areas the annual cost of controlling New World screwworm myiasis in domestic animals was estimated at USD 4.82–10.71 per head (Rawlins 1985). These flesh-eating larvae also represent a serious human-health problem (Reichard et al. 1992, Vargas-Terán 2002a, Wyss 2002a).

Gravid female flies are attracted to wounds, even those as small as a tick bite. Eggs are laid in, and around the border of, such wounds. After the eggs hatch, the larvae begin feeding on the live body tissue. As the maggots feed, they enlarge the wound, making it attractive for other female flies to oviposit and also susceptible to secondary infection. Without treatment, it is common for the animal to die.

The New World screwworm has a high reproductive rate. Each female can lay several clutches of up to 400 eggs each. Under optimum conditions, a generation or life cycle can be as short as 3 weeks. Before suppression and eradication programmes commenced, the New World screwworm occurred naturally in the south-western USA, Central America, the Caribbean, and tropical and subtropical South America. By accident, through animal movement, it spread to the south-eastern USA, where it became established (FAO 1989).

The life cycles of the Old World and New World screwworms are very similar, lasting about 21 days, and these species are a good example of co-evolution. However the Old World screwworm is smaller than the New World screwworm, and the females are less fecund, producing egg clutches of 190–250 eggs. The Old World screwworm causes myiases in Africa, Arabia, the Persian Gulf (Bahrain, Iraq, Iran), India, and South-East Asia (Spradbery 1991).

The incidence and severity of the myiasis depend on local conditions: livestock distribution and density, wildlife populations and their migratory habits, human

population density, and the effectiveness of public health services. However, most important are the climatic conditions. Screwworm populations vary during the year, being most abundant in the hot and humid season.

2. DIRECT AND INDIRECT BENEFITS OF NEW WORLD SCREWWORM ERADICATION

2.1. Benefits of Eradication in North America and North Africa

The main direct beneficiaries of the elimination of the New World screwworm are livestock producers. However, direct and indirect benefits result for the community as a whole, through the increased availability of locally produced livestock and dairy products, reduced deficiencies caused by a shortage of meat and milk, and the increased availability of draught animals and manure. At a national level, economic benefits arise due to better-integrated agriculture and livestock production, and reduced dependency on food imports. There may also be public health benefits to the community.

In North America, the economics of New World screwworm eradication programmes have been very positive, in spite of the high investment cost over the ca. 45 years of the programme (about USD 1300 million):

- USA: Cost to the United States Department of Agriculture (USDA) in 1958–1986 was about USD 650 million, in 2005 dollars (cost to producers and state governments not included) (Meyer 1994; J. H. Wyss, personal communication)
- Mexico: USD 413.5 million (FAO 2005)
- Central America: USD 268.4 million (Wyss 2002)

Estimates of annual producer benefits show the very large economic benefits that are accruing (Wyss 2000, 2002a):

- USA: USD 896.1 million
- Mexico: USD 328.6 million
- Central America: USD 87.8 million

The total annual producer benefits are USD 1300 million.

The Libyan campaign was estimated to cost close to USD 100 million from international funds, and it is a tribute to all concerned that successful eradication was achieved using less than USD 35 million, provided by a multidonor fund. This fund was established by the governments of Australia, Austria, Belgium, Finland, France, Germany, Ireland, Italy, Luxembourg, Netherlands, Spain, Sweden, UK, and USA, and institutions such as the African Development Bank, European Economic Community, International Fund for Agricultural Development (IFAD), Islamic Development Bank, Organization of the Petroleum Exporting Countries (OPEC) Fund, and the World Wildlife Fund. An independent economic appraisal showed that the programme was a remarkably profitable investment, with benefit/cost ratios of 5:1 in the infested zone, and 10:1 in the whole of Libya (Grindle 1991, FAO 1992, Vargas-Terán 2002b).

The New World screwworm was first described in 1857, infesting humans on Devil's Island in French Guinea (Coquerel 1858). Screwworms still cause

significant human morbidity and mortality in the tropical Americas, as well as dramatic effects on mammalian wildlife. It is estimated that, throughout the Americas, about 330 million people reside in New World screwworm-endemic areas. In most countries the human disease has been brought under control through strict medical surveillance and treatment, but where surveillance is relaxed, it threatens to develop into epidemic proportions. For example, before screwworm eradication in El Salvador, humans were found to be the third-most affected species (Reichard et al. 1992), and eradication relieved everyone of the personal risk of myiasis.

2.2. Importance of Livestock in South America

Livestock production in South America is based predominantly on medium- and small-sized farms raising small numbers of a variety of animal species. These animals are used for family consumption, draught power, and some for sale. However, there is also an industrial commercial sector, which is totally market-oriented, and based primarily on cattle exports. Since the general economic situation is making remarkable improvements, this sector is growing very rapidly. Therefore South American animal agriculture is developing in a complex and dynamic environment. Livestock populations are shown in Table 1.

Table 1. Number (x 1000) of animals and humans at risk of infestation by the New World screwworm (Source: FAO, FAOSTAT (1999))

Country	Bovines	Equines	Suids	Ovines	Caprines	Total animals	Humans
Argentina	55 004	3301	3200	14 707	3428	79 641	37 032
Bolivia	6556	322	2714	8574	1500	19 666	8329
Brazil	163 470	6400	27 425	18 300	12 600	228 195	170 115
Chile	3500	660	2750	4100	900	11 910	15 402
Colombia	25 614	2450	2764	2195	1114	34 137	42 321
Ecuador	5105	521	2786	2180	284	10 876	12 646
French Guiana (France)	9	-	10	2	-	21	181
Guyana	220	2	20	130	79	451	861
Paraguay	9863	400	2500	395	131	13 289	5496
Peru	4903	665	2788	13 700	2068	24 124	25 665
Suriname	102	-	25	10	10	147	417
Uruguay	10 700	500	360	15 500	14	27 074	3337
Venezuela	15 992	500	4500	780	4000	25 772	24 170
Total	301 038	15 721	51 842	80 573	26 128	475 303	345 972

2.3. Potential Economic Significance of Eradication in South America

Using data collected through surveys, and from economic studies carried out in the Caribbean region during the 1980s, Rawlins (1985) estimated the annual cost of New World screwworm surveillance and medication in various countries at USD 4.82–10.71 per head. If an average of USD 7.76 per animal per year is taken as the theoretical cost, then the annual costs of the New World screwworm in South America may be in the order of USD 3500 million (Table 2). Wyss (2002a) estimated the potential annual producer benefits for New World screwworm eradication in South America (in 2000) at USD 2800 million.

Table 2. Estimated annual losses from New World screwworm in South America

Country	USD (million)
Brazil	1770
Argentina	618
Colombia	264
Uruguay	210
Venezuela	199
Peru	187
Bolivia	152
Paraguay	103
Guyana	3
Suriname	1
Ecuador	0.17
French Guiana (France)	0.16
Chile	0

3. NEW WORLD SCREWWORM PROGRAMMES

3.1. Successful SIT Eradication Programmes in North America

The elimination of a residual indigenous screwworm population, after an intensive suppression programme, requires the area-wide application of the SIT. The SIT can most simply be described as a form of population control (Knipling 1985; Wyss 2002b; Klassen, this volume), supported by other disease-control activities including epidemiological surveillance, wound treatment, animal-movement control, and quarantine (Mangan, this volume). The eradication programmes, that over about 45 years implemented this integrated use of the SIT, have been phenomenally successful, as shown in Fig. 1 and Table 3 (Wyss 2000, 2002a; Klassen, this volume; Klassen and Curtis, this volume).
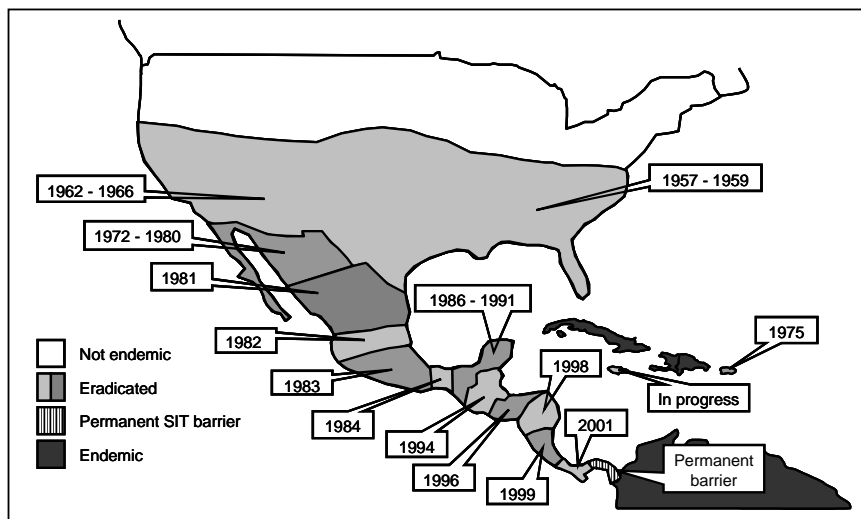
Figure 1. Progressive shift over time of eradication zones in the New World screwworm AW-IPM programmes using the SIT from the southern USA to the countries of Central America. (Map from Robinson 2002, reproduced with permission from Elsevier.)

Table 3. New World screwworm eradication programmes[1]

Country	Eradication period
USA south-east[2]	1957–1959
USA south-west[2]	1960–1966
Mexico[2]	1972–1991
Guatemala[2]	1988–1994
Belize[2]	1988–1994
El Salvador[2]	1991–1995
Honduras[2]	1991–1995
Nicaragua[2]	1992–1999
Costa Rica[2]	1995–2000
Panama[2]	1997–2000
Curaçao[3]	1954
Curaçao (reinfestation)[4]	1976
Puerto Rico[5]	1975
US Virgin Islands[5]	1971–1972
British Virgin Islands[5]	1971–1972

[1]Information extracted from [2]Wyss 2000, [3]Knipling 1985, [4]Coppedge et al. 1980, and [5]Williams et al. 1977.

3.2. Naturally Free Areas in North America

The countries and territories in the Caribbean, naturally free of the New World screwworm, are: Antigua and Barbuda, Bahamas, Barbados, Cayman Islands, Dominica, Grenada, Guadeloupe, Martinique, Montserrat, Netherlands Antilles (Aruba, Bonaire), Saint Kitts and Nevis, Saint Lucia, Saint Vincent and Grenadines, and Turks and Caicos Islands.

3.3. Remaining Endemic Areas in North America

Screwworm myiasis continues to be a serious animal and public health problem in Cuba, Dominican Republic, Jamaica, Haiti, and Trinidad and Tobago (Vargas-Terán 2002a; Klassen and Curtis, this volume). These countries pose a risk of New World screwworm reintroduction to those countries already free of the pest. Therefore the FAO and International Atomic Energy Agency (IAEA) have been providing technical assistance for New World screwworm suppression and eventual eradication

- Cuba (1995). The presence of the New World screwworm was officially acknowledged in 1995. The Cuban Government and FAO signed an agreement to establish a national suppression programme and design a project plan to implement screwworm eradication. The plan has two phases: (1) a pilot programme on Juventud Island, and (2) a full programme to cover the entire national territory. As a follow-up and part of the preparatory phase, the IAEA supported capacity building and an area-wide suppression trial on Juventud Island (García Rodríguez 2003, Méndez et al. 2005). The estimated cost of New World screwworm eradication is USD 62.5 million over 4 years. From 1995 to September 2003, 88 985 cases were reported. The animal species most affected were cattle, swine, sheep, goats, horses, dogs, and humans (FAO 1999, 2003).
- Dominican Republic (1999). Cases of the New World screwworm occur in all parts of the country, and without seasonal variation. Most neglected wounds and untreated navels of newborn animals soon become infested. Human cases are a common occurrence. In 1999, the governments of Jamaica, Haiti, and the Dominican Republic, and the FAO and IAEA, began regional technical assistance projects on capacity building and feasibility assessment of the suppression and possible eradication of the screwworm. As of February 2001, 1894 screwworm cases were diagnosed (FAO 2003).
- Haiti (1999). The disease is endemic, with a high incidence throughout the country. It causes considerable losses in domestic livestock, and affects people of all ages. A Government of Haiti and FAO technical project reported 684 myiasis cases, of which 669 were positive for the New World screwworm; seven of those cases were in humans. When the project terminated in 2001, it was expected that a joint eradication programme with the Dominican Republic would be established (FAO 2003).
- Jamaica (1998). Jamaica is one of the naturally screwworm-infested territories in the Caribbean. The high annual rainfall (over 1900 mm) and tropical climate sustain the very lush vegetation cover on the island, an ideal habitat for the New

World screwworm. Consequently the fly is widely distributed, regardless of season, altitude or ecological conditions. The island has about 400 000 cattle, 440 000 goats, and a large but unknown number of "stray" dogs. There are no wild animals, e.g. deer, rabbits, opossum, and peccaries to support screwworm infestations. According to Snow et al. (1977), the New World screwworm is the second-most important arthropod pest of livestock, exceeded only by ticks. The annual economic losses inflicted by the screwworm on the Jamaican livestock sector, in terms of animal mortality and increased production costs, in 1998 amounted to USD 5.5–7.8 million (Vo 2000). Although all ways of keeping livestock are affected by the screwworm, its eradication from Jamaica will have the greatest implication for the many subsistence farmers who depend largely on small animal holdings for their livelihood. The screwworm is also a severe human health problem, with 7 or 8 cases reported every month, and probably many more are unreported (M. J. B. Vreysen, personal communication).

In July 1998, the Government of Jamaica began a New World screwworm eradication programme, with assistance from the USDA and the cooperation of the IAEA and FAO (Robinson et al. 2000). The estimated cost was USD 9 million, and it was anticipated that 3 years would be required for completion (Vo 2000). On average, from November 1998 to October 2004, 258 screwworm cases were reported each month. Weekly treatment of the island with sterile screwworm flies began in August 1999, with the release of about 16 million sterile flies per week. In mid-2002, in response to the prevailing high number of reported cases, a new strategy was adopted (M. J. B. Vreysen, personal communication). There have been several logistical problems associated with the programme, so in spite of the new strategy, and the continuing commitment of the Government of Jamaica to eradicate the screwworm, little progress had been made as of the end of 2004 (Grant et al. 2000; FAO 2003; Box 1 in Dyck, Reyes Flores et al., this volume).

- Trinidad and Tobago. The New World screwworm is endemic, and cases are found throughout the year. Wounds left untreated usually become infested. The proximity of the islands to Venezuela, and possible immigration of flies, could complicate screwworm suppression and eradication.

3.4. Potential for Eradication in Remaining Endemic Areas of the Americas

3.4.1. Threat of Reinvasion

The Panama and US Governments have established a permanent biological barrier, in the Darien Gap in Panama, by releasing 50 million sterile New World screwworm flies per week, which contribute to the protection of the non-infested North and Central American countries. However, as long as Cuba, Dominican Republic, Haiti, and Trinidad and Tobago in the Caribbean, and several South American countries remain infested, they represent a high risk to the eradicated territories and the naturally screwworm-free countries in the Caribbean basin.

There are several examples of failure to prevent screwworm reinvasion in territories where it had been eradicated. In 1966, after eradication from the USA, a

biological barrier was established along the Mexico–US border. However, in 1972, a massive failure of the barrier occurred (due to favourable weather conditions for the insect, and intensive legal/illegal livestock trade between the two countries); 90 000 cases were detected in the USA. Nevertheless, by 1984, eradication had been achieved in Mexico down to the narrowest part of the country, and another barrier zone was established (Mexico-United States Commission 2002). Then, in 1985, several outbreaks occurred in the central and northern territories, in spite of the implementation and operation of a good quarantine network.

The threat of reinvasion of the New World screwworm increases in proportion to the area eradicated, due to the risk posed by the international trade in animals, and the movement of pets and humans. Examples of screwworm outbreaks, both actual and potential, caused by such animal movements, are as follows: 1987 — dog from Honduras to the USA; 1988 — sheep from Latin America to Libya; 1989 — man from Panama to the USA; 1992 — woman from Brazil to New Zealand and Australia; 1994 — cattle from Central America to Mexico; 1998 — woman from Trinidad and Tobago to UK; and 2001–2002 in Chiapas, Mexico, due to the introduction of flies from Central America via a small aircraft. The cost of containment varies. In the case of Libya, the programme cost USD 75 million, and Mexico's largest outbreak cost USD 8 million.

3.4.2. Caribbean

The main objective of screwworm eradication in the Caribbean will be the promotion of sustainable agricultural development and food security, and simultaneously the protection of screwworm-free countries from reinvasion.

The Caribbean programme will demand the coordinated actions of all screwworm-infested and screwworm-free countries, as well as the institutions concerned with the suppression/eradication of the disease in the region. The experience and the resources accumulated by the Central and North American governments should be transferred to the infested countries in the Caribbean region.

The governments of the affected countries, with assistance from technical international agencies, will need to further refine national assessments of the current New World screwworm status, including geographical distribution, seasonal abundance, economic impact, and on economical methods to suppress the pest. Following these feasibility studies, a multi-disciplinary mission should prepare a project proposal for the eradication of the New World screwworm from the Caribbean, to be submitted to potential donors for funding, or a strategic alliance be established with countries already screwworm-free in the region.

In the first phase of the regional programme, Trinidad and Tobago should not be included, due to its proximity to the endemic countries of South America, and the associated real risk of reinvasion. However, once suppression/eradication activities are underway in the coastal areas of Venezuela, it should be given priority consideration.

Before launching such a regional programme, the following prerequisites should be resolved by the participant countries, donors, and stakeholders:

- The governments involved will be fully committed to the New World screwworm eradication programme, and there will be no change in their policy
- Adequate funds will be available as required

- Adequate numbers of sterile insects of the desired quality will be available from the Mexican or Panamanian insect production facilities
- All infested areas in the Caribbean will be progressively treated
- All screwworm-free countries will maintain strong inspection and quarantine services to prevent the introduction of infested animals from endemic areas

3.4.3. South America

The New World screwworm is endemic throughout most of the South American continent (Vargas-Terán 2002a). All countries are infested except Chile, where the pest was last found in 1959 (although it is possible that Easter Island, a Chilean territory, remains infested). Although Chile shares borders with countries infested by the New World screwworm, it has been able to maintain its screwworm-free status as a result of strict controls imposed on the importation of animals and animal products. In South America, with the possible exception of Chile, there are no natural barriers known to prevent the spread of screwworms between countries. The screwworm situation in the Amazon is not known, and the same can be said for the presence or absence of screwworms at different altitudes in the Andes. Unless barriers can be found, for eradication purposes all of South America must be considered as one region. Once started, the programme would have to be progressive and continue until the whole continent (and thus the Southern Hemisphere) is completely free of the New World screwworm. To consider South America as a target area, considerable preparatory groundwork is needed. The governments and livestock producers in each country involved must be convinced that eradication is technically, practically and economically justified. They must be ready to commit the resources and the energy to complete the task. In addition to this groundwork, population genetic studies are being carried out (Lessinger et al. 2000) to understand more about screwworm population variation in the region, and the possible existence of isolated populations or cryptic species. There will also be a need for mating compatibility studies among the different populations in the region.

The following actions should be undertaken before the development and implementation of an eradication strategy:
- Conduct regional information campaign
- Develop baseline data on New World screwworm case incidence in each country
- Conduct studies on the economic impact of screwworms in each country
- Develop an eradication strategy
- Determine the cost of a regional screwworm eradication programme
- Determine the benefit/cost ratio
- Prepare an environmental-impact study on screwworm eradication in each country
- Identify methods of financing its implementation (Vargas-Terán and Wyss 2000)

3.5. Successful Eradication in Libya (1988–1992)

The discovery of the New World screwworm in North Africa in 1988 posed an immediate threat to Libya, the continent of Africa, and the Mediterranean region. It rapidly became clear that the price to be paid for the persistent and inevitable spread of this pest from its beachhead in the Libyan Arab Jamahiriya would be very great indeed — in terms of suffering and loss in domestic animal populations. Also the potential impact on wildlife in Africa was a grave concern to wildlife conservationists and enlightened people throughout the world (Van der Vloedt and Butt 1990, Woodford 1992).

Based on a review of Libyan livestock importations, the source of the New World screwworm outbreak was thought to be sheep (infested with New World screwworms) imported from South America. This was the first report of the New World screwworm occurring outside the Americas, and this incursion confirmed the New World screwworm as one of the most important transboundary animal-disease threats.

Immediately after official confirmation of the New World screwworm in Libya, a series of governmental and United Nations activities began. The FAO established the Screwworm Emergency Centre for North Africa (SECNA), based in the Animal Production and Health Division in Rome, to coordinate surveillance and control activities; its field programme was set up in Tripoli, Libya (Vargas-Terán 2002b). The FAO undertook the New World screwworm emergency programme in Libya on behalf of the countries threatened by the disease, and the 22 countries and agencies that provided the emergency funds (section 2.1.), including the technical support of the IAEA and other essential support provided by other United Nations agencies — IFAD and the United Nations Development Programme (UNDP).

To contain the outbreak, veterinary services in Libya provided 90 teams to undertake periodic inspections involving millions of animals in and around the infested area. Wounds were treated prophylactically, and the movement of livestock was controlled. Prevention programmes in all neighbouring North African countries concentrated on surveillance, public information, and control of animal movements.

Containment activities were successful. The infested area around Tripoli had expanded only from approximately $18\,000$ to $25\,000$ km^2 by the time field eradication activities commenced. As a consequence of the control of animal movement, no foci developed elsewhere in the region. However, the infestation had become more severe within the affected area, and more than $10\,000$ cases were recorded in the second half of 1990, compared with less than 2000 in the same period of 1989 (FAO 1991a, b).

From December 1990 to October 1992, 1300 million sterile New World screwworm pupae were transported from Mexico to Tripoli, and the emerging flies were aerially dispersed over an area of $41\,000$ km^2 around Tripoli (Lindquist et al., 1993). The last New World screwworm case was reported in April 1991 (Krafsur and Lindquist 1996). After 14 months without evidence of the parasite, and under continuous surveillance and quarantine inspection activities, Libya was declared officially screwworm-free on 22 June 1992 (Vargas-Terán et al. 1994; Klassen and Curtis, this volume).

After eradication, the emphasis was on prevention. The FAO set a priority to improve the surveillance technology and quarantine infrastructure to reduce the risk of screwworms spreading from endemic areas. This animal disease prevention approach is the background to the creation of FAO special programmes, such as the Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES), and the Regional Animal Disease Surveillance and Control Network for North Africa, the Middle East, and the Arab Peninsula (RADISCON).

4. POTENTIAL OLD WORLD SCREWWORM PROGRAMMES

The Old World screwworm is widely distributed, primarily in the Indian subcontinent and South-East Asia as far north as Taiwan and to Papua New Guinea in the south-east. It is present throughout mainland Papua New Guinea, except at altitudes of more than 2500 m above sea level, and high population densities of this screwworm have been found in the coastal swamplands adjacent to Torres Strait. The fly is present in New Ireland and New Britain, but not further into the Pacific. This screwworm species is also found in tropical and subtropical sub-Saharan Africa, and in several countries in the Middle East (Spradbery 2002). Kloft et al. (1981) reported repeated "transit infestations" of the Old World screwworm from South-East Asia to the Middle East, and a recent (1996) infestation recorded in Iraq is assumed to have originated from South-East Asia (Hall et al. 2001).

4.1. Feasibility of SIT Suppression or Eradication

At a conceptual level, any suppression and eradication programmes should first be initiated in the archipelagos of Indonesia and the Philippines, and other island nations such as Sri Lanka. Area-wide programmes could also be applied in peninsulas, such as peninsular Malaysia and southern Thailand, although a permanent buffer zone would be required at narrow interfaces between the eradicated and endemic zones, similar to the arrangement now in place for the New World screwworm in Panama. Treatment of islands should be extended progressively within a region before moving into the continental landmass.

On the Asian continent, the challenge would be much greater, similar to that of South America. Natural "ecological islands", bounded by deserts or high mountains unsuitable for screwworm survival, should be amenable to eradication programmes. However, more ecological studies on the screwworm are required before such a strategy could be implemented. There may also be a potential, at the edge of the pest's distribution, to reduce the range. This would be more feasible if the screwworm had already been eradicated in the "ecological islands" and peninsulas. If an eradication attempt were to be made on the continental landmass, it would be essential that a regional approach be adopted.

Implementation of AW-IPM programmes using the SIT should target preferably the areas where the Old World screwworm is most vulnerable, and least able to migrate back to recolonize areas from which it had been eradicated.

4.2. Preparatory Activities

In most areas where the Old World screwworm is endemic, there is a paucity of information about its population size, distribution, and possible seasonal variation; in Malaysia, monitoring showed little variation (R. J. Mahon, unpublished data). However, there was considerable variation among farms, possibly due to differences in management and closeness of supervision, and thus efficacy in detection and treatment of myiasis. Anecdotal evidence suggests that the screwworm is a relatively minor problem under smallholder livestock production systems, but it becomes a serious production-limiting pest under more extensive grazing systems. Although farm records, where available, indicate that myiasis is a major animal-health problem under extensive grazing systems, screwworm suppression in large dairy herds is effective because the animals are observed closely every day, whereas herds of cattle and flocks of sheep require special mustering for inspection every few days, requiring a high labour cost that significantly reduces the efficiency of the production system.

Information about the incidence of Old World screwworm myiasis, and its significance for livestock production and public health, is limited. While this screwworm is reported widely, there are few reports of structured studies of the economic impact of the myiasis and of suppression measures; studies in Malaysia reported annual losses of USD 4.7 million (Feldmann and Slingenbergh 2002).

Before an eradication programme is planned, however, an essential preliminary phase is the collection of comprehensive baseline data, including ecological and genetic studies to delineate the relevance of the problem (Hall et al. 2001), the infestation dynamics, and the economic, public health and environmental costs of the pest (Dyck, Reyes Flores et al., this volume). Priority areas include:

- Distribution and seasonal occurrence
- Population density
- Incidence and severity of myiasis
- Economic impact studies
- Studies on screwworm migration behaviour
- Modelling of climatic and other effects on populations
- Application of geographic information systems (GIS)
- Genetic diversity
- Risk analysis

4.3. Other Important Considerations

Countries proposing to undertake an AW-IPM programme that integrates the SIT must have a strong commitment to it. This commitment must come not only from the government, to provide resources and an operational framework, but also there must be very strong support from, and involvement of, the livestock industry and relevant private-sector groups. AW-IPM programmes require considerable input from local people to support surveillance and monitoring programmes, and to suppress outbreaks of screwworms (Dyck, Reyes Flores et al., this volume).

Clearly, the programme must be economically viable, with a favourable benefit/cost assessment, and have adequate funds. Benefits accrue from increased livestock production (better performance of animals and fewer deaths), and reduced production costs and pesticide usage.

The AW-IPM programme may also provide considerable benefits that are hard to quantify economically, e.g. environmental benefits from reduced chemical usage and impact on wildlife, and possibly public health benefits. The large mammal fauna of northern Africa and Australia are at risk from incursions of screwworms. Reductions in native fauna would impact tourism and ecosystems. In remote areas, or in areas with poor health services, there could be significant numbers of human cases. In the poorer regions of Central America, screwworm strikes in humans resulted in up to 40% mortality.

4.4. Australian Preparedness Planning for Potential Outbreaks

Australia is fortunate that neither the New World screwworm nor the Old World screwworm has become established in the country, although large parts of its northern areas are environmentally suitable for these pests (Sutherst et al. 1989).

Introduction of the New World screwworm into Australia is considered unlikely, but not impossible. In 1992, an Australian tourist returning from South America accidentally brought, in a neck wound, live New World screwworm larvae into Australia (Searson et al. 1992). This occurred in May in southern Australia, when climatic conditions are unfavourable for the survival of the insect, but it demonstrates the potential for inadvertent introduction.

The Old World screwworm, prevalent in the neighbouring countries of Papua New Guinea and Indonesia, is a substantial threat to Australia. The export of live cattle from northern Australian ports to South-East Asian nations is an important and rapidly expanding trade. In 1988 in Darwin harbour, several Old World screwworm flies were found trapped in an empty livestock vessel that had just returned to Australia after delivering cattle to Brunei (Rajapaksa and Spradbery 1989). The Old World screwworm is also present near ports in the Middle East to which live sheep are exported from various Australian ports. Both of these situations provide an opportunity for the screwworm to enter Australia as larvae, pupae or adults in empty livestock-carrying vessels. While such vessels probably represent the most likely method for the pest to gain access to Australia, accidental transport on aircraft, active myiasis in humans or companion animals, are also possibilities.

In response to the threat of the Old World screwworm to the extensive pastoral cattle-producing areas in northern Australia, for many years Australia has conducted research on this pest. From 1973 to December 1991, the Commonwealth Scientific and Industrial Research Organization (CSIRO) studied the biology and ecology of the screwworm in Papua New Guinea. In 1982 and 1986, sterile flies were dispersed from the air to evaluate their effectiveness in eradicating the Old World screwworm. It was found that sterility could be induced in a wild population (Spradbery et al. 1989, Spradbery 1990), but the efficacy of the SIT against this screwworm species was not as high as that achieved by the USDA against the New World screwworm (Wyss 2000, 2002).

From 1995 to 2000, Australia and Malaysia undertook a collaborative myiasis control research project located at the Institut Haiwan, Kluang, Malaysia. The project assisted in suppression trials of the screwworm in Malaysia, and supported research that developed and evaluated improved Old World screwworm suppression and eradication techniques. To confirm and provide confidence in the efficacy of the SIT, a demonstration to show that mass-reared and sterilized screwworms were fit and competitive in the field was made (R. J. Mahon, unpublished data).

This species was reared successfully on a hydroponics diet. Most of the rearing methods for the Old World screwworm were based on the techniques developed by the USDA for the New World screwworm (Wyss 2002b), but innovations to mass-rear larvae were developed. A small pilot mass-rearing facility was built at the Institut Haiwan, where novel production-engineering methods were applied to rearing this species (Mahon and Ahmad 2000). In its present configuration, it has a potential output of about 6 million sterile flies per week. Unfortunately, the output of Old World screwworm larvae from a given volume of diet is significantly less than is obtained in the case of the New World screwworm. There is considerable scope to improve the efficiency of mass-rearing the Old World screwworm.

In 1990, to enhance the state of preparedness, Australia's long-term Old World screwworm preparedness was reviewed and a plan developed. Models of the impact of the Old World screwworm indicated that the cost of an invasion would be high. Anaman et al. (1993) estimated that the annual cost (at 1991 values) of an endemic establishment, to beef-cattle, sheep and dairy producers in an average climate year, would be approximately USD 200 million. In comparison with the cost to the community (several times the producer losses), these costs would be trivial (McKelvie et al. 1993). The models indicated that large areas of tropical and subtropical Australia are suitable for year-round survival of the Old World screwworm, with further southern extensions in summer that would recede in winter.

Extensive cattle-grazing is the dominant industry throughout much of the northern pastoral areas of Australia. Based on experiences in the USA, it is likely that extensive cattle production, as practised in northern Australia, would not be viable if the Old World screwworm became established. Failure of the livestock industries would, in turn, impact severely on the small- to medium-sized towns servicing the industries, and, as there are few or no viable alternative business opportunities, many communities could collapse. However, by undertaking an eradication programme, an 8:1 benefit/cost ratio would be achieved (Anaman et al. 1993).

Australia's native fauna is naive to this pest, and inevitably, should an incursion occur, there would be some impact on the fauna, although it is impossible to predict the extent of it. Human cases of screwworm myiasis might also occur.

Australia has developed contingency plans to respond to a number of exotic diseases, including the Old World screwworm, involving collaboration between the commonwealth and state governments and the livestock industries. The Australian Veterinary Emergency Plan (AUSVETPLAN 1996) contains a strategy for the suppression and eradication of the Old World screwworm should it gain a foothold on the continent (Tweddle 2002). The policy is to eradicate the screwworm in the

shortest possible time, while limiting the economic impact using a combination of strategies including the following:

- Treatment of individual animals and groups to prevent or cure infestation, especially before movement
- SIT to suppress and eradicate the fly
- Quarantine and movement controls in declared areas to prevent the movement of infested animals
- Decontamination and disinfection of larvae-contaminated areas and things
- Tracing and surveillance to determine the source and extent of the infestation, and provide proof of freedom from the disease
- Zoning to define infected and disease-free areas
- Public awareness campaign to encourage rapid reporting of suspected infestations, and to facilitate cooperation from industry and communities

A fundamental plank in the eradication plan is integrating the SIT, which at present is probably the only feasible method that can eradicate an incursion of the Old World screwworm (or New World screwworm) into Australia. Being aware of earlier failures to scientifically establish its validity for this species, major elements of the preparedness strategy are to validate the SIT for the Old World screwworm in an endemically infested country, and to develop more efficient mass-rearing systems based on production-engineering principles.

It is envisaged that a facility producing 200–250 million sterile flies per week would be required. A facility with this capacity would be extremely expensive to construct, and there would be intense pressure to complete it as quickly as possible to prevent the screwworm from spreading and to minimize economic losses.

A design brief for a rearing facility (within Australia, if it were required), with a capacity to produce 250 million sterile screwworms per week, has been prepared (Phillimore 2002). Models have indicated that there is merit in constructing a facility, and then "mothballing it" until required.

A multi-species sterile insect production facility was another attractive option evaluated by Anaman et al. (1993). In the multi-insect-facility concept, the facility would be used to produce sterile insects for the suppression or eradication of endemic pests, e.g. Queensland fruit fly *Bactrocera tryoni* (Froggatt), Australian sheep blow fly *Lucilia cuprina* (Wiedemann), until an exotic pest incursion. After the exotic pest outbreak, the already-operational plant would fairly quickly be converted (in full or in part) to the production of sterile screwworms (or other exotic horticultural pests, e.g. the melon fly *Bactrocera cucurbitae* (Coquillett)). Since production would begin early in an outbreak, when the pest distribution was still restricted, a smaller capacity would be required.

The SIT could be ineffective if *Chrysomya bezziana* populations from different geographic locations proved to be a complex of sibling species (Strong and Mahon 1991; Krafsur, this volume). Studies were conducted to determine the genetic variation of *C. bezziana* samples from as many localities as possible within its geographic range (Hall et al. 2001), using a range of techniques including allozyme studies (Strong and Mahon 1991), some hybridization tests (J. P. Spradbery, unpublished data), cytogenetic studies (Bedo et al. 1994), and biochemical profiles of the exoskeleton chitin analysed by gas chromatography (Brown et al. 1998).

While differences among populations occur, the studies indicated that it is not essential that the colony used to breed sterile flies for the SIT is derived from the same location as the targeted Old World screwworm population.

Other components of the strategy are designed to minimize losses and supplement the AW-IPM eradication programme. An early warning system, part of the North Australian Quarantine Strategy (NAQS), has been established. It is based on enhanced quarantine surveillance, education, and a regular trapping programme using "swormlure", an attractant for screwworm flies.

Ivermectin and avermectin are effective systemic pesticides against the larval stages of the Old World screwworm (Spradbery et al. 1991), and would be used to treat infected animals and those wounded in the course of normal husbandry procedures. Ivermectin boluses prevent screwworm myiasis for 102 days (Wardhaugh et al. 2001). Ivermectin boluses, or another long-acting formulation, could limit the incidence and spread of the Old World screwworm in extensive pastoral areas. This would reduce the population of screwworms, and perhaps thereby the incidence of myiasis, and certainly reduce dispersal. However native and wild animals would be a problem since they cannot be treated effectively.

Public awareness and early reporting of suspect myiasis are emphasized, both prior to, and in response to, an outbreak, especially in northern Australia. A video, entitled "Recognizing exotic livestock disease number 7: screwworm fly", has been produced to train veterinarians and other health professionals. To encourage the submission of larvae from myiasis strikes, specimen collection kits have been supplied to producers (and health centres) in northern Australia. Spradbery (2002) prepared a diagnostic manual, and people have been trained in identifying the Old World screwworm and differentiating it from other species.

5. CONCLUSIONS

The parasitic stage of the New World screwworm fly, documented for its transboundary importance, is an animal and human disease that causes significant sanitary and economic damage when it enters a country previously free of the screwworm. Screwworm-free countries must be informed about its epidemiology and methods of suppressing it, and establish appropriate prevention measures to avoid introducing it. Nowadays, with international transport, the globalization of economies, and the rapid long-distance movement of animals and animal products, the risk of transporting pests and diseases has greatly increased. As a result, stronger quarantine measures are being applied, with the potential of restricting free world trade. Some risks can be mitigated if the causal factor is removed. The elimination of the New World screwworm in North and Central America has removed an obstacle to animal movement within this zone.

Although in some areas the traditional methods of suppressing screwworms apparently give good results, the use of modern area-wide approaches must be considered to eliminate the disease. In addition, other positive benefits will arise. As a direct result of the screwworm eradication programme in North and Central America, national animal-health organizations in the region are now working closely together. Also, within each country, the government animal-health sector has built

stronger bridges to producers. Since the screwworm programme directly involved producers, and depended on them to assist in the eradication, they also became part of the programme and took pride in the results; this stimulated cooperation in other programmes.

The modern area-wide approach to eradicate screwworms has proved to be successful. However, it is essential that the governments of affected countries in Africa, Asia, South America, the Caribbean, and the Middle East give political support to the elimination of the disease, and thus avoid continuous economic losses. As well, because of cutaneous myiasis in humans, screwworm eradication improves human health.

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CHAPTER 7.2.

IMPACT OF FRUIT FLY CONTROL PROGRAMMES USING THE STERILE INSECT TECHNIQUE

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SUMMARY

Measuring the impact of area-wide integrated pest management (AW-IPM) programmes, that use the sterile insect technique (SIT) to control fruit fly pests of economic significance, is complex. These programmes affect practically the whole horticultural food chain. In this chapter, the impact of the programmes is assessed by focusing only on the benefits generated to producers and traders of horticultural products, the direct beneficiaries. This is done first by describing the types of benefits accrued from these programmes, second by explaining the factors that shape programme benefits, and finally by presenting several examples to illustrate how the SIT technology, when properly applied for eradication, containment, suppression, or prevention purposes, can generate substantial direct and indirect benefits to the horticulture industry.

1. INTRODUCTION

Area-wide integrated pest management (AW-IPM) programmes, that use the sterile insect technique (SIT) to control fruit fly pests of economic significance, are designed to have a positive impact on society. They allow the production of more and better-quality horticultural products at a lower cost, and this increases the food supply, diversifies markets, creates new jobs, but still protects the environment. Measuring the impact of such programmes integrating the SIT is a complex task. This chapter will concentrate more on the benefits generated by such programmes to direct beneficiaries — the producers and traders of horticultural products, although benefits impact the whole food chain system from farmers to consumers, passing through all the intermediate links of packing, shipping, distributing to wholesale and retail markets, and selling. In addition, the benefits branch out to other related businesses, such as suppliers of raw materials and some other services.

In the past decade, several studies have assessed the economic feasibility of potential fruit fly programmes integrating the SIT. These studies have been the basis for deciding to invest or not to invest in such programmes (Reyes et al. 1991; IAEA 1995; Enkerlin and Mumford 1997; Larcher-Carvalho et al. 2001; Vo et al. 2002; Larcher-Carvalho and Mumford 2004; IAEA 2005; Mumford, this volume). However, more relevant to this chapter are similar studies to measure the economic returns and impact of ongoing programmes at different stages of implementation (USDA/APHIS 1993; Mumford et al. 2001; Kakazu 2002; Knight 2002; Mumford, this volume).

To date, no study has measured, in a comprehensive and detailed manner, the overall impact of programmes using the SIT. Therefore, this chapter assesses the impact of programmes integrating the SIT by reviewing the most common direct and

indirect benefits to the horticulture industry, in particular, to the producers and traders of horticultural commodities.

2. DIRECT AND INDIRECT BENEFITS

Inefficient pest control practices, or no control at all, result in direct and indirect losses from fruit flies that translate into direct and indirect benefits when using more effective alternative control methods such as the SIT. For example, if direct fruit fly damage would normally cause a 25% loss in fruit yield, the area-wide application of an improved technology that reduces damage by 80% would lead to a direct benefit of a 20% increase in yield. Another example is the indirect damage from secondary pest outbreaks caused by killing natural enemies with regular insecticide "cover" sprays. If an effective and environment-friendly control technology is used, the amount of insecticide applied is reduced and more natural enemies survive to suppress secondary pest populations; in this situation, a 10% indirect loss would become a 10% indirect benefit.

The direct benefits commonly used to measure the impact of programmes integrating the SIT are:
- Increase in fruit yield and quality through reduced damage
- Reduction in production costs through a more cost-effective control method

The indirect benefits commonly used to measure the impact of programmes integrating the SIT are:
- Increase in fruit and vegetable export volumes, and market retention or diversification, through effective control of quarantine pests
- Increase in export volumes through reduced rejections of commodities which do not comply with the insecticide residue levels
- Increase in fruit yield through reduced secondary pest outbreaks
- Savings in medical costs, and occasionally deaths, through reduced exposure to insecticides, and also in legal costs arising from damage to private or public property as a result of insecticide misuse
- Greater protection of beehives resulting in increased fruit yield through increased crop pollination
- New jobs created in the horticulture industry and related industries
- Better human nutrition due to a per capita increase in fresh-fruit intake
- Savings in public health and environmental costs through reduced insecticide residues in fruit, water reservoirs, and soil

These indirect benefits are very difficult to assess, and in most cases have been accounted for only qualitatively (Pimentel et al. 1992).

The impact of programmes integrating the SIT has focused mostly on the direct benefits, but attempts have been made to quantify some indirect benefits using ad hoc methodologies (Enkerlin 1997). To partially quantify benefits to the environment from using methods such as the SIT, the monetary value of savings accrued from minimizing insecticide use has been estimated (Pimentel et al. 1992). For example, in 1997, it was estimated that some Middle East countries (Israel,

Jordan, and Territories Under the Jurisdiction of the Palestinian Authority) would over 6 years, if current control practices prevailed, spend USD 46.7 million for insecticides to suppress the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). This compares with USD 5.8 million that would be spent on insecticides if pest suppression with the SIT were adopted, a benefit of USD 40.9 million in 6 years (Enkerlin and Mumford 1997).

Another example of an indirect benefit is the per capita increase in fruit production. A study (Vo et al. 2002), to assess the economic feasibility of fruit fly control in the Central American region using the SIT, indicated that the average per capita production of fruits was 17.2 kg/year, 4.3 times lower than the average annual per capita production in Middle East countries (73 kg/year). This is rather ironic, since the subtropical/tropical climate and fertile soil in Central America should be more conducive to commercial fruit production than the arid conditions and poor soil that prevail in the Middle East. Effective fruit fly control in the Central American region would reduce fruit damage and increase the annual per capita production of fruits to almost 19 kg/year, equivalent to 48 600 tonnes valued at USD 25 million/year. Although this would be an improvement, the amount is still low compared with the 73 kg per capita per year produced in the Middle East.

On a country basis, the amount of fruit (from fruit fly hosts) produced per capita per year is as follows: Costa Rica — 85 kg, El Salvador — 12.2 kg, Guatemala — 18 kg, Honduras — 11.5 kg, Nicaragua — 24.7 kg, and Panama — 16 kg. (There are no data available for Belize, but being a Mediterranean fruit fly-free country, with a traditionally friendly market under the Commonwealth umbrella, the per capita production can be assumed to be well above the regional average.) Clearly in Costa Rica, where the fruit industry is well organized and fruit production has been modernized, the annual per capita fruit production is comparable with the highest values in the world. On the other hand, in the other countries in the region, especially El Salvador, Honduras, and Panama, the annual per capita fruit production is extremely low — due to a lack of appropriate infrastructure, deficient growers' organizational schemes, and insufficient government incentives for fruit production and commercialization. Effective fruit fly control would permit the development of fruit fly low-prevalence and fly-free areas, paving the way for private investment in fruit production and commercialization for both the domestic and export markets, and leading to substantial increases in per capita production, new job creation, and stimulation of the economy (Vo et al. 2002).

In Jordan, the cost of poisonings arising from using organophosphate insecticide against fruit flies was assessed. It was estimated that USD 1.6 million/year are spent for treatment of moderate to severe poisonings, and for labour-days lost. Each year an average of 94 tonnes of insecticide, i.e. technical material, are applied to control the Mediterranean fruit fly. This represents 33.8% of the total insecticide use in Jordan. Thus, assuming that sprays against this fruit fly cause a corresponding one-third of poisoning cases, about USD 550 000 are spent each year to treat persons poisoned when spraying against the Mediterranean fruit fly. In the assessment of total benefits that would be produced should the SIT technology be adopted to

suppress this pest in Jordan, this cost was included in the indirect benefits (IAEA 2001).

More recently, a study to measure the direct and indirect benefits of Mediterranean fruit fly control in the island of Madeira, Portugal (Dantas et al. 2004), was conducted using two valuation techniques. The first involves quantifying the costs incurred by society when dealing with the externalities caused by insecticides. In this case, only those costs that can be attributed specifically to the use of insecticides for Mediterranean fruit fly control in fruit crops are included. The second method, used for environmental valuation, is called "contingent valuation". This method is based on the choices people make when they are presented with a variety of goods and services; a "willingness to pay" (WTP) indicates a positive preference. Quantification of the indirect benefits accrued from using the SIT for Mediterranean fruit fly suppression in Madeira makes economic returns positive in two out of three control scenarios analysed (IAEA 2005).

The benefits generated by programmes that control fruit flies with an "integrated SIT approach" will be determined and shaped by some obvious factors: the characteristics of the pest problem, and the programme objectives and scope set by the participating organizations and stakeholders. There are other not-so-obvious factors that will influence the benefits: the type of organizational structure used to execute the programme, the level of participation of the main stakeholders, and the strategic approach selected to achieve programme objectives. A brief explanation of how these factors affect the benefits produced by programmes applying the SIT, and hence their impact, is presented in the following section.

3. FACTORS THAT AFFECT BENEFITS PRODUCED BY PROGRAMMES USING SIT

3.1. Organizational Structure and Stakeholder Participation

To date, most fruit fly programmes integrating the SIT have been public projects, operated through a centralized federal government structure, with different degrees of participation from local governments, and with limited participation from the horticulture industry at farm level. Often these have been prevention and emergency programmes aimed at protecting the welfare of the horticulture industry, keeping out quarantine pests or eliminating outbreaks of introduced pests. A typical example of such programmes is Chile's National Preventive Fruit Fly Programme operated by the Agriculture and Livestock Service of the federal government (Olalquiaga and Lobos 1993, section 4.2.).

Other types of programmes include those where strong alliances have been formed among the federal and local governments and the fruit industry, which share capital investment, operational costs, and responsibilities. The programmes aim to control established key fruit fly pests that, by directly affecting production and commercialization, limit the development of the horticulture industry. A typical example of such programmes is the National Fruit Fly Campaign of the Mexican government (SAGAR/IICA 2001, section 4.6.).

As the SIT technology becomes more cost-effective when compared with other more-conventional methods of fruit fly control, interest in its application from the private sector will grow. Organizational schemes, where the horticulture industry and other private and/or civil organizations form alliances and partnerships for financing and operating area-wide programmes integrating the SIT, will become more common. In such cases, the participation of federal and local governments will be limited to a supporting role, e.g. creating the legal framework for smooth implementation of programme activities, setting standard operating procedures (SOPs) in the form of phytosanitary norms for the production, shipment, importation, and release of sterile flies. Also, when required, governments must enforce quarantine regulations. An example of a scheme where the private sector has become involved in a programme is the fruit fly suppression programme in the Hex River Valley, South Africa, in operation since 1997 (Barnes et al. 2004, section 4.4.).

Given today's diverse political and economic conditions, it is expected that future programmes integrating the SIT will operate under several different types of organizational structure, probably determined by the specific political and socio-economic conditions in the country. Countries such as Argentina, Brazil (Braga Sobrinho et al. 2004), Costa Rica, and South Africa will possibly embark on programmes run by government-private alliances, and even programmes operated fully by the private sector. Other countries, such as Peru, Spain, and Thailand, may conduct more government-driven programmes. A key element for success in government or semi-government programmes is that the financial resources must be available in sufficient amount and on time. They should be administered under a flexible scheme where the budget adapts to the dynamics of the programme's operations, and not the programme to the budget (Dyck, Reyes Flores et al., this volume).

The organizational structure of programmes integrating the SIT, and the participation of stakeholders, will affect the benefits produced. Government-run programmes come at low cost to the direct beneficiaries, since most of the required capital and operational inputs are fully or partially subsidized, and normally governments do not seek cost-recovery. If the programme is effectively executed, the beneficiaries start to accrue benefits or profits quickly. However, these programmes rely on political and economic stability, and the managerial and technical capabilities of governments are more exposed to failure. Since programmes integrating the SIT are financially demanding and management intensive, countries that decide to embark on such programmes must have a strong political commitment and a minimum infrastructure. Countries that begin a large-scale SIT governmental programme, with little or no participation of the private sector, and no support from international organizations, are likely to fail in the endeavour. This situation contrasts with programmes that are established as strategic alliances between government and private sector, with the support of international organizations, and where the stakeholders are actively involved in programme execution. Under this scheme, with private assets and financial capital invested in the programme, there is little or no government subsidy. Stakeholders have to wait longer for payback of the initial investment, and for profits to start flowing. However, in the long term, since

they are not so dependant on political stability, these programmes tend to be more sustainable, and normally programme management is carried out more effectively.

3.2. Strategic Options

Based on the status of the target pest in the area of interest, fruit fly programmes integrating the SIT can be grouped into four main strategic options, as follows: (1) eradication — eliminate from an area an established pest or an outbreak of an introduced pest, (2) suppression — reduce pest populations, (3) containment — apply phytosanitary and regulatory measures in and around an infested area to prevent the spread of the pest, and (4) prevention — apply phytosanitary and regulatory measures to prevent the introduction or reintroduction of a pest into a pest free area (FAO 2002; Hendrichs et al., this volume).

An example of containment is the Moscamed Programme (section 4.5.), operational since 1978 along the border between Mexico (Chiapas) and Guatemala, which protects Mexico and the USA from further northward spread of the Mediterranean fruit fly. An example of prevention is the Preventive Release Programme (PRP) in California (section 4.1.), operational since 1994, and in Florida since 1998, to protect pest free areas by the continuous release of sterile Mediterranean fruit flies that prevents any introduced flies from reproducing and establishing.

Suppression, eradication, containment, and prevention programmes provide the same types of direct and indirect benefits listed above (section 2). However, in terms of economic returns and subsequent impact, there are marked differences among the four control strategies.

The most profitable strategy is prevention. It is always much more cost-effective to proactively protect a pest free area from the introduction and establishment of a pest than having to "live with" or eradicate it. However, when the target pest is already established in an area, the options for using the SIT to control the pest are suppression, containment, or eradication.

Figure 1 shows a typical net-benefits trend for three control options — SIT suppression, SIT eradication, and insecticide-bait suppression.

Net-benefits tend to be greater in an eradication programme than in a suppression programme. After eradication has been achieved, and the area has been certified fly-free, horticultural products can be exported to fly-free high-value markets without complying with expensive postharvest treatments and other quarantine regulations. Furthermore, the ongoing cost of maintaining an area fly-free (detection networks, quarantine regulations, and in some cases the release of sterile flies), although costly, would normally be lower than the cost of a permanent SIT suppression programme. Once the eradication goal has been reached and benefits fully established, the combination of higher gross revenues at a lower cost makes the eradication approach more economical than the suppression approach (Fig. 1). However, an eradication strategy requires greater initial capital investment than a suppression strategy. In addition, after eradication has been achieved, the fly-free area will be exposed, depending on the situation, to a certain risk of reinfestation (quarantine operations

are vulnerable and subject to some degree of failure). Recurrent pest outbreaks require costly emergency programmes to eradicate the reinfestations, and the enforcement of quarantine restrictions by trading partners can last for months or even years. This cost needs to be included when calculating the total cost of an eradication programme. There are situations where the risk of reinfestation is very high, and an eradication programme is not economically justifiable; the stakeholders would be better off with a suppression or preventive programme (Mumford, this volume).
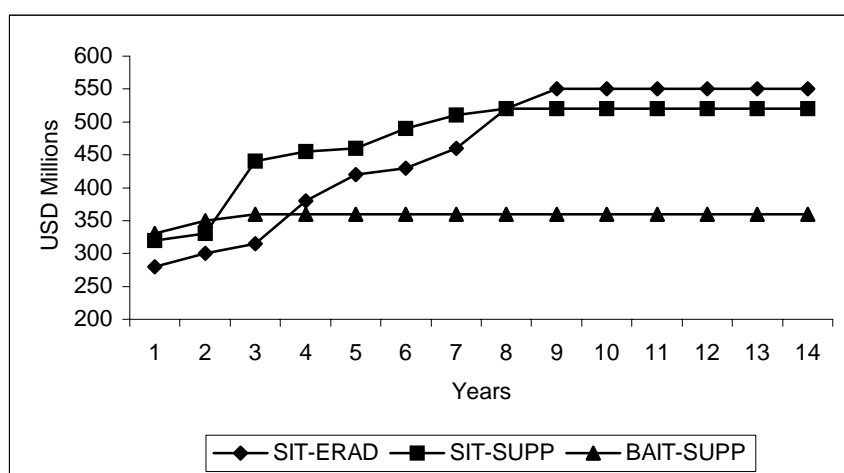
Figure 1. Typical net-benefit trend for three control options: SIT eradication, SIT suppression, and insecticide-bait suppression. (Figure adapted from Enkerlin and Mumford 1997.)

From technical and economic points of view, any one of the strategic approaches described above may, in a particular situation, be the best option. The choice depends on the conditions prevailing in the target area, such as the pest's status, the geography and ecology of the area, the available resources and overall plant protection and quarantine infrastructure, and the commitment and level of participation of stakeholders and their willingness to take risks. Therefore, it is critical that a decision to embark on one or the other approach is based on technically and economically sound analyses of the pest problem.

4. BENEFITS ACCRUED FROM FRUIT FLY PROGRAMMES USING SIT

The SIT can be a cost-effective component of the AW-IPM of some of the most important insect pests, including fruit flies of major economic significance, such as the Mediterranean fruit fly, oriental fruit fly *Bactrocera dorsalis* Hendel, melon fly *Bactrocera cucurbitae* (Coquillett), Mexican fruit fly *Anastrepha ludens* (Loew),

West Indian fruit fly *Anastrepha obliqua* (Macquart), Queensland fruit fly *Bactrocera tryoni* (Froggatt), and Caribbean fruit fly *Anastrepha suspensa* (Loew) (Nigg et al. 2004).

At least 20 AW-IPM programmes in the world integrate the SIT to control fruit flies. In Table 1, they are grouped by the strategic options used, i.e. eradication, suppression, containment, prevention, and combinations (section 3.2.; Hendrichs et al., this volume). A few programmes were selected to illustrate the benefits that have been produced.

Most of the data available on programme benefits and impact are based on quantifications of direct benefits, and in a few cases indirect benefits are also included. However, most of the indirect benefits are usually not accounted for, and therefore in most cases the benefits of these programmes are underestimated. Nevertheless, it is very evident that, when the technology is properly applied, the programmes generate substantial benefits to the horticulture industry.

4.1. Fruit Fly Prevention Programme — California, USA (Box 1)

4.1.1. Problem Definition
The Mediterranean fruit fly is a serious threat to California's economy. For the fruit industry alone, the annual value of susceptible crops in 2002 was about USD 4700 million (USDA/NASS 2002, 2003a, b; CASS 2002). The rate of introduction of this exotic pest, that can attack more than 250 different crops (Liquido et al. 1991), has been increasing with growing trade and tourism. Since 1975, more than USD 256 million (in state and federal funds) have been spent to eradicate small and large infestations, primarily in the Los Angeles Basin and the San Francisco Bay area.

4.1.2. Major Achievements
The Preventive Release Program (PRP) has resulted in a 96% reduction in the number of wild Mediterranean fruit flies caught in the treated Los Angeles basin, and more than a 96% reduction in the number of fly infestations. Before the shift to the PRP (1987–1993), an average of seven or eight Mediterranean fruit fly outbreaks occurred every year, costing the state approximately USD 33 million per year (USDA/APHIS 1992). Since the implementation of the PRP (1994–1998), the overall cost of the programme has been cut in half, and only two small Mediterranean fruit fly infestations have occurred within the boundaries of the release area. These infestations remained confined and were quickly eliminated without applying aerial sprays of toxic bait and without trading partners establishing temporary quarantines for Californian exports.

Table 1. Fruit fly AW-IPM programmes integrating the SIT, grouped by strategic option

Strategic option	Programme
Prevention	Mediterranean Fruit Fly Preventive Release Programme (Los Angeles Basin, California, USA)
	Mediterranean Fruit Fly Preventive Programme (Tampa-Miami, Florida, USA)
	Mexican Fruit Fly Preventive Programme (Rio Grande Valley, Texas, Mexico – US border)
	Mexican Fruit Fly Preventive Programme (Tijuana, Baja California, Mexico – US border)
	National Fruit Fly Control Programme (Chile)
Containment	Mediterranean Fruit Fly Programme "Programa Moscamed" (Guatemala, Mexico, USA, 1983 – present)
	Binational Chile-Peru Programme for Mediterranean Fruit Fly Eradication (1996 – present)
	Queensland Fruit Fly SIT Control Programme (South-Eastern Australia)
Eradication	Mediterranean Fruit Fly Programme "Programa Moscamed" (Guatemala, Mexico, USA, 1978–1982)
	Melon Fly Eradication Programme (Okinawa, Japan, 1982–1994)
	Oriental Fruit Fly Eradication Programme (Guam)
	National Mediterranean Fruit Fly Control Programme (Chile 1992–1995)
	Mediterranean Fruit Fly SIT Control Programme (Southern Australia)
	Mexican Fruit Fly and West Indian Fruit Fly Eradication Programme (North-West Mexico)
	Mediterranean Fruit Fly Eradication Programme "PROCEM" (Mendoza – Patagonia, Argentina, 1992 – present)
Suppression	Mediterranean Fruit Fly Suppression Programme (South Africa)
	Oriental Fruit Fly Suppression in Pilot Areas (Thailand)
	Melon Fly, Oriental Fruit Fly, and Mediterranean Fruit Fly Area-Wide Integrated Pest Management Program (Hawaii)
	Mediterranean Fruit Fly Suppression Programme "Programa Madeira-Med" (Portugal)
	Bactrocera spp. Pilot Suppression Programme (Guimaras Island, Philippines)
	Mediterranean Fruit Fly Suppression Programme (Israel/Jordan)
	Mediterranean Fruit Fly SIT Control Programme (Peru)
	National Fruit Fly Campaign (Mexico)
	Mediterranean Fruit Fly Suppression Programme (Spain)

> Box 1. *Mediterranean Fruit Fly Preventive Release Programme (PRP) in Southern California, California Department of Food and Agriculture (CDFA 2002)*
>
> After years of using the conventional reactive approach (detecting and eradicating Mediterranean fruit fly infestations at a local level), in 1994 the control strategy shifted to a proactive approach that emphasizes area-wide measures to prevent infestations from developing. This approach (a Los Angeles Basin-wide programme that includes twice weekly sterile-male releases) has become a major component of the CDFA's comprehensive pest prevention programme to prevent this pest from becoming established in California (Dowell et al. 2000). This programme is jointly coordinated and operated by county, state, and federal authorities.

4.1.3. Estimated Benefits

The impact of this programme has been measured in terms of the potential economic losses that the pest would inflict on the horticulture industry and home-garden fruit production if it became established in California. The parameters that have been used to measure the impact are the following: yield loss in agriculture and home-garden production, increased insecticide use (from 127 to 2270 tonnes/year of active ingredient), loss of export markets, and annual quarantine compliance costs (CDFA 2002). It has been estimated that a permanent Mediterranean fruit fly infestation could cost California's economy USD 1300–1900 million annually (yield loss, control costs, quarantine compliance costs, and loss of export markets, not including the social and environmental costs), with the loss of 14 000 or more jobs (Siebert and Pradham 1991).

The insecticide savings (amount of active ingredient not used) have been very large. If the CDFA had applied aerial toxic-bait sprays on the same 3800-km^2 area that was infested in 1994, 458 tonnes of malathion would have been used. In contrast, since implementing the basin-wide sterile Mediterranean fruit fly releases, only 19 kg of malathion have been applied.

4.2. Fruit Fly Prevention/Containment Programme — Chile (Box 2)

4.2.1. Problem Definition

An important component of the Chilean economy is the production and export of fruits and vegetables, worth about USD 2000 million annually. As a prerequisite to importing these commodities, some of the main commercial partners, e.g. USA and Japan, require that produce are grown in a certified fruit fly-free area. Except for Arica province at the northern tip of the country, and occasional outbreaks in other provinces, Chile has historically been regarded as a fruit fly-free country. Following various attempts to eradicate the Mediterranean fruit fly using baits sprays, the SIT was introduced, and after 8 years of an intensive programme, in 1995 the fly was eradicated in Arica, and Chile was declared a fruit fly-free country (MAG/SAG 1995).

Box 2. National Fruit Fly Programme in Chile

Chile's fruit fly-free status has allowed one of the most important export-oriented horticulture industries in the world to develop. To protect this valuable asset, in 1980 the Government of Chile, through the Servicio Agricola Ganadero de Chile (SAG) of the Ministry of Agriculture (MAG), created Chile's National Fruit Fly Programme — to prevent the introduction and establishment of any fruit fly species of economic importance, including the Mediterranean fruit fly and the economic species in the genera *Anastrepha* and *Bactrocera* (Olalquiaga and Lobos 1993).

The National Fruit Fly Programme in Chile operates through a centralized organizational structure of the Ministry of Agriculture. As part of a regional approach to the fruit fly problem, the Government of Chile has subscribed binational agreements with Argentina and Peru. Through these agreements the quarantine infrastructure and fruit fly control activities in these neighbouring countries have been strengthened, and thus the risk of introducing fruit fly pests from these countries has been reduced.

Chile has achieved its fly-free status by implementing two major strategic activities:

1. A preventive programme based on an effective national and international quarantine system (including interprovincial quarantine road stations and international quarantine at ports of entry), and an extensive and highly sensitive fruit fly trapping network to detect fruit fly introductions at an early stage. Outbreaks of exotic fruit flies, including the Mediterranean fruit fly, have been eradicated through the effective execution of an emergency eradication plan based on detecting and eradicating infestations.

2. In Arica province, the ongoing Mediterranean fruit fly AW-IPM programme that integrates the SIT functions as a containment barrier to avoid the natural or artificial spread of fly populations to the main fruit and vegetable production areas in the central and southern parts of the country.

For years, Chile has been subjected to increasing risks of pest introductions through more trade, tourism, and people coming from neighbouring countries. Consequently, there has been an increase in the rate of Mediterranean fruit fly detections and outbreaks. Nevertheless, the Government of Chile (through SAG) has strengthened its National Fruit Fly Programme by gradually incorporating state-of-the-art technology. This includes introducing an improved genetic sexing strain of the Mediterranean fruit fly into the mass-rearing facility in the Lluta Valley (Arica province), DNA-identification techniques to assess the geographical origin of introduced flies, updated fly-trapping systems, and X-ray machines at critical points of entry to facilitate detection and confiscation of fruit hosts of the Mediterranean fruit fly (Lindquist and Enkerlin 2000).

4.2.2. Major Achievements

Since its inception in 1980, Chile's National Fruit Fly Programme has, through effective eradication activities, successfully kept the country free of economic species of fruit flies. Chile's programme to remain fruit fly-free is one of the best in the world.

Strengthening binational cooperation with Argentina and Peru, and formalizing binational cooperation with Bolivia, are cornerstones that sustain Chile's fruit fly-free status.

4.2.3. Estimated Benefits

Since Chile was declared a fruit fly-free country, fruit exports have grown to an annual 2 million tonnes of fruits, mainly table grapes, apples, stone fruits, kiwis, and avocados, valued in 2002 at USD 1600 million (J. Gonzalez, personal communication).

Each year the government of Chile spends on average USD 4 million to keep the country free of fruit flies. If multiple outbreaks occurred in Chile's Metropolitan Region, from where fruits (that are hosts of the Mediterranean fruit fly) worth USD 250 million are exported each year to the USA, the industry would lose an estimated USD 78 million per year, just in market loss and compliance with US quarantine regulations. If this figure is divided by the average annual cost of the national programme, a benefit/cost ratio of 17:1 is obtained. This ratio would be significantly higher if other cost factors, e.g. control costs, yield losses, and general social and environmental costs, were included, and if the losses would be projected to a national level instead of only the Metropolitan Region (Lindquist and Enkerlin 2000).

Chile's National Fruit Fly Programme (Box 2) has been the driving force behind the expansion of the fruit and vegetable export industry, one of the main contributors to the country's gross domestic product (GDP).

4.3. Fruit Fly Eradication Programme — Japan (Box 3)

4.3.1. Problem Definition
In its current distribution range, the melon fly is the most destructive pest of cucurbit crops. It is found in Africa, India, South-East Asia, and islands in the Pacific (including Hawaii). In Japan, it was first discovered in 1919 in the Yaeyama Islands, and between 1919 and 1970 it invaded most island groups in the south of Japan. In Okinawa, this pest affected more than 40 important vegetables and fruits, inflicted substantial direct damage to fruits, and prevented the export of these infested commodities to fly-free areas.

Sugar cane was the most important cash crop in Okinawa, accounting for about 50% of cultivated land and 20% of all farm income. However, as a result of stagnant prices and productivity, as well as increased international competition, income from sugar cane production declined significantly. Okinawa's sugar industry survived only because of the government's price support programme. As a result, farmers and the local government needed to diversify from monoculture sugar cane-centred agriculture into other cash crops, e.g. flowers and tropical fruits such as mango. The other promising agricultural strategy was to produce "healthy" food, and for Okinawa to become a brand name for "healthy longevity" because of its world-renowned "healthy island" image.

Due to this situation in Okinawa, in the early 1970s the eradication of the melon fly became the top priority project at local and national levels. The problem was urgent since the fly was rapidly spreading to other islands and, unless specific eradication measures were undertaken, might spread to mainland Japan.

4.3.2. Major Achievements
- First successful use of the SIT for melon fly eradication in island communities
- Eradication was achieved with no harm to the health of the public or the environment

- Lifting of internal quarantine regulations to allow transport to non-infested areas resulted in significant expansion of major horticultural products
- Training of foreign technicians in the AW-IPM of the melon fly

4.3.3. Estimated Benefits

A post-programme economic assessment, prepared by the Research Institute for Subtropics (Kakazu 2002) using the methodology described by Enkerlin and Mumford (1997), clearly shows the benefits of melon fly eradication. The estimated net-benefits are those arising from the commercial shipments of commodities that are hosts of the melon fly. Not included in the assessment were indirect benefits for the island economies, such as human health and environmental protection, insecticide-free farming, preservation of natural enemies, savings in fumigation and quarantine costs, and above all preventing the insect from spreading to the mainland of Japan. Furthermore, due to data constraints, the indirect social benefits were not estimated. The programme proved to be economically viable, even though only the increase in commodity shipments was considered in the benefit equation.

Box 3. Melon Fly Eradication Programme in Okinawa, Japan (Kakazu 2002)

Okinawa's prefectural government, with the support of the Japanese national government and active participation of staff of municipalities (mainly in field operations) and agricultural cooperatives, operated the programme. In 1972, an experimental melon fly eradication project using the male annihilation technique (MAT) and the SIT was conducted in Kume Island. Following successful eradication, in 1979 a project was initiated that gradually covered most archipelagos in the south of Japan; it was completed in 1993. In view of the geographic closeness of the southernmost islands to Taiwan, where the melon fly is present, currently the programme operates in these islands a continuous detection, quarantine, and preventive release operation against re-establishment of the pest.

If the northward spread of the melon fly had not been stopped, the potential loss for farmers and the horticulture industry in general would have been very substantial, and the cost of an eradication programme would have risen enormously. As a result of the successful eradication programme, the production of Okinawa's high-valued niche products, such as bitter melon and mango, have risen sharply. Between 1990 and 2000, bitter melon production rose from 2720 to 6220 tonnes, a 2.3-fold increase. Similarly, over the same period, mango production increased from 278 to 1290 tonnes, a 4.6-fold increase; all together this is equivalent to USD 335 million in 10 years. (These benefits are underestimated since the savings from fumigation and quarantine costs were not included in quantifying the benefits.) This figure should be compared with the much lower total programme cost, USD 172 million, during the 10-year eradication period (1982–1991).

In 1996, 6 years after achieving eradication in the main islands, the programme reached its break-even point and repaid the initial investment. Between 1997 and 2000, the programme cost was estimated to be USD 31 million, but gross revenues were USD 167 million (from the sale of commodities shipped to mainland Japan and other countries). If the total accumulated gross revenues are divided by the total

accumulated costs during this time period, the benefit/cost ratio shows that 5.4 dollars were returned for each dollar invested. For a public investment project in Japan, it is a remarkable achievement that the programme was generating positive net-benefits only 6 years after eradication.

4.4. Fruit Fly Suppression Programme — South Africa (Box 4)

4.4.1. Problem Definition
The Mediterranean fruit fly and the Natal fruit fly *Ceratitis rosa* Karsch occur in the Western Cape province, South Africa. The Hex River Valley is a major production area for table grapes; an average of 15.5 million cartons is exported annually. The dominant and economically important species is the Mediterranean fruit fly. It causes direct damage to fruit and requires costly insecticides sprays, and infested fruit results in rejections of boxed table grapes by the phytosanitary inspectors of importing countries (Barnes and Eyles 2000).

4.4.2. Major Achievements
Growers and scientists now understand the benefits of applying the concept of AW-IPM, and the advantages to international trade of establishing low pest prevalence areas (FAO 2002). They also have effectively adopted the SIT technology and established the required infrastructure, which is now managed by the private sector. According to the growers in the valley, integrating the SIT to suppress the Mediterranean fruit fly has been very successful. From 1997 to 2002, insecticide use dramatically decreased, and fly mean population levels were reduced from 0.9–1 flies per trap per day, in the 3 years (1997–1999) before the release of sterile flies, to 0.1–0.4 flies per trap per day in the 3 years following the release (2000–2002) (Barnes et al. 2004).

These positive results have encouraged other associations of fruit growers outside the Hex River Valley to also embark on SIT activities. Negotiations are underway by the private sector to obtain local and international funding to construct and operate a large commercial rearing facility, with the possibility of also producing other insect pest species.

Box 4. South Africa Mediterranean Fruit Fly Suppression Programme (IAEA 2002)

In 1997, a pilot project to control fruit flies using the SIT was implemented in 10 000 hectares (100 km^2) in and around the Hex River Valley. The goal was to suppress, in a cost-effective and environment-friendly manner, the Mediterranean fruit fly populations to below the economic threshold, and then create an internationally recognized low pest prevalence area (FAO 2002).

The organizational structure of this project is rather unique. It is a partnership between Infruitec/Nietvoorbij (a branch of the Agricultural Research Council (ARC), a parastatal body, with a mandate to conduct research, technology development and transfer) and the Hex River Valley Research Services Trust (which represents the deciduous fruit growers). Through an export carton levy, the growers raise funds to support programme operations (Barnes and Eyles 2000). The sterile male production, initially established by the ARC, is now managed by the private sector, and growers manage the fly release and other field operations.

4.4.3. Estimated Benefits

By using environment-friendly technology, and reducing production costs and increasing revenues, the Mediterranean fruit fly suppression programme increased the profits of the table-grape industry in the Hex River Valley. By replacing insecticide applications with a combination of aerial and ground releases of sterile male flies at hot spots, the reduction in control costs was substantial, from USD 350 000/year with chemical control to USD 130 000/year with the SIT. Rejections, due to fruit fly infestation, of exported cartons of table grapes from the valley were reduced approximately 50%. In 2000, a reduction of 60% in rejections of cartons by phytosanitary inspectors of importing countries represented savings of USD 150 000. For the 2001/2002 season, the direct benefits totalled USD 370 000/year, at a cost of USD 130 000, which is equivalent to a benefit/cost ratio of 2.8:1.

The expected medium-term impacts of the area-wide project include:

- Expansion of export markets by meeting sanitary and phytosanitary restrictions
- Creation of new jobs in agriculture (not only for large producers, but also emerging small producers) and related industries
- Strengthening regulatory, research, and development support of the fruit industry
- Protecting the environment and the health of farm workers

4.5. Fruit Fly Containment Programme — Mexico and Guatemala (Box 5)

4.5.1. Problem Definition

The Mediterranean fruit fly was introduced into Costa Rica in 1955. It spread across Central America, causing devastating effects on fruit production, and limiting the development and growth of the fruit industry (section 2). The pest became established in Guatemala in 1976, and in 1977 was detected in the border zone between Guatemala and Mexico. By 1979, the fly had spread to the Mexican states of Chiapas and Oaxaca, gone beyond the coffee belt along the South Sierra Madre Mountains, and threatened the states of Campeche, Tabasco, and Veracruz. If the pest had advanced beyond the Isthmus of Tehuantepec, 600 km into Mexican territory, the US government threatened to close its border to imported Mexican fruits and vegetables, and fly eradication would have been practically impossible (Schwarz et al. 1989).

Gutierrez (1976) estimated that preventing the establishment of the Mediterranean fruit fly in Mexico translated into annual savings of at least USD 2000 million in direct damage (including yield loss and cost of insecticide treatment) and indirect damage (including loss of the price differential obtained from selling the produce in export markets). In addition, if the pest became established, thousands of jobs across the production chain would be lost, and substantial environmental costs would be generated by the tonnes of insecticides that would have to be sprayed to keep the pest under control.

Box 5. Mediterranean Fruit Fly Containment Programme "Programa Moscamed"

Due to the threat that this pest posed to horticulture industries in Guatemala, Mexico, and the USA, in 1976 and 1977 the governments of the three countries, with the support of several international agencies (including the Food and Agriculture Organization of the United Nations (FAO) and International Atomic Energy Agency (IAEA)), subscribed cooperative agreements to create the "Moscamed Programme", a Mediterranean fruit fly containment programme using the SIT, with the following objectives:

- Eradicate the Mediterranean fruit fly in infested areas of the state of Chiapas, Mexico

- Establish a containment barrier at the Mexico-Guatemala border, and continue eradication activities in Guatemala to create a fly-free buffer area between the containment barrier and the leading edge of the infestation in Guatemala

- Eventually eradicate the Mediterranean fruit fly throughout Central America and Panama

Since then, under these agreements, the respective plant protection organizations of these countries have jointly operated the programme through special commissions.

4.5.2. Major Achievements

After 4 years of intensive eradication activities (1977–1982), the first programme objective was met — the Mediterranean fruit fly was eradicated from an infested area of 640 000 hectares (6400 km^2) in the state of Chiapas, Mexico, using an AW-IPM approach that included legal measures (e.g. quarantine regulations), chemical, mechanical, and cultural control methods, and the SIT. This was the first time that a tephritid fruit fly population was eradicated at a continental level, in a region of difficult topography, high ecological diversity, and using an environment-friendly technology (Hendrichs et al. 1983).

Since eradication was achieved in 1982, for 23 years (1982–2004) the programme has successfully maintained a sterile fly containment barrier. This barrier has prevented the northward spread of the pest, protecting the horticulture industries in Mexico and the USA (worth thousands of millions of dollars) (Orozco et al. 1994, Villaseñor et al. 2000). In addition, the area of Peten (northern part of Guatemala) has been kept free of the Mediterranean fruit fly. This fly-free status has been recognized officially by the Mexican and US governments, and benefits small-scale papaya fruit growers in Peten who can now export their produce to fruit fly-free countries. Moreover, other Guatemalan fly-free areas have been certified — Quetzaltenango, where peaches are produced commercially, and Laguna de Retana, where tomatoes are produced; both commodities can now be exported to Mexico without quarantine restrictions.

The programme has continued to develop and adapt technologies to large-scale conditions — new and more cost-effective mass-rearing, release and field technologies for Mediterranean fruit fly control, including the temperature-sensitive lethal (*tsl*) male-only fly strain (Cáceres et al. 2000; Cáceres et al. 2004; Franz, this volume), the more environment-safe insecticide spinosad (Rendón et al. 2000, USDA/APHIS/PPQ 2000), and more sensitive female-biased trapping systems (Heath et al. 1995, IAEA 1999).

These technological advances, and continuing commitments from the governments of the three countries involved, have been the basis since 1999 for a

renewed Mediterranean fruit fly eradication programme. The aim is to push the infestation front to the southern part of Guatemala (Tween 2004). This would create a large buffer zone between the fly-free areas in southern Mexico and the leading edge of the infestation in Guatemala, greatly reducing the risk of the pest spreading into Mexico and the USA.

4.5.3. Estimated Benefits

The primary impact of the Moscamed Programme has been in protecting the horticulture industries of Mexico and the USA. As a result, and under the framework of the North American Free Trade Agreement (NAFTA), Mexico's gross revenues from horticultural products have tripled since 1994 to more than USD 3500 million per year (Economist 2004).

For Mexico, if we add the projected yearly value of savings in direct damage of USD 2000 million (Gutierrez 1976) over 24 years (1977–2000), and divide the savings by the total cost of the programme in Mexico and Guatemala (estimated at USD 288 million) (Programa Moscamed 2002, Enkerlin et al. 1989), the programme's economic return over these years is remarkable — 167 dollars return in crops and control costs saved for each dollar that was invested. The return would be substantially higher for the USA, where the value of the fruits and vegetables that would be affected by the fly is several times higher than for Mexico. In addition, these returns are even underestimated, since no monetary value has been placed on the social and environmental savings obtained by preventing establishment of this pest.

The economic impact of the Moscamed Programme on the local economies of Chiapas (Mexico) and Guatemala has been substantial. The Moscamed Programme (which began operations in Chiapas) has permanently employed an average of 400 people (who sustain an estimated 2000 family members), apart from the hundreds that have been hired for temporary work in the factory and field operations. In 24 years, from the total programme budget, at least USD 124 million have been spent in Chiapas, mostly for wages and the purchase of services, supplies, and equipment (Programa Moscamed 2002). Over the past 25 years, the Moscamed Programme is one of the main organizations injecting cash into the Chiapas economy, and this has helped one of the poorest states of Mexico, especially at a time when the local economy was severely affected by reduced world prices for coffee (coffee is a major plantation crop, and a source of employment and income in the region.)

Indirect benefits of the programme include:

- Strengthening the plant protection infrastructure in both Mexico and the USA, including the national and international quarantine, and national surveillance, systems — systems that protect the two countries from other exotic fruit fly pests, and also from other pests of quarantine importance that are intercepted through the same quarantine infrastructure and resources
- Recognition of the programme, by regional and international organizations, as an International Fruit Fly Training Centre — contributing greatly to technology transfer in more than 40 countries of Latin America and other parts of the world

(including the Caribbean, Africa, and Asia) through the hands-on training of hundreds of professional scientists

- Transfer of the SIT technology, that has been improved and validated on a large scale, to other fruit fly AW-IPM programmes integrating the SIT in other parts of the world

Keeping Mexico and the USA free of the Mediterranean fruit fly has not only protected the horticulture industries of these countries but, for Mexico, it has also created an opportunity to develop the industry into a multimillion dollar export-oriented enterprise (Economist 2004).

4.6. Fruit Fly Suppression/Eradication/Prevention Programme — Mexico (Box 6)

4.6.1. Problem Definition

Mexico has more than 1 million hectares planted to fruit crops, with an estimated annual production value of more than USD 2500 million (SAGAR/IICA 2001). The fruit industry is significantly hindered by four fruit fly species: Mexican fruit fly, West Indian fruit fly, guava fruit fly *Anastrepha striata* Schiner, and sapote fruit fly *Anastrepha serpentina* (Wiedemann). The annual direct damage that these fruit flies cause is more than USD 230 million (Reyes et al. 1991). This amount does not include the cost of insecticide applications, and the losses due to restrictions in fruit commercialization. These restrictions prevent the industry from benefiting from price differentials, and more importantly from market diversification, negatively affecting the general development of the industry. Some other losses are also not included, e.g. cost to human health from moderate and acute poisoning arising from applying insecticides, shortage in the supply of fruits, and negative impact on the environment.

4.6.2. Major Achievements

- Fruit flies of economic importance have been eradicated in more than 35 000 hectares of commercial plantations of citrus, mango, apple, and peach in north-west Mexico, completely freeing from fruit flies of economic importance the States of Chihuahua, Sonora, Baja California Norte, and Baja California Sur (SAGARPA 2001).
- In the north-east region, SIT suppression activities have reduced fruit fly populations to low-prevalence levels in parts of the more than 30 000 hectares of commercial citrus production.
- By creating federal legal instruments in support of the campaign, the construction of additional interstate quarantine checkpoints, and installation of X-ray equipment at specific ports of entry, have been possible. This has strengthened the international and national quarantine system, providing greater protection from exotic fruit flies and other pests of plants and animals.
- Through training courses and workshops, a work force of hundreds of professional scientists, specialized in the large-scale and area-wide operation of phytosanitary campaigns, has been deployed throughout the country.

Box 6. Mexico National Campaign Against Anastrepha *Fruit Flies*

Anastrepha spp. fruit flies are major pests in Mexico. After a thorough economic feasibility study (Reyes et al. 1991), in which the returns of integrating the SIT into the control of fruit flies in major commercial fruit production areas were assessed, the Mexican federal government in 1992 approved the National Fruit Fly Campaign (Campaña Nacional Contra Moscas de la Fruta (CNCMF)), with the following objectives:

- To suppress, and in selected areas eradicate, fruit flies of economic importance in fruit production areas, using an AW-IPM approach including the SIT
- To protect Mexico from the introduction and establishment of other economic fruit fly species that threaten the country

The CNCMF is part of the Plant Protection General Directorate of the Ministry of Agriculture, Livestock and Rural Development (SAGARPA). The CNCMF operates through state governments and fruit-grower associations under compliance agreements subscribed to by the three parties (federal and state governments, and industry).

The resources required to operate the programme are contributed in equal parts by the federal and state governments and the fruit industry, in financial and in-kind contributions. The federal government facilitates the legal instruments for smooth implementation of the programme, supplies the sterile flies, and operates international quarantine stations at ports of entry. The state governments, through their plant protection infrastructure, are responsible for distributing and releasing the sterile flies, and for conducting field activities outside the fruit production areas to assure area-wide control of the pest. Responsibilities also include operating quarantine interstate road stations. At the farm level, the programme is conducted by the fruit industry, through Plant Protection Committees, that link the producer associations with the state and federal governments. Activities of producers in orchards include trapping, applying bait sprays, and releasing sterile flies (Reyes et al. 2000).

4.6.3. Estimated Benefits

In the first 4 years after 1997, when fruit fly eradication in north-west Mexico was officially declared, the direct benefits (reduced fruit fly damage and increased yield) amounted to USD 25 million. In addition, in the same time period, the benefits obtained from the price differential paid by export markets, and savings in postharvest treatments, totalled approximately USD 35 million. Thus, the total benefits in the fruit fly-free areas over 4 years amounted to USD 60 million (SAGAR/IICA 2001).

A more specific case is citrus production in the fly-free area of the state of Sonora in north-western Mexico. The state grows 10 000 hectares of citrus, and more than 90% of the production is for the export market with no phytosanitary restrictions. In 6 years, the total amount exported was more than 130 000 tonnes, with an estimated value of USD 10.3 million. The crop generates 2000 jobs per year, equivalent to USD 3.2 million (CNCMF 2002).

The eradication of fruit flies, and subsequent maintenance of the fruit fly-free status, have opened the possibility in the north-western states of expanding the area planted to fruit crops to 50 000 hectares. No doubt this has resulted in substantial economic and social benefits to that region of Mexico.

5. SUMMARY OF ECONOMIC RETURNS

The benefit/cost ratio (BCR) achieved in some major fruit fly AW-IPM programmes integrating the SIT is high, ranging from 2.8 to 400, clearly showing that the SIT technology, when properly integrated with other methods and applied on an area-wide basis, is economically feasible (Table 2). Even when the types of benefits accrued from these programmes are similar, the economic returns (BCR) vary widely, even among programmes with common fruit fly pest problems, and similar objectives and strategic approaches. This is due to the different intrinsic characteristics of each programme, e.g. the magnitude of the pest damage, size and value of the crops being protected, commitment of the main stakeholders, resources available to execute the programme, and efficiency in programme management.

6. CONCLUSIONS

The high economic returns from some fruit fly AW-IPM programmes that integrate the SIT are possible primarily because of the environment-friendly and area-wide nature of the SIT technology. This technology allows cost-effective suppression, and in selected cases eradication, of insect pest populations, and also prevents the establishment of important fruit fly species in pest free areas through the use of pest risk-mitigation measures, such as SIT containment and prevention programmes. Such programmes protect, at a relatively low cost, high-value horticulture industries, such as those in Argentina, Australia, Chile, Mexico, and the USA. This is a major advantage of the SIT technology when compared with more conventional pest control methods such as insecticides (Enkerlin 2003). In contrast, the worldwide benefit/cost ratio of insecticides has been estimated at 4:1, if indirect costs are excluded, and only a 2:1 ratio if indirect environmental and public health costs are included (Pimentel 1991). As the use of the SIT technology for certain pests and crops expands, it is expected that the release of sterile insects will gradually reduce the application of insecticides. Eventually, the insecticide industry will view the SIT as an opportunity for diversification rather than as a competing technology; this has already happened with other control methods that are biological in nature.

One of the unique features of the integrated application of the SIT is that, since its application is area-wide, the benefits spread beyond commercial fruit and vegetable producers to backyard gardens and subsistence farms in poor rural areas. In the future, the SIT will contribute even more to improved food security worldwide by increasing fruit and vegetable production in a cost-effective, environmentally clean, and sustainable manner.

7. ACKNOWLEDGEMENTS

Table 2. Estimated Costs, Benefits, and Benefit/Cost Ratios (BCR) for some Major Fruit Fly AW-IPM Programmes Integrating the SIT

Programme	Parameter used to measure benefits	Avg annual cost[1] (USD million)	Avg annual benefit (USD million)	Benefit/ cost ratio (BCR)[2]	References
Mediterranean Fruit Fly Containment Programme "Programa Moscamed"	Protection of horticulture industries in Mexico and USA (includes only potential production and market loss)	12	1800	150	Gutierrez 1976, Reyes et al. 1991, USDA/ APHIS 1993
Melon Fly Eradication Programme in Okinawa, Japan	Export of fruit commodities (mainly mango and bitter melon) after eradication was achieved	7.7	41.7	5.4	Kakazu 2002
National Mediterranean Fruit Fly Control Programme in Chile	Export of horticultural products affected by fruit flies	4	1600	400	MAG/SAG 1995, Lindquist and Enkerlin 2000
South Africa Mediterranean Fruit Fly Suppression Programme	Savings in chemical sprays and table-grape rejections during the certification process	0.13	0.37	2.8	IAEA 2002
Mexico National Fruit Fly Campaign	Savings in direct damage and value of fruit exports from the fly-free areas in north-west Mexico	2	15	7.5	SAGAR/ IICA 2001
Mediterranean Fruit Fly Preventive Release Programme in Southern California, USA	Protection of California's horticulture industry from yield loss in agriculture and home-garden production, increased insecticide use, loss of export markets, and annual quarantine compliance cost	13	1300–1900	100–146	CDFA 2002
Control of the Mediterranean Fruit Fly in Israel/Jordan	Export of vegetables from the Arava Valley	0.8	8	10	Cayol et al. 2004

[1]Includes fixed and variable operational costs, but not capital or financial costs.
[2]Assuming the whole benefit is lost in the event of fruit fly establishment and widespread infestation.

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CHAPTER 7.3.

IMPACT OF MOTH SUPPRESSION/ERADICATION PROGRAMMES USING THE STERILE INSECT TECHNIQUE OR INHERITED STERILITY

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SUMMARY

Numerous lepidopteran species have been investigated as candidates for control using the sterile insect technique (SIT) or inherited sterility (IS). However to date only two programmes are operational — the pink bollworm containment programme in the San Joaquin Valley, California, USA, and the codling moth suppression programme in British Columbia, Canada. Both of these programmes have been highly successful in controlling the pest populations, reducing insecticide use, and improving interactions between growers and the general public. However, other benefits, including the positive economic impacts of these programmes, have not been fully quantified. Methods to reduce the cost of lepidopteran

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programmes might include combining the SIT/IS with other pest control tactics such as mating disruption or the release of natural enemies, the development of genetic sexing strains, or the application of molecular technologies to develop genetic markers and genetic sterility. In future the greatest potential for impact of lepidopteran SIT/IS programmes may be in combating key invasive threats such as the eradication of an outbreak of the painted apple moth in New Zealand.

1. INTRODUCTION

Lepidopterans are among the most devastating agricultural and forest pests in the world. In the United States of America (USA) seven of the eight most serious insect pests of agricultural crops are lepidopterans (Peters 1987). According to a recently developed list of the 37 worst invasive insect pest threats to US agriculture and plant resources, 19 are lepidopteran species (ESA 2001). Control of these pests relies largely on insecticides, and the development of resistance is becoming a serious problem for many species, e.g. the codling moth *Cydia pomonella* (L.) (Varela et al. 1993), and the diamondback moth *Plutella xylostella* (L.) (Shelton et al. 1993). The latter has also developed resistance to the microbial insecticide *Bacillus thuringiensis* Berliner (=*Bt*) (Tabashnick et al. 1990). In addition, the indiscriminate use of pesticides has had a significant negative impact on the environment. Of particular importance to agriculture is the destruction of crop pollinators and natural enemies that keep secondary pests in check (Edwards 2000). It is therefore probably not surprising that, following the successful eradication of the screwworm *Cochliomyia hominivorax* (Coquerel) on the island of Curaçao (Baumhover et al. 1955, Lindquist 1955), more lepidopterans than any other group of insects have been investigated as potential candidates for control using the sterile insect technique (SIT) or inherited sterility (IS), a variation of the SIT that involves the release of partially sterile insects (North 1975, LaChance 1985, Bloem and Carpenter 2001). Carpenter et al. (this volume) list more than 30 species for which radiation biology studies have been conducted and where IS has been documented.

In spite of the tremendous impact that area-wide control of key lepidopteran species could have, and the fact that numerous lepidopterans have been investigated in laboratory and field-cage studies for their suitability as candidates for SIT/IS programmes, field trials have been performed on only a limited number of species. Of the 11 lepidopteran species that have been investigated in the field (*C. pomonella,* carob moth *Ectomyelois ceratoniae* (Zeller), oriental fruit moth *Grapholita molesta* (Busck), corn earworm *Helicoverpa zea* (Boddie), tobacco budworm *Heliothis virescens* (F.), gypsy moth *Lymantria dispar* (L.), Asian corn borer *Ostrinia furnacalis* (Guenée), European corn borer *Ostrinia nubilalis* (Hübner), pink bollworm *Pectinophora gossypiella* (Saunders), *P. xylostella*, and painted apple moth *Teia anartoides* Walker, only two area-wide SIT/IS programmes have progressed to the operational stage, one against the pink bollworm in California, USA, and one against the codling moth in British Columbia, Canada. In both cases, partially sterile moths are released in the context of area-wide integrated pest management (AW-IPM) programmes that use a combination of tactics.

Although studies on *G. molesta, O. nubilalis, O. furnacalis* and *P. xyllostella* generally reported positive results from releasing sterilized insects (Rosca and Barbulescu 1996, Apu 2002, Genchev 2002, Maung 2002, Wang et al. 2002, Yang

et al. 2002), detailed information concerning the size of treatment areas, release rates, release methods, and methods for evaluating efficacy was not provided. Programmes for *H. virescens, H. zea,* and *L. dispar* were deemed economically impractical following a number of large-scale field studies.

The release of partially sterilized male painted apple moths was recently added to an eradication effort in Auckland, New Zealand, that had been relying on aerial applications of *Bt* and an intensive trapping programme for this introduced pest (Suckling 2003). The carob moth is a major pest of dates in North Africa, and the use of *Bt*, parasitoids, and post-harvest fumigation does not provide sufficient pest control to allow the industry to reap the potential economic benefits from increased exports. Laboratory and field studies have been initiated in Tunisia and Algeria, with a view to integrating the SIT into AW-IPM programmes for date and pomegranate plantations. A mass-rearing system was established, and small-scale releases of partially sterile moths have been made (Dhouibi et al. 2000).

The primary impact of lepidopteran SIT/IS programmes that has been reported has been the degree of pest suppression or extent to which establishment of the pest has been prevented in the treatment area. Quantification of other benefits, e.g. lower commodity production costs, access to new markets, and fewer farm worker health and safety problems or decreased ground water contamination as a result of reduced insecticide use, for the most part has not taken place. Therefore, rather than limit this discussion to the little information available on accrued benefits from the two lepidopteran operational programmes, the major achievements of each of the lepidopteran field programmes that have been undertaken are more broadly discussed to include their impact on the target pest population, the involved stakeholders, and the advancement of the SIT/IS as a tactic for lepidopteran control.

2. GYPSY MOTH

The gypsy moth was accidentally introduced into the USA in 1869 near Boston, MA. It is now an important forest defoliator that periodically builds to outbreak levels resulting in serious economic, environmental, and public nuisance problems (Liebhold et al. 2000). More than 32.8 million hectares of forests have been defoliated by the gypsy moth since 1924 (USDA Forest Service 2001). The area in North America infested by the gypsy moth is confined to the north-eastern USA (behind an advancing front slowly moving in a south-westerly direction) and eastern provinces of Canada (Fig. 1) (Sharov et al. 2002a). The potential of using the SIT to manage leading-edge populations, and isolated outbreak areas resulting from the accidental transportation of egg masses and other life stages through commerce and recreation, was recognized long ago, and radiation biology studies were initiated in 1957 (Godwin et al. 1964). Based on a number of criteria, the gypsy moth appeared to be well suited for population management with the SIT — it is univoltine, females do not fly, males may mate several times, and females mate only once and produce an egg mass in the fall from which larvae hatch the following spring (Reardon and Mastro 1993). However, after nearly 35 years of intensive study, it was determined that the SIT/IS was not cost effective for the gypsy moth, and this technology was never employed on an operational basis.
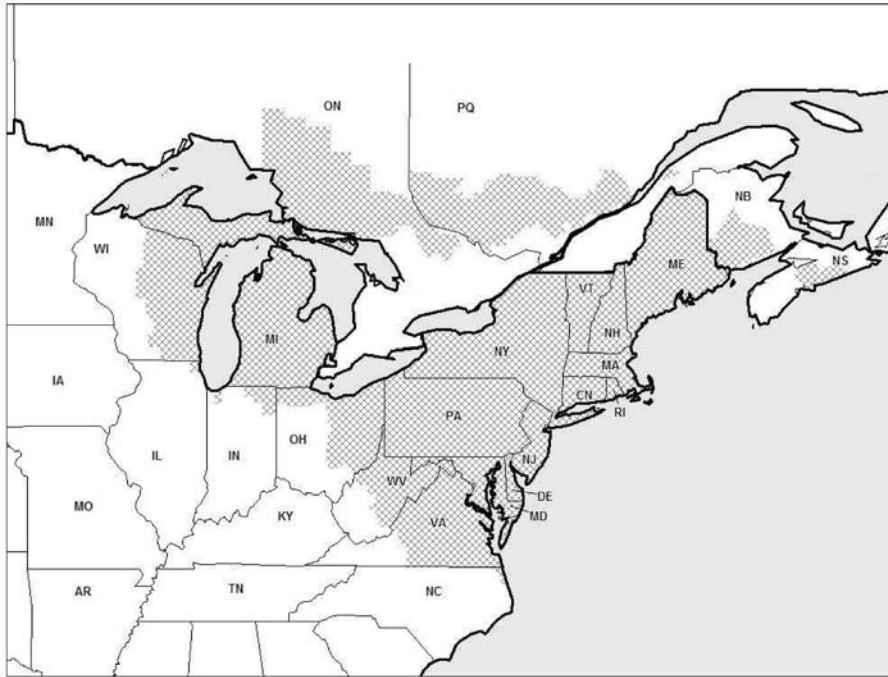
Figure 1. Map of north-eastern USA and eastern Canada (names of states and provinces abbreviated) showing areas (cross-hatched) with established breeding populations of the European gypsy moth. (Map is a composite derived from publications of the USDA/APHIS and the Canadian Food Inspection Agency/Agricul.ture and Agri-Food Canada, was drawn by B. Sterling, Cartographer, APHIS, and is reproduced with permission.)

Mastro et al. (1981) and Reardon and Mastro (1993) provided good reviews of the research that was conducted throughout the 1960s, 1970s, and 1980s. This research defined the sterility effects of various doses of radiation when applied to different gypsy moth developmental stages, and quantified the impact of releasing sterile and partially sterile insects. Three different release techniques were investigated (Reardon and Mastro 1993): (1) field-placement (from the ground) of male pupae (sexed visually by size and form) treated with at least 150 Gy that emerged as fully sterile adults, (2) deployment (from the ground) of substerilized male pupae treated with 80–100 Gy, and (3) broadcast release (from the ground or air) of diapausing F_1 sterile egg masses produced from males treated with 80–100 Gy and mated with untreated females. Schwalbe et al. (1991) discussed the gypsy moth problem in North America and the rationale for focusing on the use of F_1 sterile egg masses to eradicate isolated infestations. Unfortunately the experimental design and procedural details of the various field trials that were conducted are lacking, with the exception of a few key pilot tests (Berrien County, Michigan, USA, 1980–1982, using fully sterile male pupae; Horry County, South Carolina,

USA, 1982, using partially sterile male pupae; and Bellingham, Washington, USA, 1984–1985, using sterile F_1 egg masses — Mastro and Schwalbe 1988, Mastro et al. 1989, Schwalbe et al. 1991, Reardon and Mastro 1993).

What little additional information exists on gypsy moth SIT is contained principally in a series of unpublished annual progress reports by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) Laboratory, at Otis, MA, USA. However, in a report to the USDA, Agricultural Research Service (ARS), National Technical Advisory Board, LaChance (1976) provided some information on early gypsy moth field tests.

Small SIT field trials to suppress the gypsy moth also were conducted in Yugoslavia in 1969–1971 (Maksimović 1971, 1972, 1974). Even though Maksimović concluded that the release of sterile males reduced wild population levels, an assessment of the actual impact of these releases is difficult given the experimental design that was used (LaChance 1976).

In general, the release of irradiated pupae was effective in eradicating isolated infestations and in suppressing low-density populations along the leading edges of spreading infestations. However, despite the technique's effectiveness, several operational difficulties were identified, and the technique was judged to be impractical for large-scale use (Mastro et al. 1989). Some of the problems were: (1) pupae were fragile and required special care in packaging and shipping (Reardon and Mastro 1993), (2) pupae had to be released in emergence cages that were expensive to deploy and maintain (Reardon and Mastro 1993), (3) pupae were sometimes subject to intense predation, (4) overcrowding in emergence cages resulted in poor quality adults (LaChance 1976), and (5) since released males lived only 2 or 3 days, frequent releases were necessary to maintain high ratios of released to wild insects (Schwalbe et al. 1991, Reardon and Mastro 1993). In addition, since male flight activity lasts only about 4 weeks, a mass-rearing facility would be under-utilized for most of the year (Mastro 1993, Reardon and Mastro 1993).

As a result of the difficulties in releasing pupae, research efforts were refocused on the release of F_1 sterile egg masses. Male pupae were irradiated with 100 Gy, the eclosing males were mated to fertile females, and the F_1 egg masses were stored in diapause until needed for release the following spring. In this way, in theory, F_1 sterile larvae would emerge and develop in the field in synchrony with the wild population. This technique had the advantages that the F_1 sterile egg masses were easier than pupae to handle and ship, could easily be produced and stockpiled in diapause throughout the year using a relatively small rearing facility, and required only a single release per season (Schwalbe et al. 1991, Reardon and Mastro 1993). In spite of these advantages, the additional population suppression that IS offers (suppression by the release of partially sterile males followed by field production of their F_1 sterile progeny) could not be taken advantage of when this approach was used. Also, because a feeding stage (larvae) was being released, concerns were raised about causing excessive plant damage (Mastro et al. 1989, Mastro 1993).

Although the early use of gypsy moth F_1 sterile egg masses was successful in eradicating an isolated population in Bellingham, Washington, USA (Mastro and Schwalbe 1988, Mastro et al. 1989, Schwalbe et al. 1991, Reardon and Mastro 1993), similar results were not consistently obtained in subsequent trials against

isolated outbreak populations, and the technique proved inadequate for managing gypsy moth populations along invading fronts of established infestations (Reardon and Mastro 1993). The low efficacy and inconsistent results of releasing F_1 sterile egg masses were due to the fact that the resulting fully sterile adults that developed in the field were less competitive than the wild population, and were actually less competitive than laboratory-reared males irradiated and released as pupae. Nonetheless, between 1988 and 1992, eight isolated populations of the gypsy moth were treated with F_1 sterile egg masses with apparently "generally positive results" (Reardon and Mastro 1993). Between 1980 and 1992, infestations in a total of 2385 hectares were eradicated using sterile gypsy moth releases of all types (USDA Forest Service 2001).

Current programmes for gypsy moth suppression rely on the use of *Bt* variety *kurstaki*, a virus, diflurbenzuron, and the synthetic sex pheromone disparlure (Sharov et al. 2002b). Although sterile gypsy moths are now used only to calibrate trapping grids and to determine parasitoid host preferences, the investigations associated with the development of the SIT for this pest led to a better understanding of its biology and behaviour that is being utilized in ongoing management activities (Mastro et al. 1989). Suckling et al. (2002) also credited the gypsy moth programme with providing invaluable background to the development of IS for painted apple moth eradication in New Zealand. In Europe, investigations are in progress on the release of F_1 sterile egg masses in advance of imminent gypsy moth outbreaks; this allows natural-enemy populations to build up early and thus reduce the magnitude of an outbreak.

3. PINK BOLLWORM

The pink bollworm was first reported in North America from Mexico in 1911, probably entering on cotton seed shipped from Egypt (Noble 1969). The first reported infestation in the USA was in 1917 in Robertson County, Texas (Scholl 1919). By 1926 this highly invasive insect had spread from Texas through New Mexico and into eastern Arizona, and quickly established itself as one of the major pests of cotton in south-western USA and northwestern Mexico (Burrows et al. 1984). Management of the pink bollworm has relied on the extensive use of broad-spectrum insecticides, and growers have experienced significantly increased production costs and reduced yields (Watson and Fullerton 1969, Burrows et al. 1982, 1984). Ingram (1994) provided a worldwide perspective on the pest status and management of the pink bollworm, and Henneberry and Naranjo (1998) reviewed its status and the various integrated management approaches used for its control in the south-western USA.

The only cotton-growing area in the south-western USA that is not infested with the pink bollworm is the San Joaquin Valley, California. The prevention of pink bollworm establishment in this valley is attributed to an ongoing SIT containment programme that has been in continuous operation since 1968. Stewart (1984) described the mass-rearing of the pink bollworm. Staten et al. (1993) summarized the history and operational details of the programme. Miller et al. (2001) reviewed recent efforts to enlarge the rearing facility in Phoenix, AZ, and to mechanize many

steps in the rearing process. These changes dramatically increased sterile insect production capabilities at reduced costs, and opened up the possibility that sterile moths could be integrated into other AW-IPM programmes in south-western USA and north-western Mexico.

Several field-cage studies evaluating the potential of the SIT to control the pink bollworm showed positive results, but many of the early open-field trials failed or were only partially successful. These early failures were attributed to a lack of isolation from migrating moths into experimental areas and the low competitiveness of mass-reared sterilized male moths that necessitated high (more than 60) sterile to wild overflooding ratios (Bartlett 1978). Henneberry and Keaveny (1985) provided detailed information on a large-scale SIT field trial in St. Croix, US Virgin Islands. Henneberry (1994) made a thorough review of all sterile moth release trials for pink bollworm control, including the St. Croix project and the San Joaquin Valley programme.

The objective of the pink bollworm AW-IPM programme in the San Joaquin Valley is containment rather than suppression or eradication (Henneberry 1994; Hendrichs et al., this volume). This is achieved by the release of large numbers of sterile moths each year relative to the number of immigrating wild moths. The fact that the pink bollworm has not become established in the Valley, despite the annual immigration of moths from infested cotton-growing valleys to the south (Staten et al. 1993) and the demonstrated ability of the moth to successfully overwinter in the area (Henneberry and Keaveny 1985, Venette and Hutchison 1999), shows that the programme has been effective.

The release of sterile moths protects a cotton crop with an annual value of approximately USD 1000 million, and is a joint effort by California cotton growers, California Department of Food and Agriculture (CDFA), and USDA/APHIS. Upon its inception in 1968, programme costs were shared equally by the two government agencies. However, over time, the California cotton growers have gradually taken up the financial responsibilities, and now pay approximately 95% of the programme's budget. Funding is obtained through an assessment on each bale of cotton that is ginned in the state. In 2002 this assessment fee was set at USD 2.00 per bale. Based on an average yield of 6.3 bales per hectare, the direct cost to growers is about USD 12.50 per hectare per season. This is substantially lower than the amount that growers in the southern desert valleys of California and Arizona pay to control this pest, where chemical costs are about USD 216 per hectare per year (5-year average, 1990–1995) (E. Miller, personal communication). Although fewer generations and a lower pest population development potential would be expected in the San Joaquin Valley than in the southern desert areas (Henneberry and Naranjo 1998), it has been estimated that grower pest control costs would increase by USD 175–200 per hectare if the current containment programme were not in place, and an additional 7.94 kg/hectare or 2.22 million kg of pesticide would have to be used every year (J. Rudig, personal communication).

The success of the San Joaquin Valley containment programme, coupled with the development of other biorational tools such as pheromone mating disruption and later *Bt*-cotton, created the opportunity to test the feasibility of using combinations of these tools to manage established pink bollworm populations on an area-wide

basis. The first test was conducted in 1986–1989 involving 30 cotton fields in the Coachella Valley, California, using a high-rate pheromone disruption system and sterile insects. During the 4-year project, pink bollworm populations were maintained at low densities and major reductions in conventional insecticide use were achieved (average of 7.3 insecticide applications per field per year decreased to 1.2) (Staten et al. 1993, Henneberry 1994).

A second area-wide pest suppression trial, combining the SIT with mating disruption, *Bt*-cotton, and cultural controls, was conducted in the Imperial Valley, CA, from 1994 to 2000. Walters et al. (2000) reviewed the strategic objectives and results of this integrated management trial for the period of 1994–1998. During 1994–1996, sterile moths were released 6 days per week in all fields at variable rates (70–560 moths per hectare per day) calculated to deliver an overflooding ratio of at least 60:1 as measured by trap captures. Sterile moth releases were supplemented with mating disruption if a 60:1 sterile to wild moth ratio was not maintained. In 1997 the cotton planted in the Imperial Valley consisted of 81% *Bt*-cotton, 17.5% conventional or non-*Bt*-cotton protected with mating disruption, and 1.5% conventional untreated cotton as a refuge. Sterile moth releases were reduced to 40 insects per hectare per day, 3 days per week, throughout the Valley. The trial was expanded in 1998 to include the Blythe and Palo Verde Valleys, CA, where there also was widespread use of *Bt*-cotton. The trial was finally terminated in 2000 after having achieved a high degree of suppression of established pink bollworm populations in all areas using essentially no pesticides.

Based on the success of these trials, and a more complete understanding of both the long-term impact of *Bt*-cotton on pink bollworm populations and the potential benefits of integrating sterile moths with *Bt*-resistance management, a proposal was developed to eradicate the pink bollworm from all cotton-producing areas of the USA and adjacent areas of northern Mexico (NCCA 2001, El-Lissy et al. 2002). Each of three programme areas would use grower-planted *Bt*-cotton and pheromone applications for mating disruption for 1 or 2 years to lower the pest population levels. Then, in the following 2 or 3 years, sterile insect releases would be included to complete the eradication process. In 2001, phase I of the programme was initiated in south-western USA and north-western Mexico. Although the full impact of this programme has yet to be realized, it is estimated that USA cotton producers lose over USD 32 million each year from yield losses and control costs related to the pink bollworm (El-Lissy et al. 2002).

4. CODLING MOTH

The codling moth is the key pest of apples and pears in the western USA and in most regions of the world where pome fruit is grown (IAEA 2000). The larval stage burrows into the fruit and renders it unmarketable. As a consequence, organophosphate pesticides have traditionally been applied to kill larvae as they emerge from eggs and before they can penetrate fruit. Control failures due to the development of insecticide resistance, and concerns about the impact of insecticides on the environment, have over the last 50 years led researchers in different parts of the world to make numerous attempts to use the SIT/IS against the codling moth.

Notable examples include the work of scientists at the USDA/ARS laboratory in Yakima, Washington, USA (Hutt et al. 1972; Butt et al. 1973; White et al. 1976a, 1976b; Hutt and White 1979), and the research conducted in Switzerland using diapaused F_1 sterile larvae released into small pome-fruit orchards (Charmillot et al. 1973, 1976a, 1976b, Charmillot 1977). However, only in southern British Columbia, Canada, at the Agriculture and Agri-Food Research Centre in Summerland, did the investigations (Proverbs 1962, 1969, 1974, 1982, Proverbs et al. 1973, 1982) lead to the implementation of an operational AW-IPM programme that integrated the release of irradiated moths (Dyck et al. 1993, Bloem and Bloem 2000).

The Sterile Insect Release (SIR) Program in Canada was launched in early 1992 (Dyck et al. 1993), more than a decade after Proverbs et al. (1982) demonstrated in a 3-year (1976–1978) pilot project that eradication of the codling moth was possible. Unfortunately, at that time, the cost of delivering this technology was about 2.4 times greater than the use of conventional pesticides (Proverbs et al. 1982). Between 1978 and 1992 several benefit/cost analyses were conducted at the request of growers to reassess the economics of implementing the SIT (Holm 1985, 1986, Jeck and Hansen 1987). Following more positive outcomes of these studies (benefit/cost ratios ranged from 1.1–1.4), an implementation plan was developed (DeBiasio 1988). A 2-year clean-up or sanitation phase (phase 1) was to be followed by 3 years of sterile moth releases (phase 2) at an initial overflooding ratio of 40:1. Two zones, each containing about 4000 ha, would be treated sequentially, and urban trees were to be included in the programme. Sterile codling moths would be released indefinitely along the USA-Canada border (phase 3 — containment) (Hendrichs et al., this volume), which was considered the only plausible route for reinfestation. Research on the codling moth published by M. D. Proverbs and colleagues included sterilization using gamma radiation (Proverbs and Newton 1962a, 1962b, 1962c), development of an inexpensive, agar-free meridic diet (Brinton et al. 1969), design of a rotating oviposition cage to house the reproductive colony (Proverbs and Logan 1970), and the design and testing of ground-release devices to distribute chilled moths in the orchard (McMechan and Proverbs 1972). All of these components, with only slight modifications, are currently in use in the SIR Program (Dyck et al. 1993, Bloem and Bloem 2000), which is a great testament to the focus on implementation that Proverbs gave to his research programme.

Construction of a mass-rearing facility at Osoyoos in the Okanagan Valley was begun in August 1992 and completed in March 1993. Sterile moths were released into orchards for the first time in the spring of 1994. Unfortunately clean-up activities during 1992 and 1993 were not entirely successful, and a higher-than-anticipated wild population resulted in poor overflooding ratios and poor control. In 1995 growers received a one-time compliance grant of USD 115 per hectare to combine aggressive pesticide applications with the release of sterile moths to help turn the programme around and reduce the wild population to a level that was more in line with the sterile moth production capacity, i.e. one that would allow a 40:1 sterile:wild overflooding ratio to be achieved. The programme also made several other strategic changes including tougher enforcement of codling moth control bylaws, an expanded communications campaign, and the adoption of a new timeline

that included three rather than two treatment zones (Bloem and Fielding 1996, Bloem and Bloem 2000).

Average wild codling moth captures in pheromone traps in the zone 1 treatment area were reduced from 13 moths per trap per week during the first generation and 2.5 during the second generation in 1995 to 0.08 moths per trap per week during both first and second generations in 2000 (Figs. 2 and 3). The amount of codling moth fruit damage at harvest also has decreased significantly. At the end of 1995, 42% of orchards in the treatment area had no detectable level of codling moth damage at harvest. This percentage increased to 91% in 1997, and minimal damage has been reported ever since (Fig. 4).

Equally important, insecticide use (based on kg of product sold in the zone 1 release area) has been reduced by 82% of pre-SIR Program amounts (Fig. 5). In 1991, 18 903 kg of organophosphate insecticide, including azinphos-methyl, phosmet and phosalone, were sold in the south Okanagan Valley; in 2001, only 3403 kg were sold (Jerry Vakenti, British Columbia Ministry of Water, Land and Air Protection, Penticton, Canada). Although probably other factors than just the SIR Program have contributed to this reduction in pesticide use, the decreased reliance on insecticides for codling moth control has resulted in local packing houses encouraging their apple growers to consider switching to certified organic production or following new "Growing with Care" production practices where no pesticides are applied in orchards between blossom and fruit harvest. In addition, negotiations between the organic growers association, the Canadian Food Inspection Agency, and Japanese officials were recently initiated to permit the entry of British Columbia certified organic apples into Japan.

The Canadian federal and British Columbia provincial governments paid the initial capital costs (USD 5.33 million) of the programme. Five regional governments in the province agreed to levy a parcel tax on commercial apple and pear growers receiving sterile moths (estimated at USD 72 per hectare) and a tax on all property owners in the treatment area (USD 0.13–0.26 per 1000 dollars of property value or about USD 4.32 per household) to fund the yearly operating costs. Unfortunately, soon after the SIR Program initiated operations, it became obvious that there had been a significant underestimation of the effort and costs involved, particularly in delivering the programme to urban areas and for the removal of wild trees and derelict orchards (Bloem and Fielding 1996). As a result, the cost of the programme to both growers and homeowners gradually increased between 1992 and 1997. In 1997 the federal and provincial governments each contributed an additional USD 1.44 million to help stabilize the annual cost of the programme. In 2002 the operating budget was USD 3.38 million, with pome-fruit growers paying USD 169 per hectare and homeowners USD 0.195 per 1000 dollars of assessed land value. The total estimated SIR Program cost for the years 1992–2006, after which a long-term maintenance programme is scheduled to begin, is about USD 40.3 million.

The sanitation phase for commercial orchards in zones 2 and 3 commenced in 1998 using a combination of mating disruption and insecticide sprays. Releases of sterile moths in these zones began in 2002. It is expected that by the end of 2005 all zones will have achieved minimal codling moth population levels similar to those existing in zone 1. Efforts are now underway to determine the most cost-effective

long-term maintenance programme for use post 2005 when SIR Program activities will be reduced (Dendy et al. 2001).
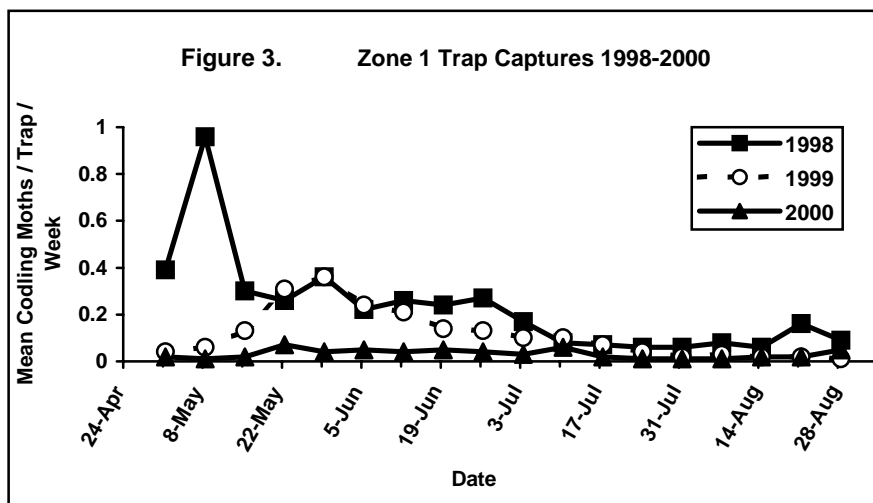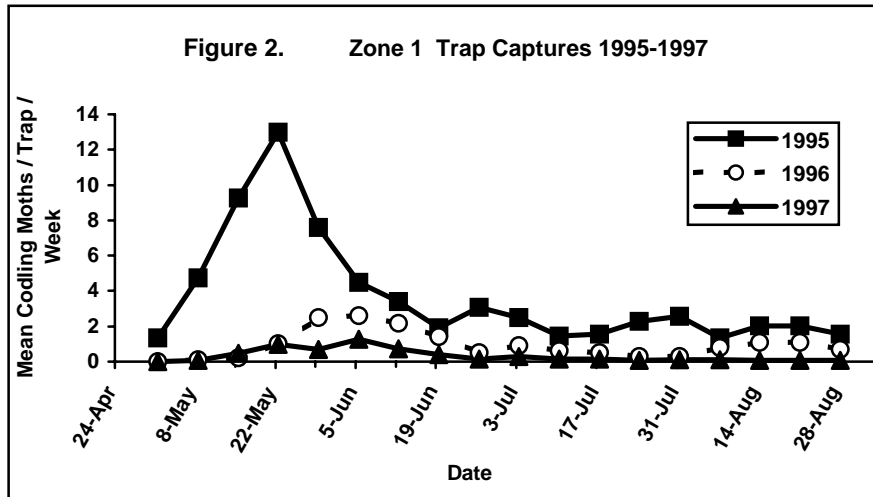
Figures 2 and 3. Mean weekly captures of wild codling moth Cydia pomonella *males in pheromone-baited sticky traps deployed in apple and pear orchards in the treatment area (zone 1) receiving sterile codling moths in British Columbia, Canada. Note the difference between Figures 2 and 3 in the scale of the y-axis for trap captaures.*

Figure 4. Percentage of Zone-1 Orchards with No Codling Moth Damage at Harvest

Figure 5. Sales of Organophosphate Insecticides (Kg) in the SIR Zone-1 Treatment Area
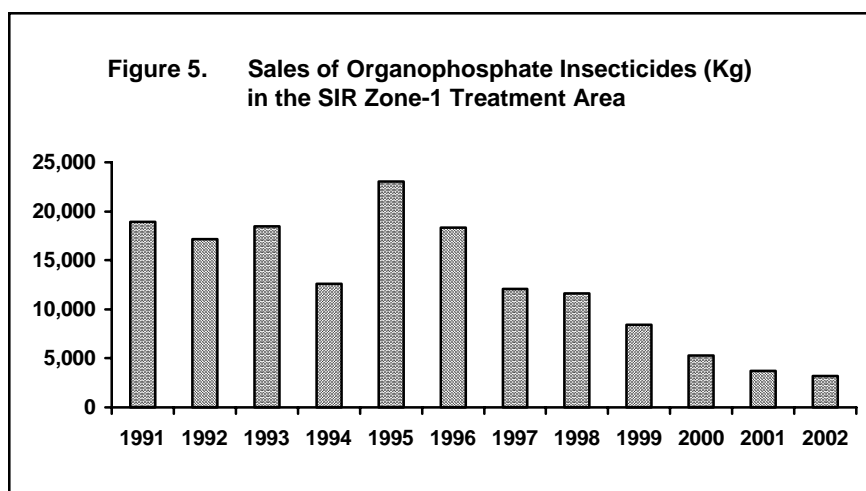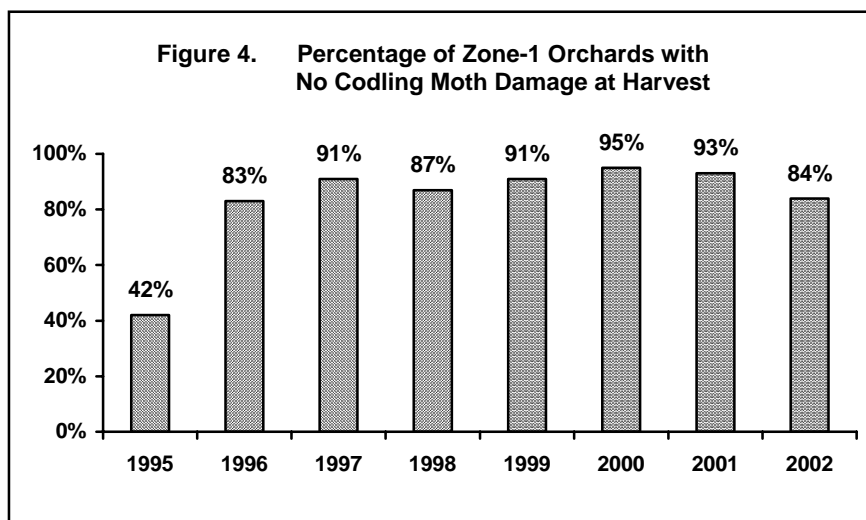
Figures 4 and 5. Some measurable impacts of the codling moth Sterile Insect Release (SIR) Program initiated in 1994 in British Columbia, Canada. Figure 4 shows the percentage of zone 1 orchards that had no detectable level of codling moth larval damage when fruit sampling was conducted at the end of each season. Figure 5 shows the decrease in quantity of organophosphate insecticides purchased in the zone-1 treatment area since the implementation of the SIR Program (J. Vakenti, British Columbia Ministsry of Water, Land and Air Protection, Penticton, Canada).

The SIR Program has faced many challenges since its inception. Managers continue to struggle with the concept of eradication, which would require quarantine measures and restrictions on the movement of fruit and bins, versus the current area-wide suppression strategy, and with the programme's long-term financial, political, and operational demands. However, as conventional pest control methods continue to become more expensive, less effective, and less tolerated, international interest in the Canadian programme has grown.

Based on the impact of the programme in Canada, Argentina and South Africa are currently developing plans to conduct codling moth SIT/IS pilot projects. In South Africa, interest in codling moth SIT/IS has also generated interest in using the SIT/IS for control of the false codling moth *Cryptophlebia leucotreta* (Meyrick), a key citrus pest (Bloem et al. 2003).

Finally, one of the lasting impacts of the Canadian SIR Program could be to serve as a model of how to garner support from the general public to implement more environment-friendly AW-IPM programmes. From its inception, home and business owners were encouraged to recognize the value that apple growers brought to the community — in terms of quality of life and economic benefits through agriculture and tourism. As a result, area residents took responsibility for helping growers implement a mutually beneficial AW-IPM programme by paying a portion of the annual budget and actively participating in activities such as the removal of unmanaged host plants.

5. TOBACCO BUDWORM

The tobacco budworm is a key pest of cotton and tobacco, and has developed resistance to most insecticides used for its control (Harris et al. 1972, Elzen et al. 1992). In an attempt to develop an alternative control strategy for the tobacco budworm, Laster (1972) discovered hybrid sterility in F_1 male moths when male *Heliothis virescens* were hybridized with female *Heliothis subflexa* (Guenée). The F_1 (hybrid) females produced from this cross, when backcrossed to *H. virescens* males, produced sterile male and fertile female progeny. This male sterility persisted in all subsequent backcross generations. Genetic studies of tobacco budworm backcross sterility discovered abnormalities in the sperm of hybrid and backcross males (Richard et al. 1975, Goodpasture et al. 1980). LaChance (1985) and Laster et al. (1988) reviewed in detail the different biological mechanisms responsible for sterility in the hybrid, including the early backcross and subsequent backcross generations. The potential use of backcross sterility was examined using population models (Makela and Huettel 1979, Levins and Parker 1983) that predicted a decline in a natural population of tobacco budworm following the release of backcross insects. Following encouraging results from many studies of host plant preference, mating preference, and mating competitiveness (Laster et al. 1988), a pilot release programme was planned for the island of St. Croix, US Virgin Islands.

The objectives of the pilot programme on St. Croix during 1977–1981 were to introduce a measurable amount of backcross sterility into the natural population of tobacco budworm, and to evaluate the population suppression and the level of backcross sterility in subsequent generations. This project was a cooperative effort

between the USDA and the Mississippi Agricultural and Forestry Experiment Station. During 1979 and 1980, four separate releases were made (Proshold et al. 1983). Male sterility in tobacco budworm wild populations continued to increase as long as backcross insects continued to be released. During the last 6 weeks of 1981, 94% of wild tobacco budworm males were sterile (Proshold 1983). However, as the backcross frequency declined following the last release, tobacco budworm populations returned to pre-release levels. Proshold and Smith (1990) were not able to detect the backcross phenotype five years after the last release, presumably because of genetic drift and selection.

Following the pilot release programme on St. Croix, a pilot test was conducted during 1991–1993 in the central delta of Mississippi, USA, to study the effects of released backcross insects on natural tobacco budworm populations in a typical agricultural production area (Laster et al. 1993, 1996, Hardee and Laster 1996). Backcross moths were released by placing pupae in emergence boxes at the test location, a 16.7-km^2 area in Washington and Sunflower Counties, Mississippi, in 1992, and in Bolivar County, Mississippi, in 1993. Control areas of the same size were designated for each year. The backcross to budworm overflooding ratio achieved during 1992 was 3:1. After releases had ceased, this ratio declined to 1.3:1 during June, and to 1:2.3 during July. The backcross:budworm ratio in the same area in 1993 was 1:2.2 (29.9% sterility) for the overwintering generation. Releases in Bolivar County during 1993 achieved a backcross:budworm ratio of 2.6:1. After releases had ceased, this ratio declined to 1:1.6 in June, 1:3.6 in July, and 1:4 in August, and produced a 12.1% sterility carryover in 1994. Hardee and Laster (1996) concluded that backcross release results were favourable. However, considering the survival and migration potential of the tobacco budworm, higher overflooding ratios of released to wild insects must be used to achieve the best results.

6. CORN EARWORM

The corn earworm is a major pest of maize, cotton, and many other field crops in the Western Hemisphere. Due to the importance of this pest, a method to mass-rear the corn earworm was developed (Burton 1969), and several attempts to eradicate this pest using the SIT from St. Croix, US Virgin Islands, were made (Snow et al. 1971, Laster et al. 1988). The first eradication trials were conducted for 3 months in 1968, and 6 months in 1969, with a second campaign being conducted from 1972 to early 1974.

Many problems were encountered during 1968, including an unexpected increase in the area planted to maize, inconsistent releases of irradiated insects, and poor and inconsistent insect production resulting from disease contaminants in the laboratory colony. Shipping and disease problems were reduced in 1969, but still caused periodic slumps in the supply of sterile corn earworms. Nevertheless, when there was no slump in the insect supply, releases of about 1700 corn earworm males per day treated with 320 Gy resulted in sterile to wild overflooding ratios ranging from 10:1 to 15:1. As a result of the 1969 programme, there was a reduction in the field in the number of fertile corn earworm eggs, rather than an increase in the number of sterile eggs. It was concluded that this reduction in oviposition was caused by a high

incidence (50%) of locking (failure of mating pairs to disengage upon completion of copulation) between released and wild adults (Snow et al. 1971, Laster et al. 1988).

For the second eradication campaign, changes were made in the rearing system to improve insect quality and the reliability of the supply of insects for release. In general, only males were released, and the radiation dose used to sterilize males was reduced from 320 to 225 Gy (Hamm et al. 1971, Young et al. 1976). However, several times during the course of the campaign, changes were made in the radiation dose actually used, and in the sex of the insects actually released. Eradication of the corn earworm was not achieved during either campaign, but much knowledge and experience were gained concerning the operation of an area-wide programme against a lepidopteran pest. As a result Laster et al. (1988) concluded that improved rearing techniques and more competitive insects were critical needs, and suggested that using a lower radiation dose would improve the efficacy of future AW-IPM programmes against the corn earworm.

To assess the influence of released males treated with a substerilizing dose (100 Gy) of gamma radiation, and to measure the level of IS induced in wild populations of the corn earworm, Carpenter and Gross (1993) conducted a pilot test in small mountain valleys in western North Carolina, USA, from 1986–1990. They found that the number of wild males captured per hectare was positively correlated with the distance from the release site of the substerilized moths. Analyses of seasonal population levels of wild corn earworms, calculated from mark-recapture data, indicated that seasonal increases of wild males were significantly delayed or reduced (or both) in mountain valleys where substerile males had been released. The incidence of corn earworm larvae with chromosome aberrations (indicating they were progeny of irradiated, released males) collected from the test sites during the growing seasons demonstrated that substerile males were competitive with wild males in mating with wild females, and were successful in producing sterile F_1 progeny that further reduced the wild population. These significant reductions (73.5%) in populations of the corn earworm resulted even though the average overflooding ratio of irradiated to wild males (5.3:1) was low compared with that of other programmes that release sterile insects.

The use of the SIT has not been implemented into operational programmes for the control of either *H. virescens* or *H. zea*. Although hybrid backcross sterility and F_1 sterility suppressed pest populations in the field, the cost of rearing the insects, the highly mobile nature of these species, and the development and adoption of effective *Bt* transgenic varieties, make it economically impractical at the present time to use the SIT to control these pests. However, releasing sterile moths when wild populations are naturally low and have a limited dispersion, e.g. at overwintering sites, would be an opportunity to apply IS (Hardee et al. 1999).

7. PAINTED APPLE MOTH

The painted apple moth is an Australian species, with flightless females. An outbreak of this invasive pest was eradicated in Auckland, New Zealand, by the Ministry of Agriculture and Forestry (Suckling et al. 2002). In New Zealand, larvae of the pest feed on many different plants of importance to horticulture (e.g. apple),

forestry (e.g. plantation pine), and native ecosystems. It was estimated to have a potential cost to New Zealand of USD 52–203 million. An eradication programme was approved, with a budget of up to USD 52 million (including communications and human health monitoring costs), and operations began in January 2002, initially involving helicopter and fixed-wing aerial spraying of *Bt* var. *kurstaki*. In February 2003, releases of partially sterile males irradiated as pupae at 100 Gy (Suckling et al. 2002, Wee et al. 2005) were initiated at three sites with known or suspected painted apple moth breeding populations. By May 2003, 45 000 males had been released, and recapture ratios in virgin female baited traps averaged ca. 100:1 of sterile to wild males.

Earlier modelling of the impacts of the *Btk* spray programme on the insect population suggested that a protracted programme would be needed with this tactic alone. The addition of IS to eradication efforts was welcomed immediately by public factions that opposed the spray applications. However assessment of the full benefit of the addition of IS to the successful eradication of painted apple moth remains to be determined. Nevertheless, because virgin females were being used extensively as lures in a trapping grid across Auckland, the additional costs of rearing, sterilizing, and releasing males were relatively minor (less than USD 145 000 per year). All indications from a preliminary analysis of the IS programme were positive, including a highly favourable benefit/cost analysis and no public resistance (D. M. Suckling, personal communication). As a result the SIT/IS is now being evaluated as a potential tactic to help eradicate other exotic pests such as the Asian gypsy moth, which was discovered as a single male in a pheromone trap in New Zealand in 2003 (Suckling 2003).

8. IMPACT, CHALLENGES, AND FUTURE DIRECTIONS

The long list of pestiferous lepidopterans and their tremendous impact on agriculture and forestry dictate that we continually look for more efficient, economical, and environment-friendly ways of dealing with these pests. The SIT/IS is a species-specific technique that is compatible with all other tactics, including ground or aerial spraying of insecticides, mass trapping, habitat removal, host-plant resistance, and biological control (Mangan, this volume), and is likely to be especially effective when combined with other inversely density-dependent tactics such as mating disruption (Suckling 2003). The overall effectiveness of the SIT/IS at reducing lepidopteran pest populations has been demonstrated effectively in the operational programmes for the pink bollworm in the USA and the codling moth in Canada.

However, a number of serious challenges still need to be met to make the SIT/IS for Lepidoptera more cost effective relative to other pest management tactics. Lepidopteran meridic diets are complex, the insects are generally large and developmental times long, the larvae are often cannibalistic, sanitation measures to prevent pathogen contamination (fungi, viruses, microsporidia) must be stringent, and the insects are fragile (which cause irradiation, transportation and release costs to be higher). Another challenge in rearing large numbers of lepidopterans is the presence of scales and setae that can be potent allergens (Davis and Jenkins 1995; Parker, this volume), requiring specialized equipment and air-filtration systems to

reduce/mitigate allergic reactions suffered by workers. The fact that only a couple of SIT/IS programmes for Lepidoptera have become operational has unfortunately also meant that they have not been able to take advantage of shared learning experiences in the way that the many fruit fly programmes have, and the beneficial impacts of such programmes are less well documented and accepted.

Methods to reduce the cost of lepidopteran programmes might include combining the SIT/IS with other pest control tactics such as the release of natural enemies. In this instance the cost of rearing might be reduced by using the same facility to rear both insect species, while the efficiency and effectiveness of a combined programme can help meet objectives in a more timely fashion through synergistic action of both tactics (Knipling 1992; Carpenter 1993; Carpenter et al., this volume). As it has for dipteran programmes, the development of genetic sexing strains (Marec et al. 2005) would greatly reduce the costs of rearing in lepidopteran programmes. F. Marec and colleagues in the Czech Republic demonstrated genetic sexing in the Mediterranean flour moth *Ephestia kuehniella* Zeller, using a mutant-male strain that is trans-heterozygous for two lethal genes (Carpenter et al., this volume). The application of molecular technologies might be used to develop genetic sterility and reliable genetic markers for the released moths (Peloquin et al. 2000; Miller et al. 2001; Marec et al. 2005; Robinson and Hendrichs, this volume).

The SIT/IS has special attributes that make it a desirable and unique insect pest management tool. However, because this technique requires a large start-up investment and a management-intensive support system, candidate pest species to be controlled with the SIT/IS should be selected carefully. A decision process to evaluate the suitability of the SIT/IS for controlling a pest lepidopteran should consider many factors including: (1) key pest status, (2) economic importance, (3) mass-rearing costs and technical feasibility, (4) favourable radiation biology, (5) migration ability, (6) potential for the treatment area to become reinfested, (7) host specificity, (8) availability of monitoring tools, (9) stakeholder and customer support, and (10) the availability and effectiveness of other control options for the target pest (IAEA 2000).

In addition to carefully selecting the most appropriate candidate species to be controlled with the SIT/IS, the type of programme should also be evaluated carefully. Since prevention is far more cost effective and environmentally desirable than measures that have to be taken once the introduction of an exotic invasive species has occurred, future considerations for selecting target lepidopterans should emphasize key invasive threats, similar to the SIT preparedness model being used in Australia for the Old World screwworm *Chrysomya bezziana* (Villeneuve) (IAEA 1999; Tweddle 2002; Vargas-Terán et al., this volume). For example, the cactus moth *Cactoblastis cactorum* (Berg), the false codling moth, the leek moth *Acrolepiopsis assectella* (Zeller), the European grape vine moth *Lobesia botrana* Denis and Schiffermüller, and the Central American potato moth *Tecia solanivora* Povolny, are all serious invasive threats to the USA. The proactive development of the SIT/IS technologies offshore at the point of origin offers the following advantages: (1) capitalization on contributions by foreign counterparts due to their interest in controlling the same pest, (2) reduction in pest pressure at the point of origin (off-shore risk mitigation) and thereby the risk of the pest being accidentally

introduced elsewhere, and (3) reduction in response time to implement an SIT/IS eradication program should the pest become established in a new location while pest populations are still restricted geographically and at low densities. The ability of biosecurity scientists in New Zealand to make use of existing knowledge, and quickly provide decision makers with ready-response capabilities for the inclusion of IS in the eradication programme against the painted apple moth, is a good example of how this approach can be successfully applied (Suckling 2003).

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CHAPTER 7.4.

POTENTIAL IMPACT OF TSETSE FLY CONTROL INVOLVING THE STERILE INSECT TECHNIQUE

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SUMMARY

Hunger and poverty persist in rural sub-Saharan Africa. Many affected communities could produce enough food for themselves, and even for sale, if they had the basics — livestock and crops. In most of these communities, the presence of tsetse flies and the disease they vector, trypanosomosis, prevents optimal productive livestock-keeping and mixed farming, resulting in inadequate local food production. Since a vast majority of the rural communities depends on agriculture, the removal of a key development problem like tsetse and trypanosomosis (T and T) will permit increased local agricultural production, socio-economic and market development, and alleviate hunger and poverty. A sustained alleviation, if possible a complete, lasting removal of the T and T problem, is therefore considered a prerequisite to rural self-sufficient agriculture, in which productive livestock can provide milk, meat, draught power to cultivate the land, and eventually generate higher income and market opportunities. Hence the removal of such a key problem would catalyse overall development in rural areas. However, the poverty and food security status of communities in Africa is rather heterogeneous, and reflects the impact of various constraining factors, including T and T on the current agricultural production process and human well-being, as well as on the overall development potential. Correspondingly, the benefits to sustainable agriculture and rural development (SARD), resulting from an elimination of the T and T problem, will also vary from area to area. In view of the substantial funding required over the next decades to address this key problem, and the need for early "success stories" that show tangible benefits, it is important that the initial T and T control areas are carefully selected according to technical feasibility, and to the predicted potential in the context of SARD. Trypanosomosis is a major, but technically solvable, development problem, and the effectiveness of the sterile insect technique (SIT), as a component of area-wide integrated pest management (AW-IPM) programmes to create tsetse-free zones, has been demonstrated in Zanzibar and other locations. This chapter (1) outlines the causal relationship between the T and T problem and food insecurity, malnutrition, poverty, and related disease and development constraints, (2) describes the impact of the problem on African rural communities and the overall economy, and (3) indicates the potential benefits of a reduced T and T burden, or even of its zonal elimination from selected priority areas in support of sustainable rural development.

1. INTRODUCTION

Tsetse flies are the cyclical vectors of trypanosomosis [trypanosomiasis], a disease occurring mostly in rural areas and affecting agro-pastoral activities in rural communities. The fly infests an area of 8.7 million km² in sub-Saharan Africa. In humans the disease is called human African trypanosomosis (HAT) or "sleeping sickness", and in livestock African animal trypanosomosis (AAT) or "nagana". Besides the human suffering and death from sleeping sickness, rural people have to cope with nagana, a major constraint to livestock production. The resulting food insecurity is a major problem for people in this region of the world. The severe limitations on health, food production, and even survival, imposed by these vectors and transmitted diseases, make the tsetse and trypanosomosis (T and T) problem one of the most severe that the people face.

Sleeping sickness is a potential threat to some 60 million people living in the infected areas, and in 1997, up to 350 000 people were assumed to be infected. Even if, as at present, the prevalence has been reduced dramatically, some areas may still be highly infected, and a large flare-up would occur if control activities slackened. An appropriate level of disease surveillance would require that some 70% of the

people at risk be screened on an annual basis, at a cost of USD 35 million, not including a similar expense for treating infected people (Feldmann and Jannin 2001).

Considering the overall impact (large direct and indirect losses), and the causal relationship between poverty, food insecurity, and the tsetse-related rural development constraints, it is inevitable that, in the long term, the suppression of vector populations, and better yet the creation of tsetse-free zones, are both economical and a moral imperative. If the T and T constraint were removed in an area, thereby making it feasible for rural people in subtropical and tropical Africa to produce food for their subsistence through mixed livestock-crop farms, they will obtain better nutrition and higher incomes, and the poorest region of the world will experience improved economic and social development.

Although some livestock producers apply methods of controlling tsetse flies and trypanosomosis with reasonable success, the benefits are partial and usually last only a short time. The rural poor cannot generally afford and sustain enduring T and T-control efforts. Therefore, it appears more appropriate to identify those T and T-infested areas that have a high rural-development potential, and isolated or at least geographically confined target tsetse fly populations, and implement programmes aiming at removing vector populations in a phased, integrated, and sustainable manner by applying — where feasible and justifiable — the sterile insect technique (SIT) as part of an area-wide integrated pest management (AW-IPM) programme (Feldmann 2004). The SIT lends itself to be the final component of an integrated programme for the creation of sustainable tsetse-free zones. The potential to eliminate the disease in a sustainable manner may open a new dimension of benefits, derived from introducing upgraded livestock breeds and cross-breds, and changing to more productive agricultural farming practices.

Although important for economic development and conservation of the environment (Ford 1973; Reichard 2002; Nagel and Peveling, this volume), the potential for changes in land use resulting from tsetse control is not discussed here.

2. POVERTY AND HUNGER

In 2001, the International Fund for Agricultural Development (IFAD) stated that:

> . . . progress in reducing rural poverty has stalled. In the 1990s, it fell to less than one-third of the rate needed to meet the United Nations' commitment to halve world poverty by 2015. It was six times less in sub-Saharan Africa (IFAD 2001).

Between 1981 and 2001, the percentage drop in the gross domestic product (GDP) per capita in sub-Saharan Africa was 15%, and the percentage increase in the number of people living on less than USD 1 per day was 91% (Time 2004). Reducing poverty in poor countries, especially in sub-Saharan Africa, is now the primary objective of development programmes. In his Millennium Report (UN 2000a), the Secretary-General of the United Nations pointed out that:

> . . . extreme poverty affects a higher proportion of the population in sub-Saharan Africa than anywhere else.

An initiative by the World Bank (WB) and the International Monetary Fund (IMF) to help the Least Developed Countries (LDC) (34 of 49 are in Africa), and especially the Heavily Indebted Poor Countries (HIPC) (34 of 42 are in Africa), to obtain debt relief, has led to these countries preparing Poverty Reduction Strategy Papers (PRSPs) (UN 2000b, 2001) which hopefully will emphasize food security and agriculture (FAO 2001a).

The depth of hunger in the world is clearly the greatest in sub-Saharan Africa, where the prevalence of undernourishment stands at about 34%, with no dramatic reductions expected in the years to come (FAO 2001c, UN 2001). It is the only region of the developing world where, over the past 40 years, the regional average of food production per person has been declining (UN 2001).

Boosting poor-country agriculture is critical to reducing hunger (Economist 2002), and the sustainable removal of this key development problem appears to be a prerequisite for tackling poverty and food insecurity.

> A sharper focus on hunger and agricultural development is needed within the broader objective of poverty reduction (FAO 2001b).

Every year, 6 million children, under the age of five, die as a result of hunger and malnutrition (FAO 2002a). Hunger leads to reduced productivity, to environmental degradation, and to conflict at national and international levels (FAO 2002a, Sachs 2004, Diamond 2005). Sickness reduces productivity, quantifiable through the Disability-Adjusted Life Year (DALY) Index (Murray 1994, Murray and Lopez 1994).

At the 1996 World Food Summit, a goal was set to eradicate hunger in all countries, and to halve the number of chronically undernourished people in the world (from about 840 to 420 million) by the year 2015 (FAO 1996). The target, later called one of the United Nations' Millennium Development Goals (MDG), was to achieve sustainable food production and security for all. The Food and Agriculture Organization of the United Nations (FAO) has predicted that, in 2015, sub-Saharan Africa will be the region furthest from reaching the MDGs, and the actual number of hungry people may increase rather than decrease.

Programmes that feed the hungry with imported food, as important as they are at critical times, provide only temporary relief, and, if wrongly implemented, they can have a devastating effect on rural people whose livelihoods depend on the production and sale of staple crops (Clark 2001). The role of agriculture, in generating food supplies and incomes necessary for access to food, is paramount in developing countries, especially the Low-Income Food-Deficit Countries (LIFDCs) (FAO 2001e).

Mattioli et al. (2004) estimated that 85% of the African poor are located in rural areas, out of which over 80% rely on agriculture for their livelihood. Success in raising small-farmer productivity will lead to improvements in household food security, level of nutrition, and income. Efforts to eradicate hunger need to focus on empowering families to achieve inclusive food security, encouraging a maximum of self-reliance (FAO 2001d).

Smallholder production, and production of food staples, play a critical role in the livelihoods of the rural poor, and labour-intensive approaches are especially appropriate to rural-poverty reduction. Over two-thirds of the income of the rural poor is from farming, and most of the rest depends for growth on linkages to farming. Small farms employ more people per hectare than do large farms (IFAD 2001).

3. LIVESTOCK AND CROP PRODUCTION

A major barrier to significantly improving agriculture in sub-Saharan Africa is the lack of productive livestock. Productive livestock are the key to agricultural improvement since they: (1) provide food (milk and meat), (2) aid in crop production, especially of staples and cash crops (draught power to cultivate the land for crops and manure for fertilizer, and can live off crop residues as well as grazing), (3) provide manure for fuel, hides for leather and power for transport, (4) act as a form of savings, a "walking bank", and reduce risk, and (5) provide a vital, and often the only, source of income for the poorest and most marginal of the rural poor, such as pastoralists and those living on poor land, sharecroppers and widows (Delgado et al. 1999).

Livestock, for example a few cattle and goats, owned by each rural family are the means to food production on a continuing basis.

An estimated one-third of all children, and perhaps a higher share of pregnant or lactating women, in developing countries suffer from mild to moderate protein-energy malnutrition (FAO 2001e). People in developing countries typically consume annually only one-fifth to one-sixth the milk, and one-third to one-fourth the meat, of those in industrial countries (FAO 2000). In 1995–1997, people in developing countries obtained an average of only 11% of their calories, and 22% of their protein, from animal food products, compared with more than twice these figures (25% and 49%, respectively) in developed countries (FAO 2001e).

These low consumption levels in developing countries indicate the potential for growth in the consumption of animal food products without adverse consequences on human health. Increased consumption, of even a relatively small additional amount of meat and milk, would supply the necessary protein and micronutrients, as well as needed additional calories, especially for children (FAO 2001e).

Farming families with small holdings must be enabled, through mixed farming, to increase agricultural productivity for their own immediate benefit.

The integration of livestock and crop operations is the main avenue for sustainable intensification of agriculture in many regions of the developing world (FAO 2000). Zimbabwean smallholders, who combine livestock and crop production, have incomes twice as high as those with only crops (IFAD 2001).

Traditional agriculture in Africa is based on crops grown on land worked with a hand-hoe. According to the FAO, in sub-Saharan Africa, 89% of all primary cultivation is carried out by hand (Budd 1999). However, the hand-hoe is not enough to produce sufficient crops to feed rural Africa! Animal draught power enables a family to cultivate at least twice as much land as can be done by hand (2 hectares with a team of oxen compared with 1 hectare with a hoe) (Swallow 1999). Animal

traction has been shown to generate higher returns on land (25–45%) and labour (140%) than the hoe, partly from the advantage of using manure as a fertilizer (Swallow 1999).

If the land is not suitable for crops, then livestock can still be grazed on it. In the case of natural disasters, such as drought and floods, livestock provide longer-lasting food security, and more risk-free agriculture, than crops. Even landless people can own cattle or small livestock, which graze on communal-pasture land, or are kept indoors with feed brought to them (zero grazing).

4. TSETSE AND TRYPANOSOMOSIS (T AND T) PROBLEM

4.1. Human African Trypanosomosis (HAT) (Sleeping Sickness)

The tsetse-transmitted human African trypanosomosis, sleeping sickness, is a major burden on African people in rural areas between northern Angola (Independent Online 2004) and southern Sudan. If left untreated, the disease is inevitably fatal; it has been estimated that each year 50 000–100 000 people die from the disease (Shaw 2004). The number of infected persons has been increasing for the past 40 years (Fig. 1). Some 45 000 new cases were reported annually until 2000. Although, since 2001, the number of new cases is decreasing, many infected people remain undiagnosed and die. Even though 60 million people in 20 countries are at risk, only 3–4 million are covered by active surveillance (Feldmann and Jannin 2001, Shaw 2004).
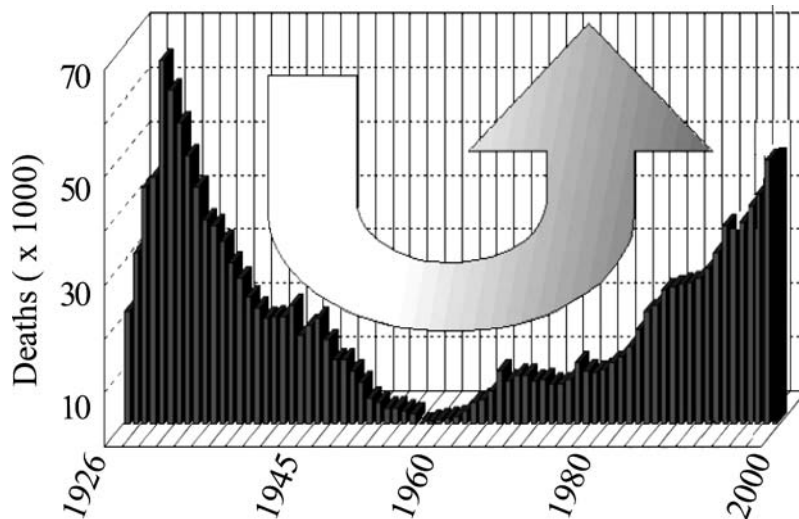
Figure 1. Number of deaths from human sleeping sickness in Central Africa from 1926 to 2000. (Figure from J. Jannin, reproduced with permission.)

The DALY Index, an indicator to quantify the burden of disease, includes the impact of both the duration of life lost due to premature death and the duration of life lived with a disability. The annual burden of sleeping sickness is estimated at 2 million DALYs (Shaw 2004). Since the disease tends to affect economically active adults, the total cost to a family with a patient is about 25% of a year's income (Shaw 2004).

4.2. African Animal Trypanosomosis (AAT) (Nagana)

4.2.1. Problem of African Animal Trypanosomosis

The disease is also devastating to livestock, with 45–50 million animals at risk in T and T-affected countries (Kamuanga 2003, Shaw 2004); the vast majority of the animals are crowded into the few tsetse-free areas in these countries. In some areas, there is evidence that the problem is exacerbated by an expansion in the distribution of tsetse flies (Leak and Mulatu 1993, Stevenson 1998).

As stated in ILRI (1999),

> Trypanosomosis is probably the single greatest health constraint to increased livestock productivity in sub-Saharan Africa,

and it significantly impairs agricultural development (Swallow 1999). When not lethal, trypanosomosis in livestock leads to a chronic debilitating condition that reduces fertility (calving rates), weight gain, meat and milk offtake by at least 50% (USD 2750 million per year), and the work efficiency of oxen used to cultivate the land (Budd 1999, Swallow 1999, DFID 2001, Shaw 2004). However, if left untreated, it is often fatal (especially for calves), with at least 3 million cattle and other domestic livestock dying each year (Hursey and Slingenbergh 1995). The problem is getting worse rather than better.

Besides the direct impact of the disease on livestock, there are also indirect negative effects. It discourages using more-productive exotic and cross-bred cattle, depresses the growth and affects the distribution of livestock populations, reduces the potential opportunities for livestock and crop production (mixed farming) through less draught power to cultivate land and less manure to fertilize (in an environment-friendly way) soils for better crop production, and affects human settlements (people tend to avoid areas with tsetse flies) (Shaw 2004).

T and T are enormous barriers to raising productive cattle in most of the humid and sub-humid zones of Africa (FAO 2000), especially exotic breeds and cross-bred cattle, which are much better milk and meat producers than local breeds but more susceptible to the disease. At present, cattle (especially cross-breds) can only be raised relatively satisfactorily outside (or around the periphery) of the tsetse zone, or by giving regular treatments of expensive trypanocidal drugs, in addition to other health care. Therapeutic chemical treatments are expensive (35 million doses used per year, each costing USD 1) (DFID 2001), and must be administered regularly. This practice elicits drug resistance in the trypanosomes. Consequently, treatments are no longer effective, and the future of cattle production in these areas may become problematic.

The use of trypanotolerant cattle in West Africa shows some promise as a way of coping with the disease threat, but these animals are rather small (DFID 2001) and thus are less suitable as draught animals. Shaw et al. (2004) compared trypanotolerant, Zebus and cross-bred (trypanotolerant x Zebu) cattle in herd models. Benefits with and without T and T, expressed per head of cattle over a 20-year period, were highest with cross-bred cattle.

Current methods of controlling tsetse, and the disease in humans and livestock, are by no means perfect. The current situation is untenable, with risk to the environment arising from high pressure on natural resources in areas not infested. The problem remains a major barrier to meeting the basic livelihood needs of the rural poor.

4.2.2. Benefits of Controlling African Animal Trypanosomosis

Everyone agrees that trypanosomosis

> . . . is sufficiently important for virtually any intervention to be beneficial (Shaw 2003).

Livestock Production. The benefits of T and T control are reduced disease prevalence or even disease elimination, reduced mortality rates and treatment expenses (and thus more money available for other things), and improved health and agricultural productivity. These benefits can be estimated using a dynamic herd model, which includes animal traction among the herd outputs. The reduced livestock production cost (38% and 25% reduction per tonne of producing milk and meat, respectively) would result in an increase in the amount of milk and meat supplied by farmers, and a lower price to consumers. For tsetse-free areas, milk and meat production would almost double, increasing by 83% and 97%, respectively. Benefits from traction power and manure production have been estimated to be, on average, 34% of the total value of livestock production (Kristjanson et al. 1999). The monetary values of losses and benefits are summarized in Box 1.

The 36 countries (10 million km², population 260 million) that have some level of tsetse infestation have about 172 million cattle, but only 45 million of them are kept in tsetse-infested areas, while the remainder are forced to crowd around the periphery, leading in some instances to land degradation (Swallow 1999). The overcrowding of cattle and people in tsetse-free areas is a problem for both good land-use practices, in terms of sustainable utilization of natural resources, and long-term community development activities.

Box 1. Monetary Values — Estimates of Losses from T and T Problem, and Potential Benefits of Controlling African Animal Trypanosomosis

Hursey and Slingenbergh (1995) valued the direct annual losses in cattle production due to AAT at USD 1000–1200 million.

Shaw et al. (2004) created maps that show the estimated potential benefits from T and T control in Benin, Burkina Faso, Ghana, Mali, and Togo. The research modelled cattle density, animal draught work, rate of herd growth, and rate of cattle spread. If the disease were removed, the total benefits after 20 years (in the form of a present value discounted over 20 years) ranged from less than USD 500 to well over 5000 per km² (Fig. below). Benefits included milk, meat, draught power, the extra cattle present, and the spread of cattle.

The potential benefit of enhanced trypanosomosis control in livestock in Africa is USD 700 million per year, arising from increased milk and meat productivity (Kristjanson et al. 1999). If one includes other potential benefits that would accrue from trypanosomosis elimination, such as animal traction and manure,

> . . . the total cost of the disease is likely to be well in excess of USD 1338 million per year (Kristjanson et al. 1999).

In 10 fully infested countries alone, the impact of T and T on the agricultural GDP was estimated to amount to 10% below a theoretical T and T-free GDP level (Swallow 1999), which corresponds to USD 1000 million in monetary terms.

Estimates of the overall annual lost potential in livestock and crop production range from USD 1950 to 4500–4750 million (Budd 1999, DFID 2001, Shaw 2004, Gooding and Krafsur 2005).
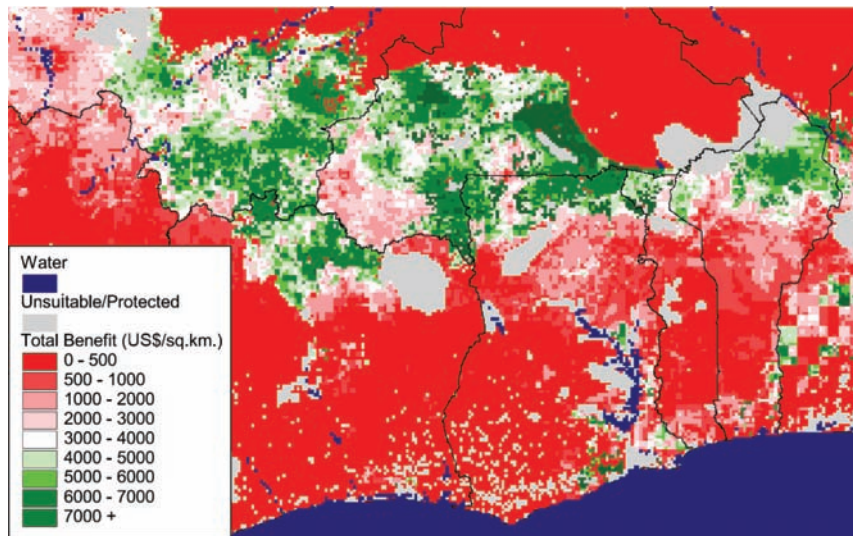
Total calculated potential benefit (USD per km²), after 20 years, of removing trypanosomosis (benefit range USD 0–500 [dark red] to 7000+ [dark green] per km²). (Figure from Shaw et al. (2004) for the countries Benin, Burkina Faso, Ghana, Mali, and Togo; reproduced with permission.)

If T and T were removed, the number of cattle would increase (Kristjanson et al. 1999, Swallow 1999, Shaw 2004); estimates range from 33 to 95 million (Kamuanga 2003). Using computer models, the increase in cattle density can be predicted (Gilbert et al. 2001) (Fig. 2).

However, it is expected that the increased cattle populations would not be low-productive cattle kept in limited areas but instead in many cases be high-productive cross-breds kept at a lower average density per unit of land (with more rural families owning cattle) than is the case today. Therefore, the creation of sustainable T and T-free zones, and the resulting rural development and the opportunities for profitable investment into productive agricultural and livestock systems, are not expected only to be major contributions in the fight against food insecurity and poverty, but also reduce the environmental risks of a potentially large increase in the number of cattle.
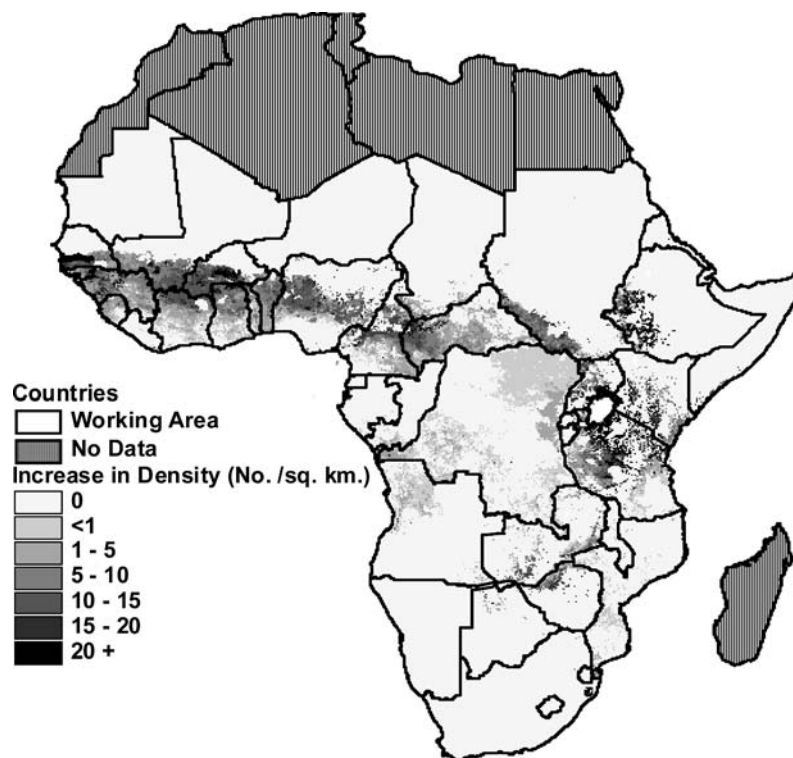
Figure 2. Predicted impact of tsetse removal on cattle density. (Figure from PAAT 2004, reproduced with permission.) (Gilbert et al. 2001)

Crop Production and Mixed Farming. In sub-Saharan Africa, livestock and crops are usually separated due to T and T, but there is a huge potential benefit if livestock and crop production can be integrated (Box 1).

In total, about 820 million hectares in Africa are classed as cultivable land (but half of it is marginal) (Harrison 1996). Up to 600 million hectares, especially in Central and southern Africa, are not cultivated for various reasons (Ford 1973). The best potential for fodder production lies in the humid zones, 18% of Africa's land area, but only 6% of the livestock are found there (Harrison 1996). In some cases, tsetse flies deny access to fertile, arable land, and their removal makes it accessible to livestock-agricultural production.

> African animal trypanosomosis constrains agricultural production in the areas of Africa that hold the continent's greatest potential for expanded agricultural production (Swallow 1999).

4.2.3. T and T, Hunger, and Poverty

Evidence for the causal relationship between the T and T problem and the prevalence of hunger and poverty is presented in this chapter. It cannot simply be a coincidence that most of the 37 tsetse-infested countries of Africa are also poor, debt-ridden, and underdeveloped. A comparison between a map highlighting the tsetse-infested areas (FAO 1985), and a map highlighting the 34 Heavily Indebted Poor Countries in Africa (WB 2001) (32 of which have tsetse flies), reveals the striking similarity of the two highlighted areas (Fig. 3). It is concluded that the T and T problem is a major contributor to hunger and poverty.

Of course there are other problems creating food insecurity, such as limited water supply, severe human diseases, lack of credit, poor governance, civil unrest and wars, as well as other livestock diseases, and these are also important, and some are interconnected and interdependent. Solutions to these problems must be found as well. However, to achieve rather quickly the objective of reducing hunger and poverty in such a needy region as sub-Saharan Africa, it is vital first to adopt the solution that will bring about the biggest change in the fundamental problem, and for which strategies and technology packages already exist and need to be integrated into the overall context of sustainable agriculture and rural development (SARD). It is a matter of giving a high priority to a problem that is fundamental, and to a solution that will have a great impact in the near future. This approach is part of the comprehensive policy of the Programme Against African Trypanosomiasis (PAAT), an international alliance comprising the FAO, African Union/Inter African Bureau for Animal Resources (AU/IBAR), International Atomic Energy Agency (IAEA), and WHO. The removal of T and T, a key problem, is expected to have a catalytic effect on overall rural development initiatives aimed at food security and poverty reduction.
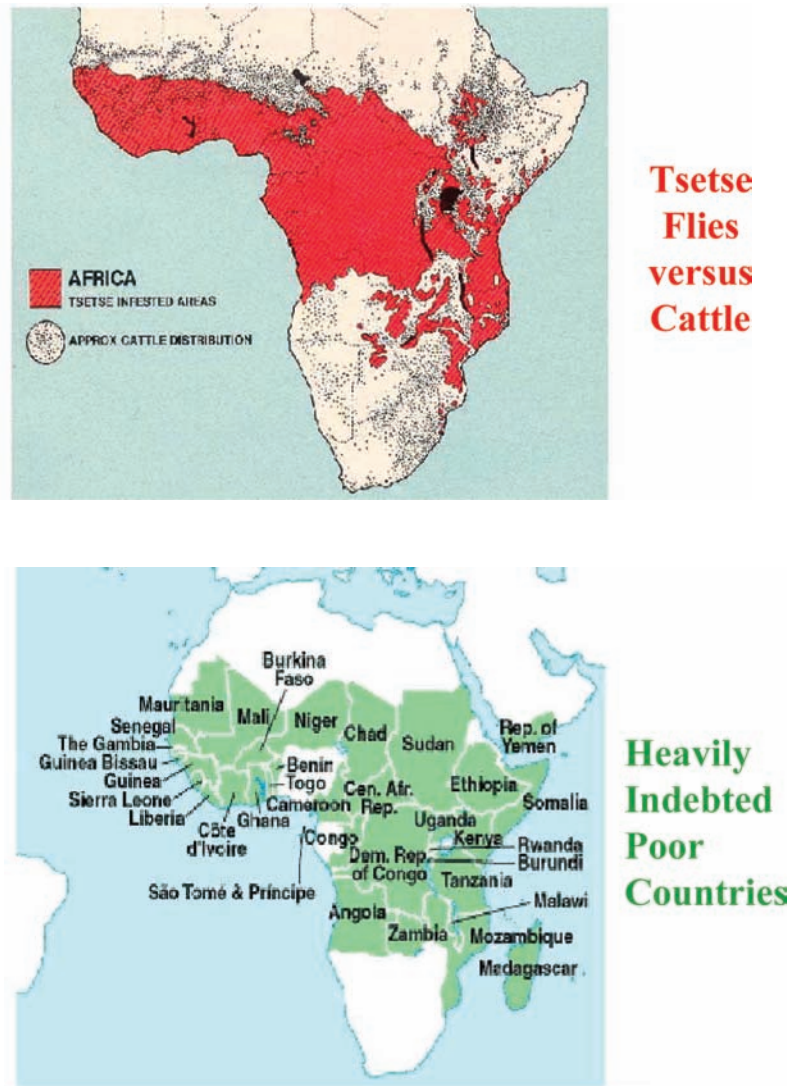
Figure 3. Comparison of tsetse-infested areas and Heavily Indebted Poor Countries in Africa. Due to tsetse, cattle tend to be distributed (black dots) outside the tsetse-infested areas (red). (Tsetse-cattle map: FAO, reproduced with permission; HIPC map: World Bank (2001), reproduced with permission.)

4.2.4. Why is the T and T Problem so Hard to Solve?

Why has the barrier of T and T remained so strong, and prevented a breakthrough in livestock and crop production? The T and T problem is a rural problem, and most political leaders, policy makers, and planners are probably already overwhelmed by various needs and deficiencies requiring immediate attention, ranging from water supply, infrastructure issues like roads and schools, to medical and other services. Actions against a problem like T and T are expected to result in benefits only in the medium to long term, and thus may receive a low or inadequate level of attention and political support. Since the T and T problem is chronic, the attention of donors and the media tends to focus on other acute, novel, or emerging problems. Since several efforts in the past to control the T and T problem did not lead to a sustainable improvement, it is difficult now to generate the necessary awareness and commitment among decision-makers. Also several influential scientists maintain the view that tsetse flies and the disease trypanosomosis cannot be eliminated, and that there is no alternative except to "live with the problem" at the lowest possible level of disease transmission. Many people have spoken out against "eradication", and many are pessimistic, believing that the "curse of tsetse" must simply be endured. This prevalent fatalistic attitude has led to a resigned acceptance of the seemingly inevitable need to suffer in hunger and poverty, with no hope of change. This attitude and approach to the T and T problem has prevented farmers, governments, and donors from trying to create, and consistently and gradually expand in a sustainable manner, zones that are free of the problem.

In past years, several community-based programmes to reduce fly populations were initiated enthusiastically, and relatively successfully suppressed tsetse populations and consequently the disease risk. However, enthusiasm waned, suppression was neglected, and the flies and disease returned (Jordan 1995, Barrett and Okali 1998). Unfortunately, even during phases of good tsetse suppression, the flies were still efficient vectors, and therefore continued to discourage the introduction of productive livestock breeds.

4.2.5. T and T, and Sustainable Agriculture and Rural Development (SARD)

Large-scale AW-IPM programmes against T and T should focus on priority development areas in affected countries. Regarding the selection of the initial priority areas for T and T intervention, in the context of SARD, and in view of the need for substantial funding over the next decades, initial efforts should focus on areas where the required investments are expected to result in maximum benefits. FAO (2002d) provided some criteria and guidelines for the selection of initial priority intervention areas.

To generate impact and benefits for the affected communities, it is essential that control measures against the T and T problem be firmly embedded in overall development efforts towards SARD. A programme called Farming in Tsetse Controlled Areas (FITCA), which was completed in 2004, aimed to improve the welfare of people through sustainable rural development, and to increase livestock productivity by improving animal health through community-based T and T suppression. FITCA had anticipated that the generation of integrated crop/livestock

production systems would increase food production (Daily Nation 2003, AU/IBAR/FITCA 2004). However, it remains uncertain if a way can be found to sustain such community-reliant methods of T and T suppression beyond the phase of funded institutional assistance. Unfortunately it is expected that, after the successful introduction of community-based tsetse suppression and a discontinuation of donor support, very few farmers would pursue the work and pay for the traps, insecticides, etc. Whenever an approach, that necessitates continued action, is not sustained, no breakthrough is possible. Under such scenarios, sub-Saharan Africa will likely remain a "green desert", preventing sustainable self-sufficiency in food for the rural poor.

4.2.6. PATTEC

An historic decision (OAU 2000), by the African Heads of State and Government, at the 36th Ordinary Session of the Organization of African Unity (OAU) (now called African Union (AU)) summit meeting in Lomé, Togo, in July 2000, recognized the seriousness of the T and T problem. The summit noted that this problem is one of the greatest constraints to socio-economic development in Africa, severely affecting human and livestock health, limiting land use, causing poverty, and perpetuating underdevelopment. The year 2001 was declared as the "Year of the Control of Tsetse Fly" to mark the beginning of renewed efforts to suppress tsetse flies and trypanosomosis. This new campaign, initiated by the OAU, is called the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) (PATTEC 2000, Pesticide Outlook 2002). The PATTEC Plan of Action to be implemented was approved at OAU's Lusaka meeting in 2001 (OAU 2001). T and T-affected countries anticipate that this new African-owned and African-led initiative will be a very useful contribution to reducing rural hunger and poverty.

5. TSETSE AND TRYPANOSOMOSIS-FREE ZONES

5.1. Concept

To implement the breakthrough solution, plans to create and progressively expand T and T-free zones, in areas with potential for livestock-agricultural development, are urgently needed. The problem is complex. A major effort is required that necessitates full ownership, and a high, consistent commitment by the affected countries. Long-term partnerships with local, national, and international stakeholders, non-governmental organizations (NGOs), and the private sector will be instrumental in the gradual creation of zones free of the most important tsetse species in priority areas for rural development. The very large benefits of complete disease control will only be realized if the disease threat is virtually non-existent, which would encourage poor producers to take the risk of investing in productive cattle. Instead of continuous efforts to suppress the vector fly populations and alleviate the disease, the creation and progressive expansion of T and T-free zones in a sustainable manner (Feldmann and Jannin 2001, UN 2001) will provide a quantum

leap forward, and "break the bottleneck" that for so long has prevented the rural poor from producing enough food. Establishing such tsetse-free zones would permit a significant increase in livestock productivity (Feldmann 2004).

It is neither necessary nor desirable to start applying this concept to the whole of sub-Saharan Africa, or to suggest that tsetse SIT would be of immediate use in all T and T-problem scenarios. As experience with aerial-spraying operations in northern Nigeria (Spielberger et al. 1977) and Botswana (Allsopp and Phillemon-Motsu 2002, Tsetse News 2002) has demonstrated, solely insecticide-based methods of tsetse suppression may also result in long-lasting solutions for some species or in some situations, provided that confined or isolated fly populations are attacked, following (intentionally or not) the principles of AW-IPM, and/or the campaign capitalizes on favourable agro-ecological or other relevant trends or developments.

While efforts to create T and T-free zones are initiated in priority areas (obviously other zones need to be screened for control actions), T and T suppression needs to be pursued in other areas. This is particularly true for areas with sleeping sickness. Although it is recognized internationally that vector-control strategies should be a component of long-term sleeping-sickness prevention measures, the control of sleeping sickness will continue, for the foreseeable future, to depend on disease surveillance and treatment as the principal priority, with vector suppression as a complementary tool. In animal trypanosomosis, tsetse suppression has a greater role to play in immediate (but with long-term perspectives) problem alleviation in priority areas, and as an important forerunner of creating tsetse-free zones (PAAT 2000).

The concept of creating and progressively expanding T and T-free zones, when and where needed and necessary, will require substantial funding over many decades. Initial resources must be focussed on a few selected priority areas — with strong demographic pressures, and high agricultural, livestock, and overall development potential — and must achieve good progress with early successes. An advantage that should be utilized is the fragmented distribution of tsetse fly species, with discrete populations in isolated or well-confined habitats. The initially targeted areas will be those with such isolated or confined fly populations. Progressively, over time, the size of infested areas will be reduced. The transboundary nature of fly infestations requires a regional approach, based on the AW-IPM concept, to reduce substantially the risk of fly reinvasion.

The international T and T community has agreed that efforts to create and subsequently expand T and T-free zones should be initiated in areas with a high potential for agricultural development, e.g. Southern Rift Valley in Ethiopia, and the Moist Savannah Zone in West Africa (Swallow 1999, Feldmann and Jannin 2001, FAO 2002b, PAAT 2002, Hendrickx et al. 2004). There may be opportunities to embark on projects for creating T and T-free areas in several other countries, but further evaluations are needed, e.g. South Africa, Botswana, Kenya, Uganda, and Tanzania (IAEA 2001). In some of these areas, particularly where other vector suppression techniques face technical limitations, or for environmental or other reasons can only be applied as a temporary measure, the SIT may play a role.

Regarding the economics of investing in integrated tsetse control programmes, an

> . . . economic analysis indicates that the cost of controlling trypanosomosis through controlling the tsetse fly populations will be covered several times by the benefits of tsetse-free status (Budd 1999).

Area-wide interventions against the T and T problem appear more efficient and profitable if sufficiently large areas, with high numbers of cattle, can be covered.

To minimize ecological disturbances, and ensure environmentally appropriate utilization of natural resources, components of the environment, and land-use practices, must be monitored before and after removal of the vector population (Feldmann and Hendrichs 2001). Even though there is no factual basis for the fear that tsetse eradication will lead to ecological imbalance (AU 2002; Nagel and Peveling, this volume), it is wise to monitor continuously several components of the biological and physical environments to permit, at an early stage, the identification and correction of undesirable developments in the field.

5.2. Potential Role of SIT

The SIT could contribute, and, when integrated with other suitable tsetse suppression methods in a phased manner, has been demonstrated in the field to be effective and efficient (Cuisance et al. 1986, Clair et al. 1990, Oladunmade et al. 1990, Vreysen et al. 2000). The scientific technology has been well studied in Africa, and it is a proven, internationally accepted technology against tsetse flies (Offori 1993, Feldmann and Hendrichs 2001, Feldmann and Jannin 2001, Feldmann 2004). The technology benefits all segments of rural society, including poor farmers, because, instead of protecting selected animals or farms, entire economically important populations of tsetse flies are targeted. Furthermore, as the programme aims at a public good, local people would usually not be charged for the SIT component. Since the launching of PATTEC in October 2001, the concept of removing the T and T problem in sub-Saharan Africa, by systematically creating and expanding sustainable tsetse-free zones, has received increased support, both at the level of T and T-affected countries and internationally. This approach will involve the temporary area-wide application of insecticide-based methods of tsetse suppression, and, where necessary, feasible and justifiable, be followed by the integration of the SIT to remove completely the entire relic target vector population.

The SIT is most efficient at low pest densities, and therefore is applied when the pest density is naturally low or due to prior suppression. When the SIT is integrated as a final component into an area-wide pest management system, the treatment area will become a tsetse-free zone (Vreysen et al. 2000).

Regarding the environmental considerations of such an integrated area-wide campaign (Nagel and Peveling, this volume), there are several insecticide-based and other suppression techniques, e.g. trapping, that are environmentally acceptable, provided they are not applied continuously. Even after the application of the sequential aerosol technique (SAT) (repeated application of ultra-low-volume formulations of non-persistent insecticides, usually from aircraft) for tsetse

suppression in the Okavango Delta in Botswana, Perkins and Ramberg (2004) showed that terrestrial non-target invertebrates, and the vast majority of aquatic non-target invertebrates, recovered within 1 year to the pre-spray composition and abundance. After the temporary use of such techniques to suppress efficiently the vector population to a very low level, the application of the SIT to "mop up" the relic tsetse population involves using the only vector-control method that has no known side effects on non-target organisms (Müller and Nagel 1994, Feldmann and Hendrichs 2001).

The weekly cost of tsetse SIT per km², i.e. USD 2.7–9.4, is in the range of, or lower than, the required expenditure (weekly, per km^2) in AW-IPM programmes integrating the SIT against other insect pests of agricultural and veterinary importance, e.g. codling moth *Cydia pomonella* (L.) (USD 100), Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (USD 80), and New World screwworm *Cochliomyia hominivorax* (Coquerel) (USD 3.5) (Hendrichs et al., this volume). Therefore, when feasible and justifiable, integrating the SIT into area-wide programmes, and removing a target tsetse fly population, may be cost effective. If programmes to create and expand T and T-free zones are focused, and relatively small incremental steps can be taken, financing field projects in African countries should be feasible. The ongoing recurrent expenditures for tsetse fly suppression and treatment of the disease could be redirected towards eradication projects.

6. BENEFITS FROM TSETSE ERADICATION

6.1. *Predictions for Ethiopian Southern Rift Valley*

Regarding the anticipated benefits of removing the T and T problem in the Ethiopian Southern Rift Valley, in a preliminary benefit/cost analysis, Knight (2001) pointed out that there are significant benefits to be gained from controlling tsetse flies by involving the SIT. For investments made to create a tsetse-free zone in the Southern Rift Valley, the break-even point should be reached in year 5 or 6, and the net present value (NPV) over 12 years is estimated at between USD 36.6 and 52.7 million. This represents an internal rate of return (IRR) of between 33 and 43%.

6.2. *Zanzibar Experience*

In 1994–1997 it was demonstrated in Zanzibar, Tanzania, that, after pest suppression with insecticides, the SIT completely removed the *Glossina austeni* Newstead population from Unguja Island of Zanzibar (Vreysen et al. 2000); subsequently, trypanosomosis in local cattle could no longer be found. This successful integrated and area-wide programme created significant opportunities to improve livestock and crop farming, and farmers are already taking advantage of them and obtaining benefits (Tambi et al. 1999, Mdoe 2003). Even though Unguja Island, due to poor soil in many areas, is probably not a perfect example of the agricultural benefits that would accrue from eliminating the T and T constraint on the African mainland, it

illustrates the range of agricultural and socio-economic benefits that can be expected after removing this constraint. (Sleeping sickness does not occur in Zanzibar, so no direct human health benefits were assessed.) It is important to recognize that the benefits began accruing as soon as the tsetse fly population and disease transmission were reduced, in some areas starting in 1985, using insecticides, and continued until, and especially after, the target fly population collapsed in 1996 as a result of applying the SIT. Subsequently no reinvasion of *G. austeni* has occurred (Saleh et al. 1999).

Tambi et al. (1999) and Mdoe (2003) collected local data, interviewed farmers soon after eradication occurred, and assessed relevant socio-economic parameters for 1985, 1997, 1999, and 2002. Even though most of the benefits are probably yet to come, and it takes time for resource-poor smallholder farmers to invest in and adopt improved or new technologies, the following summary of benefits indicates the kind of benefits that can be obtained after the T and T threat no longer exists:

- Increase in the contribution of the livestock sub-sector to the agricultural GDP, from 12% in 1986 to 34% in 1997.
- Increase in domestic food production, and consequent decrease in food imports, due to increases in livestock and crop production.
- Increase in the relative proportion of farms raising indigenous cattle, from 31% in 1985 to 94% in 2002.
- Increase in the proportion of farms with improved cattle breeds (mainly cross-bred) from 2% in 1985 to 24% in 2002. From 1985 to 1999, the number of indigenous cattle increased by a factor of 1.5, while that of cross-bred cattle increased 7 fold. The average number of cross-bred cattle per farm in 2002 was 0.41. While 38% of the farmers preferred local breeds of cattle, the majority (61%) preferred improved breeds, primarily because of their high productivity. The number of farmers acquiring improved breeds continues to increase, but the relatively high cost of such breeds, coupled with the low financial resources of farmers and limited availability of cross-bred animals, are slowing down this development.
- Increase in the proportion of farms milking indigenous cows, from 15% in 1985 to 74% in 2002, and increase in milk yield, from 1 to 2.5 litres per cow per day in 1985 and 2002, respectively. Milk production increased by a factor of 2.67 between 1985 and 1999. Improved cattle provided 35% of the total milk production in 1999, compared with only 10% in 1985. Since the milk yield of the local cattle breed, Zebu, is about 2 litres per cow per day, compared with 8 litres per cow per day for cross-bred cattle, an increase in the number of cross-bred cows resulted in a major increase in milk production. Milk production in recent years increased by 3% per annum.
- Increase in the number of farmers selling milk, and the quantity of milk being sold. Milk processing is not well developed in Zanzibar, with most of the milk being marketed in its raw form, but producers have little difficulty in finding buyers. In 1985 only 11% of farmers sold milk from indigenous cows, but in 2002, 62% of farmers marketed milk, and most of them (78%) sold to vendors. Yoghurt is now available in the local market. Nevertheless milk production in

Zanzibar is still able to meet only 25% of the local demand, the remainder being imported as processed products.

- Increase in milk production has probably also contributed to an improvement in the nutritional status of rural households. About 20% of the milk produced per farm is consumed at home as raw milk, and about 1% as fermented milk.

- Increase in meat production. The proportion of domestic versus imported cattle slaughtered for meat doubled (29% to 66%) between 1978–1985 and 1986–1995, indicating an increase in slaughter cattle obtained domestically. Between 1999 and 2001, the production of beef increased by 7%.

- Increase in crop productivity in mixed-farming systems, resulting from a greater capability to use animal power (stronger and healthier animals) for ploughing and transporting farm products. The proportion of farmers using oxen for ploughing increased to 5% in 2002, but in future 60% intend to use animals for ploughing. In 2002 about 21% of farmers used animals to transport their own products.

- Increase in crop-livestock integration through more farmers using manure for crop production. About 51% of farmers in 1999, and 54% in 2002, grew crops on fields fertilized with animal manure. From 1999 to 2002, the increase in productivity of cassava, rice, maize, coconut, and vegetables may be attributed to the use of improved seed varieties and manure for crop production. In addition, more farmers are using crop by-products to feed their animals. In 1999 only 13% of farmers were feeding crop by-products to livestock, but in 2002 this figure had increased to 22%. Also farmers are increasingly feeding improved fodder to their cattle. Some 92% of dairy farmers raising improved breeds zero-grazed their cattle.

- Increase of 30%, from 1999 to 2002, in average income per month of farming households. The proportion of households with an income over USD 25 per month increased from 69% to 86%, and the proportion with an income over USD 50 per month increased from 22% to 36%. It is logical to associate this increase with T and T eradication since a strong correlation was observed between household income and milk yields, milk sales, and use of manure and animal power for cultivation and transport.

- Increase in confidence that investing in livestock produces more food and more income for the family, even though, of course, farmers in Zanzibar still have to cope with the other challenges in keeping livestock.

- Increase in the number of Zanzibar red colobus monkeys *Pilicolobus kirkii*, an endangered and protected species found primarily in forests (also former major habitat of *G. austeni)*, from 1000–1500 in 1991 to more than 2500 in 1999 (Masoud et al. 2003). Therefore, subsequent to eradicating *G. austeni* in Zanzibar, there was no decline in the monkey population. This contradicts the conventional wisdom that eradication of tsetse flies will inevitably lead to habitat destruction followed by a reduction in wildlife populations.

7. CONCLUSIONS

Keeping livestock, and especially operating a mixed farm, is fundamental to the rural poor being able to produce their own food. There are many problems of food security that, even though they limit the availability of adequate food supplies, are problems that can be solved, step-by-step. However, if approached from the viewpoint of conventional pest suppression, the T and T problem remains an insurmountable barrier to productive agriculture. A partial solution is really no solution. Without breaking this barrier to enable the introduction of higher productive agriculture and livestock systems, and without the resulting development and profitable investment opportunities, there is no hope that the hungry in sub-Saharan Africa will become food self-sufficient within a reasonable time period. The poor farmers of Africa, because of where they live, are helpless in the face of a problem that, even though they may be able to alleviate it temporarily, on their own they cannot remove it in a sustainable manner.

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PART VIII

FUTURE DEVELOPMENT OF THE STERILE INSECT TECHNIQUE

CHAPTER 8.1.

PROSPECTS FOR THE FUTURE DEVELOPMENT AND APPLICATION OF THE STERILE INSECT TECHNIQUE

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SUMMARY

Science-based modern agriculture and international trade in agricultural commodities have achieved that, even though the world population has doubled in the last 40 years, the absolute number of people in poverty and hunger has been falling steadily. The major challenge in the immediate future is to consolidate these positive gains, while simultaneously expanding environment-friendly agricultural practices. Within this context, the sterile insect technique (SIT), as part of area-wide integrated pest management (AW-IPM) programmes, will continue to gain momentum for application against certain key insect pests. This is in response to the demands for cleaner food and a better environment, the need to facilitate increasing international trade by overcoming pest related trade barriers to the movement of agricultural commodities, and the imperative of dealing with the increasing invasion of exotic pests. As the use of the technology increases, changes will continue to be made to improve the overall efficiency of the technique for those species where the SIT is already being used, and to expand the use of the technique to new key species. Modern biotechnology may also contribute to improving efficiency and, even though there are as yet no transgenic strains of pest insects that could be used in AW-IPM programmes, transgenic technology may eventually benefit these programmes in terms of strain marking, genetic sexing, molecular sterilization, and disease refractoriness; however, first the regulatory hurdle to allow their use will have to be overcome. There appears to be much promise in improving sterile male performance by exposing male insects to hormonal, nutritional, microbial, and semiochemical supplements. Furthermore, the management of mother colonies will be significantly improved to reduce the effects of colonization and to slow down mass-rearing effects on key behavioural parameters that often result in rapid colony deterioration. Progress will also need to be made in the cost-effectiveness of all components of SIT implementation, from cage design to facility design, and from programme planning to evaluation. The trend of increasingly using sterile insects for routine pest suppression rather than eradication, particularly in commercially important commodities, will favour the involvement of the private sector and hence accelerate these improvements. Commercial producers of beneficial insects will probably be the natural investors, in view of the complementarities with sterile insects, experience in managing living organisms, and understanding the biological control market. As programme implementation is logistically complex, management will remain the key issue determining the success or failure of any area-wide approach to insect control. Thus, in spite of the many successes achieved and to be expected, in many least-developed countries the SIT may be a technology that is "ahead of its time" and beyond the animal and public health as well plant protection infrastructures. Failures in SIT application, mostly confined to such countries, have not been due to science but the implementation of systematic large-scale operations. Increased involvement of the private sector in such countries probably would assure effective implementation.

1. INTRODUCTION

It is likely that the use of sterile insects in area-wide integrated pest management (AW-IPM) programmes will gain momentum as increasing constraints are placed on the use of chemical pesticides responding to the public's growing demands for a cleaner environment and residue-free food. Global food chains increasingly link agricultural production with powerful supermarket chains that compete to offer their customers higher quality food in terms of maximum residue levels of insecticides. Even without these constraints, the cost of development and regulatory approval of new insecticides will continue to increase. Added to this, more species-specific

methods to control key pests, such as mating disruption or sterile insects, will play a more important role to complement the increasing use of biological control agents, which are often disrupted by insecticide applications. Furthermore, the international standards that regulate global agricultural trade, including measures for pest management where these affect trade (under the World Trade Organization's (WTO) Agreement on Sanitary and Phytosanitary Standards (SPS), are having a major impact on agricultural production and, this is creating an environment in which area-wide approaches to the management of insect pests have a comparative advantage.

With this expansion in the use of the technology, the basic components of the sterile insect technique (SIT) — mass-rearing and release of sterile insects — are hardly likely to change. However, changes will continue to be made to improve the overall effectiveness and efficiency of the technique for those species where the SIT is already being used, and research needs will be addressed to expand the use of the technique for new key species. For some potential candidate species, technical bottlenecks have already been identified that prevent the development of the technology, e.g. artificial rearing systems, which could be addressed by a concerted research effort, whereas other pest species, because of their biology, are not amenable to SIT application (Lance and McInnis, this volume).

Expanding globalization will inevitably lead to an increase in the invasion of exotic or alien insect pests into new areas. The concept of using the SIT against such pests to preclude their establishment will probably gain increasing support. This can take the preventive approach of the California model where sterile Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) are continuously released in areas of high risk of establishment (due to the regular introductions into the region (Dowell et al. 2000; Enkerlin, this volume). Alternatively, it can take the form of the Australian approach where the technology, expertise, and contingency plans are developed to be able to respond should an introduction of the Old World screwworm *Chrysomya bezziana* (Villeneuve) occur (Tweddle and Mahon 2000). The trend to counter the impact of increasing globalization, by moving plant protection "offshore" for risk mitigation of exotic pest introductions, will open up new opportunities to integrate the SIT into the process of creating pest free or low prevalence areas under a systems approach (FAO 1999, 2004), from which agricultural products can be safely exported.

Currently there is no interest from pesticide companies to become involved with the SIT technology, probably because it is not impacting on their market to any significant extent. Even with continued expansion, the SIT will remain a niche market. Commercial producers of pollinators and other beneficial insects will probably be the natural investors, in view of their experience in managing living organisms and their understanding of the biological control market. Furthermore, such companies can offer a complete "biological package", i.e. the SIT is used to manage some of the major key pests, while the reduced insecticide applications allow a significant expansion of the sales of parasitoids and predators to control secondary pests. The use of sterile insects for routine pest suppression rather than eradication, thus creating a sustainable demand analogous to the current use of augmentative biological control, will favour the involvement of the private sector

(Hendrichs et al. 1995). However, for commercial companies to become involved in the SIT, issues related to intellectual property rights (IPR) and regulation will need to be addressed (although see section 2.6.).

Programmes with the strategic goal of eradication can suffer from the price of success, e.g. maintaining staff in the face of a successful but declining programme. It is important to have a long-term vision — alternative uses of the rearing facility and a programme for another pest. The deliberate and malicious removal of fertile insects from a facility for release into an eradicated area is a constant threat, and needs to be addressed, e.g. biosafety protocols, staff relations, and strains with genetic markers and/or conditional lethal mutations.

For future expansion of the SIT, a major constraint that will always need to be addressed is the significant upfront cost of constructing a facility. Of course, plants producing and formulating insecticides represent larger investments, and compared with the costs of insecticide development, the costs of SIT development are minimal. Nevertheless, raising funds is not easy, and so far most rearing facilities have been constructed with public funds, and the beneficiaries often pay, partially or fully, for the sterile insects and/or the field operations. (The costs to beneficiaries usually do not include costs for constructing the facility; this is in contrast to the costs of insecticide where the development costs are recovered in the pricing policy of the company.) In some situations, true commercialization may be a way out of the dilemma (section 2.1.). Another constraint that must be overcome at the start of an area-wide programme is to involve, and get the commitment of, all stakeholders in the programme. Again this is a problem not faced in the pesticide situation; an individual farmer can make his/her own decision, and be approached on an individual basis by chemical companies.

Quality control protocols, both for the product, i.e. the sterile insect, and the process, i.e. the procedures used to produce, sterilize, and distribute the sterile insect, will need to be further improved, and in some way standardized. This has already been done for sterile fruit flies, where an internationally agreed set of protocols is used to monitor the quality of the sterile insect (FAO/IAEA/USDA 2003). It is reasonably straightforward to develop the protocols and to update them regularly. This is a pre-requisite for commercialization. Producers and users require harmonized protocols to measure sterile insect quality, and the increasing transboundary trade in sterile insects will gradually ensure that all programmes implement them (Enkerlin and Quinlan 2004).

Management remains the key issue determining the success or failure of any area-wide approach to insect control. Since the SIT is logistically complex and management intensive, its implementation requires flexible procedures and non-bureaucratic management structures. In general, the scientists who have been closely involved with developing the technology should not be responsible for programme implementation, as other skills are needed. Also research activities should not be part of an operational programme; instead, a separate unit (but associated with the programme) is needed for problem-solving and continuous fine-tuning of procedures; technology can always be improved. The "secret" is to find a balance between sterile insect quality and operational stability, predictability, and innovation.

2. UTILIZATION OF SIT TECHNOLOGY

2.1. *Commercialization*

Almost all of the field programmes integrating the SIT described in this book have been carried out by public-funded organizations, with or without some financial support from the direct beneficiaries. Although area-wide programmes often address a public good, in the long run this is not sustainable, and continued expansion of the technique will be facilitated when commercial enterprises become involved. Even if public funds continue to be used, governments may, to increase implementation efficiency, subcontract private companies to operate the whole programme or parts thereof. However, thus far commercialization of the SIT has been a difficult concept to promote, for the following reasons: (1) there are no equivalent models through which commercial confidence can be generated, with the possible exception of the biological control industry, (2) currently there are no international agreements or regulations for the production and transboundary shipment of sterile insects (but see section 2.6.), (3) only a very few detailed business plans for the production and use of sterile insects have been developed (Quinlan et al. 2002), (4) the initial capital cost of constructing a rearing facility is rather high, and (5) public-funded programmes have been providing sterile insects at subsidized prices, thus undercutting competition from private sector companies that have to sell at real prices that include the cost of capital (Bassi 2005). In spite of this list of difficulties in initiating the commercialization of sterile insect production, rather paradoxically there is currently no shortage of customers who would purchase sterile insects if they were available. The use of sterile insects only for eradication of pest populations was not an attractive proposition for commercialization, but the widespread use of sterile insects for suppression, containment, and prevention programmes provides some continuity in the need for sterile insects.

Commercialization of the SIT could involve the delivery of a complete package, or more likely be partitioned into several different components, depending on the type of programme. A commercial company could be responsible for rearing, shipping, emerging, and releasing sterile insects, and charge customers (who would only manage the field monitoring) according to the number of insects released or the area treated. This would be suitable where there are large agricultural areas growing mainly one crop, or where governments contract such turnkey operations to deal with pests of animal or public health importance. In an alternative strategy, a grower cooperative could buy sterile insects from a supplier, manage the monitoring and fly emergence, and subcontract the release activities; the Mediterranean fruit fly programme in Patagonia, Argentina, already follows such an approach.

Another strategy that lends itself to commercialization is the production of eggs in a large facility for satellite facilities (maybe even in another country) which then simply rear, sterilize, and release the insects. This removes the necessity for every programme to manage mother colonies and to maintain a large egg production colony, an expensive and highly skilled operation, especially if genetic sexing strains are being used (Caceres et al. 2004, sections 3.1.1. and 3.1.2.).

2.2. Exotic Pest Introductions

Globalization, increases in the movement of agricultural products, and changes in climatic conditions will all lead to increases in the movement of pests and in their ability to establish in new locations. This increasing problem of alien or exotic invasive species is causing much concern; often no good remedial actions can be taken when such a pest enters a new area. A good example of how such problems can be addressed is the accidental introduction into Libya in the 1990s of the New World screwworm *Cochliomyia hominivorax* (Coquerel). In the case of this incursion, fortunately a solution was at hand; the technology was already available for this pest, and sterile pupae could be brought from Mexico. For the eradication in 1990–1991, 40 million irradiated pupae pre-packed in boxes for release were air-freighted from Mexico each week in aircraft especially outfitted for long-distance transport of chicks. The insects were released in Libya, and the incursion was successfully eradicated (Lindquist et al. 1992). Of course, the major drawback to this approach is that a monitoring and rearing/release system must be in place, and the procedures must be implemented rather quickly.

The Australian government has taken this proactive approach and developed offshore a mass-rearing system for the Old World screwworm; should there be an incursion of this devastating pest into the country, the needed expertise and experience are available (Mahon and Ahmad 2000; Vargas-Terán et al., this volume). The recurrent introductions (through cargo ships) of the Asian gypsy moth *Lymantria dispar* (L.) into British Columbia, Canada, and north-western USA, but also as far as New Zealand, are also potential targets for integrating the SIT with repeated aerial *Bt*-sprays, increasing the effectiveness of ongoing efforts to eradicate such outbreaks (Suckling 2003).

Countries or regions may decide to identify their most important exotic insect threats, and then in advance develop the required pest-specific technology and expertise, as well as the general legal and physical infrastructure, that provide some degree of remedial action (Suckling 2003). This could include the SIT; it has a unique ability to eradicate incipient outbreaks of exotic insects in an environment-friendly way and with minimum public resistance. For example, one of the most feared exotic pests is the false codling moth *Cryptophlebia leucotreta* (Meyrick), still largely confined to the southern part of the African continent. To prepare for any future outbreak, the US government has been supporting in South Africa the development of the SIT against this major polyphagous pest (Bloem et al. 2003).

2.3. SIT Application to Environmental Problems

Since the SIT has virtually no environmental impact, it is an ideal tool for use in protected areas such as natural parks and biological reserves where other kinds of control are prohibited. Examples are some national parks in Africa where conventional tsetse control is no longer acceptable, but sterile insects can be released without any impact on biodiversity (Nagel and Peveling, this volume). Similarly, the SIT plays a special role in the large organic coffee production areas of Central

America where suppressing the Mediterranean fruit fly using conventional bait sprays is now prohibited.

Exotic insects, as well as causing problems for humans, their livestock and crops, can have a devastating impact on the environment. A textbook example of successful classical biological control is the introduction of the cactus moth *Cactoblastis cactorum* (Berg) to Australia to control invasive *Opuntia* cacti. However, after its arrival in North America (where *Opuntia* cacti are endemic), *C. cactorum* has become an invasive pest. It has spread from the Caribbean to the south-eastern USA, attacking endangered cacti. If this pest reaches the American southwest and Mexico, it is expected that it will have a devastating effect on whole ecosystems based on *Opuntia* cacti. In response, radiation biology, mass-rearing, and pheromone studies have been carried out on *C. cactorum*. The aim is to apply SIT/inherited sterility to contain this environmental pest, in its westward spread along the Gulf of Mexico, before it reaches the south-western USA (Zimmermann et al. 2004).

SIT/inherited sterility also has considerable potential to assess under natural conditions the environmental impact of introducing exotic herbivores as biological control agents of invasive weeds (Greany and Carpenter 2000). There is increased awareness of possible risks of non-target effects. To obtain importation and release permits for such species, increasingly stringent host specificity testing is required. Tests carried out under unnatural quarantine conditions often overestimate the host range, leading to rejection of effective candidates. Releasing sterile herbivores can be another risk-management tool for host-range assessment under natural conditions. There can be no leakage of genetic material into the gene pool because there is no native population present, and in the case of inherited sterility, released females are fully sterile. The survival of F_1 larvae under various abiotic and biotic conditions can be assessed, and if non-target species are attacked, releases can be suspended with no risk of permanent establishment.

This concept is being developed in Florida for an herbivore of the Brazilian peppertree *Schinus terebinthifolius* Raddi (Anacardiaceae), a major invasive exotic weed that is altering native plant communities in many subtropical regions. The South American leaf-rolling moth *Episimus utilis* Zimmerman (Tortricidae) has been imported into Florida for quarantine evaluation, and radiation biology studies have already been conducted to determine the dose that results in full female sterility (Moeri et al. 2005).

2.4. New Target Species

The biological criteria, to be considered before embarking on an AW-IPM programme integrating the SIT for a particular species, are described by Lance and McInnis (this volume), but the external conditions under which a pest population can become a candidate for the SIT are changing continually. For example, recent problems in the citrus trade between Spain and the USA have led to the development of an area-wide programme, and the incorporation of the SIT, against the Mediterranean fruit fly in large citrus growing areas in Valencia. Increasing

concerns about food safety and residues will also impact positively on the economics of using sterile insects.

An expansion in the area planted to crops expressing *Bt* toxins will influence candidate species for the SIT. For example, the use of *Bt*-cotton will largely remove insect pests of this crop from consideration as potential SIT targets. Nevertheless, in the south-western USA, the low pink bollworm populations (resulting from the extensive growing of *Bt*-cotton) have motivated the upgrading of the existing containment programme to an eradication programme (Henneberry 2005).

Pest lepidopterans constitute a major threat to many agricultural crops, resulting in the use of large amounts of insecticide. Currently there are only two operational programmes being implemented against this group of pests (Bloem et al., this volume), although in the past much research has been conducted. The number of lepidopteran species, for which SIT technology will be developed, will probably increase, but this will be only gradual since its application is usually more expensive compared with dipteran species, transgenic crops primarily target moth pests, and mating disruption is most effective in Lepidoptera pests (Cardé 2005).

Insect vectors of malaria and other human diseases continue to exact a huge toll in human lives and livelihoods, and in the 1970s many SIT pilot projects against vectors were implemented with varying degrees of success, including a very successful field trial in El Salvador (Weidhaas et al. 1974; Klassen and Curtis, this volume). Since that time, many technologies related to SIT development and implementation have changed dramatically, and the use of this technology against an important vector of malaria in Africa is being re-evaluated (Benedict and Robinson 2003). The technical problems to suppress *Anopheles* spp. mosquito populations using the release of sterile males are quite formidable, but there are situations where an assessment of the feasibility of this technology is warranted. The problems in developing and implementing the SIT are significantly smaller for *Aedes aegypti* (L.) populations, hence the first effective mosquito programmes will probably be against this species.

2.5. Integration with Other Measures

The SIT is always applied in combination with other methods of pest management, and often it is the final component of an integrated sequential approach (Klassen, this volume; Mangan, this volume). Knipling (1992) suggested that the combinatorial approach involving the simultaneous release of natural enemies together with sterile insects may be extremely efficient in population suppression and eradication. Theoretical modelling (Barclay, this volume) has confirmed the effectiveness of this integration. Although there has been limited experimental work to assess the veracity of this prediction (exceptions are some studies in inherited sterility in Lepidoptera, and the release of parasitoids) (Carpenter et al. 1996, Bloem et al. 1998), an expansion of this approach can be foreseen.

Today the control of mosquitoes relies heavily on the use of insecticide-treated bednets that target female mosquitoes during their host-seeking behaviour. This technology is very compatible with the release of sterile male mosquitoes; they do not seek the host for feeding but rather target the mate-seeking behaviour of female

mosquitoes. Similarly, success in New World screwworm eradication programmes requires the simultaneous treatment of wounds with insecticides, which are directed at the immature stages, and the systematic release of sterile insects, which are directed at the adult stage.

There are increased efforts to achieve field sterilization through deploying chemosterilant-baited traps (Hargrove and Langley 1990, Charmillot et al. 2002, Navarro-Llopis et al. 2004). Such approaches attempt to achieve autosterilization and dissemination through slow-acting compounds (such as insect growth regulators and chitin synthesis inhibitors) that are ingested with the bait and spread in the target population through intraspecific interactions. In most instances, these non-mutagenic materials are ingested with the bait and spread in the target population through intra-specific interactions; however, the boll weevil *Anthonomus grandis grandis* Boheman has been sterilized by spraying fields with a chitin synthesis inhibitor (Taft and Hopkins 1975). This effect can complement the release of sterile insects because, unlike wild insects, they are not affected. Nevertheless, the potential for large-scale application is limited; high-density trap deployment is not practical for area-wide implementation, and the environmental impacts on non-target species must be thoroughly assessed.

2.6. Regulatory Issues

Future expansion of the SIT will require a regulatory framework to facilitate the commercial production, trade, shipment, and release of sterile insects. Historically, since they are not self-replicating, sterile insects were not considered to be biological control agents, and therefore not included in the regulatory framework provided by the International Plant Protection Convention (IPPC) in the form of International Standard for Phytosanitary Measures (ISPM) Number 3 "Code of Conduct for the Import and Release of Exotic Biological Control Agents" (FAO 1996), which specifically deals with the import and release of biological control agents. Notwithstanding this lack of a regulatory framework, over the last 50 years there have been significant transboundary shipments of sterile insects, totalling about 960 000 million insects (Enkerlin and Quinlan 2004). This significant transport of live insect pests testifies to the inherent safety of radiation-induced sterility and its general acceptance by the international plant protection community.

The increasing interest shown by some commercial companies in producing sterile insects has emphasized the importance of having a regulatory framework. During the ongoing revision process of ISPM Number 3 by representatives of national and regional plant protection organizations, the definition of biological control agents has been broadened, sterile insects have been specifically included as "beneficials", and the SIT is now officially accepted internationally as a type of biological control (FAO 2005a). In addition, the terms "sterile insect" and "sterile insect technique" are being defined for inclusion in the ISPM Glossary of Phytosanitary Terms (FAO 2005b). Thus, the application of sterile insects as part of the integrated management of plant pests is now recognized by the IPPC, and this should facilitate their transboundary shipment and use, especially in terms of their commercial use. This framework would only cover those cases where plant pests are

involved; the use of the SIT against insects of veterinary or public health importance will require other regulatory action, possibly through the Office Internationale des Épizooties (OIE) and the World Health Organization (WHO).

2.7. Resistance to SIT

The increased use of sterile insects to suppress pest populations may bring with it concerns about the development of resistance (Itô et al. 2003; Lance and McInnis, this volume; Whitten and Mahon, this volume). As suppression programmes by definition are long-term, this will provide opportunity for natural selection to select individuals that can, in some way, differentiate between a released sterile male and a fertile wild male. This may be especially relevant where an island population is targeted on an area-wide basis; this represents an isolated genetic environment (Itô and Yamamura, this volume). The danger for resistance to occur is probably more remote when a population is targeted for eradication, although the development of resistance in suppression programmes will be modulated by the occurrence of refugia from where "susceptible" individuals re-enter the target population.

For resistance to develop and be selected, three conditions must be fulfilled: (1) a recognizable difference or differences between a sterile male and a wild male that a wild female can recognize, (2) a fitness cost to the wild female if she mates with a sterile male, and (3) a genetic basis for the recognition of the sterile male by the wild female. It is not inconceivable that, for certain species, all these conditions could be met. For condition (1), routine quality control procedures should identify any anomalies, allowing correction through the introduction of a new strain; for (2), as the fitness of a wild female mating with a sterile male is zero, this condition will always be met in a programme integrating the SIT, and for (3), for most species this is largely unknown. The development of resistance in a target population can be dealt with by re-colonizing a population from the field and replacing the original colony. This becomes slightly more problematic when specialized strains, such as genetic sexing strains, are used for release (Robinson et al. 1999). Field cage tests under semi-natural conditions, to continuously monitor the effectiveness of sterile males when competing with wild males to mate with wild females, become a very critical component of all programmes.

In those programmes where sterile females are also released, tests need to evaluate the ability of wild males to discriminate between sterile females and wild females. If wild males mate only with wild females, but sterile males mate with both types of females, modelling indicates that a doubling of the sterile insect release rate is required to overcome this wild male discrimination against sterile females (Vreysen et al. 2006).

3. TECHNICAL IMPROVEMENTS

3.1. Improving Insect Quality

A critical factor determining the success or failure of a programme that includes an SIT component is the ability of the released sterile males to effectively inseminate wild females with sperm that is competitive with normal sperm (although in some species aspermic sterile males can also inhibit female remating at the same rate as normal matings involving sperm transfer (Itô et al. 1993)). This ability is generally termed "competitiveness", and is determined by many factors related both to the treatment of a particular cohort of released insects and the developmental history of the mass-reared strain used to produce the cohort for release. Both sets of factors can impact negatively on the competitive ability of released insects, and efforts will continue to be made to minimize these negative effects. As the "production philosophy" still tends to dominate decision-making, even though theoretical models continue to stress the importance of insect quality over the number of insects released (Itô and Yamamura, this volume), more efforts to educate decision-makers are needed. A major component of any quality improvement strategy is to have in place a series of quality control protocols that can be used as a baseline to evaluate the effect of any experimental changes on insect quality (FAO/IAEA/USDA 2003).

As quality refers only to a field parameter, then it would seem appropriate to develop field-based protocols for its assessment, unless laboratory-based protocols can be identified that correlate directly with field competitiveness. In the past, probably too much attention was focussed on laboratory-based protocols that had very little predictive value in terms of field performance. Quality is relative; it is dependent on the space and complexity of the testing arena. In a restricted space, for example in a small laboratory cage with a reduced distance between individuals, sterile males are often more effective than wild ones, but in larger cages the effectiveness of the same sterile males decreases significantly, and in field cages with vegetation it decreases even further (Soemori et al. 1980, Miyatake and Haraguchi 1996). It is also of little value to develop quality control parameters using only mass-reared insects, especially for species with a mating system in which the females choose among mating partners (Lance and McInnis, this volume). In other words, the quality of mass-reared insects must increasingly be measured directly under semi-natural conditions and in competition with wild insects (FAO/IAEA/USDA 2003; Calkins and Parker, this volume).

The key field parameter to assess the quality of mass-reared sterile males is the amount of sterility induced in wild females, and protocols need to be developed that provide information on this parameter. In tsetse flies and screwworms, the opportunity to monitor the fertility of a field population under challenge from sterile insect releases is a tremendous advantage to programme evaluation (Vreysen, this volume). In fruit flies, where it is not yet possible to monitor this parameter in the field, a standard field-cage system, including host plants, has been developed, where mass-reared sterile insects have to compete with wild insects. A similar system has also been developed for tsetse flies (Mutika et al. 2001). This type of approach needs to be expanded and refined to include semi-field cage experimental systems that

contain a cycling wild population, and in which sterile insects can be reared and their competitiveness assessed. This has already been done for the Mediterranean fruit fly (D. O. McInnis, personal communication) and for mosquitoes (Knols et al. 2002).

To a limited extent, compensation for low insect quality is possible by releasing a larger number of sterile insects. However, there is a limit to this in terms of the overall economy of a programme, and there is a biological limit in that, below a certain quality value, increasing the number of released insects will simply not be effective (Barclay, this volume; Itô and Yamamura, this volume). In future, much more attention should be paid to quality as opposed to quantity. The more expensive it is to produce an insect, the more important it is that quality be maintained.

The introduction of a colony mass-rearing protocol based on the filter rearing system, currently used to maintain stability in genetic sexing strains (section 3.1.2.; Parker, this volume), can also make a major contribution to the quality of mass-reared insects.

3.1.1. Colony Initiation and Maintenance

There are virtually no procedures or protocols on how to establish an initial colony of a species for future mass-rearing (McDonald 1976). Questions about the number of insects that should be sampled, where they should be sampled, and when the sampling should be done, remain largely unanswered. Even if guidance could be given on the above points, there is still the well-known phenomenon of a "bottleneck" which occurs during the first one or two generations of laboratory rearing, and which can negate the effects of any science-based approach to field sampling (Cayol 2000a; Parker, this volume).

The answer to the question, "How many insects should be sampled?' is generally, "As many as possible". Of course this is not very satisfactory, and the number of insects used is usually influenced by the logistics involved in collecting insects in the field, coupled with the known size of the bottleneck that occurs during initial colonization. If the aim of the initial sampling of the field population is to sample the available genetic variability, then, based on population genetic studies of natural populations, samples of 20–100 insects generally reveal most of the genetic variability present in a field population, indicating that a sample in the hundreds is probably sufficient for most situations, provided that most individuals reproduce in the laboratory. Then the major task becomes the preservation of that variability during initial colonization and subsequent mass-rearing in the laboratory.

From where in its distribution should the field population be sampled? Here again logistics tend to determine the location of sampling sites and hence the parts of the insect distribution that are sampled. A related question to this concerns the strengths and weaknesses of using a single mass-reared strain for all regions where a particular species is a pest. This is especially important for species that are distributed worldwide, e.g. the codling moth *Cydia pomonella* (L.) and the Mediterranean fruit fly, and even for other species showing very discontinuous distributions. To a point, this can be tested by conducting mating compatibility studies, using the target populations from the different regions and the mass-reared strain (Cayol 2000b). Even if tests reveal no mating problems between the two

populations, still programme managers often request that a particular genetic background be backcrossed into the strain to be used, despite the absence of any evidence that this is beneficial. In biological control circles, there has been much discussion on whether to sample marginal or central populations, but some of the hypotheses proposed to answer this question are, in practice, very difficult to test (Mackauer 1976).

The underlying assumption made during this discussion is that the level of genetic variability, per se, in the mass-reared insects is correlated with the overall quality of an insect once sterilized and released in the field, and hence is important to maintain. Krafsur (this volume) challenges this assumption, and suggests that inbreeding, i.e. reduction of overall variability during colonization and mass-rearing, may not always affect competitiveness in the field. At the moment, probably there are insufficient data to make a general conclusion regarding the correlation of genetic variability in the mass-reared colony with competitiveness of the insects in the field. As suggested by Krafsur, in the future quality control procedures should be expanded to include routine measurements of genetic diversity in the colony over time, coupled with effective tests to monitor the competitiveness of the insect in the field. In the colonization strategy for the New World screwworm, procedures have been developed that involve the establishment of isofemale lines, followed by their evaluation and subsequent hybridization to establish a strain for mass-rearing (Mangan 1992).

The decision-making procedure, as to when a strain needs to be replaced, has to be improved. Since strain deterioration is normally correlated with the number of generations under selective mass-rearing conditions, at present programme managers usually establish a time-based replacement schedule. Better tools need to be developed to monitor the quality of released sterile insects in the field. A science-based and data-driven approach to strain replacement is needed (but see next section). Also, colony maintenance procedures that slow strain deterioration should be developed, thus extending the life of a strain under mass-rearing. These will probably include a combination of the following actions:

- Holding mother colonies under relaxed rearing conditions (see filter rearing system below),
- Making simple modifications to the holding conditions of the mass-reared colony that reduce the selective pressures on some of the key behavioural processes (Liedo et al. 2006),
- Implementing an "active" quality control programme that establishes heritabilities and genetic correlations between desirable traits such as mating behaviour and traits that can be counter-selected during routine mass-rearing (Miyatake and Yamagishi 1993, Miyatake et al. 1997), and
- Maintaining founder/back-up strains under cryopreservation to guard against genetic drift (Leopold 2005).

3.1.2. Strain Replacement Procedures and Filter Rearing System (FRS)

For all insect species that are mass-reared, the artificial environment of a facility presents the insect with a tremendous challenge, i.e. to adapt to the artificial conditions, both biotic and abiotic. Successful colonization will inevitably result in

selection for adapted genotypes, and this can impact negatively on the competitiveness of a sterile insect once it is released in the field (Miyatake and Haraguchi 1996, Briceño and Eberhard 2002). Most mass-rearing protocols follow the principle of large cycling colonies, whereby a proportion of the production is used for sterilization and release while the remainder is returned to maintain the production colony. In this system, over time there is an inevitable accumulation of highly selected genotypes that can significantly compromise the quality of the insects released in the field. This fact has prompted most operational programmes to replace their strains regularly, and to establish improved quality control procedures that monitor the overall competitiveness of the mass-reared strain. Strain replacement is a major logistical exercise, and invariably the new strain requires considerable time before it is as productive as the old strain. It is not clear how much of the overall quality of a strain is lost during this adaptation process, and how often a strain needs to be changed. Improved methodologies to monitor strain quality are needed.

As discussed by Franz (this volume) and Parker (this volume), the use of genetic sexing strains in Mediterranean fruit fly programmes incorporating the SIT required the development of a filter rearing system (FRS) to maintain the integrity of the sexing procedure (Fisher and Caceres 2000; Caceres et al. 2004). The FRS relies on the maintenance of a mother colony that each generation is checked for unwanted individuals (which are then removed). Eggs from this colony are harvested as required, and following 3–4 generations of amplification (during mass-rearing) the males are sterilized and released. In the FRS, no insects that have been through mass-rearing are returned to the mother colony, and therefore there is no accumulation of highly selected genotypes in the colony.

The FRS can, of course, be used for purposes other than maintenance of the integrity of genetic sexing strains, and can make a major contribution to the overall competitiveness of mass-reared strains. The mother colony can be kept under more natural environmental conditions, at reduced adult and larval densities, and with reduced selection pressure for genotypes adapted to mass-rearing conditions. In addition, a more natural environment, preferably under greenhouse conditions with hosts and natural light, could help address the major problem of loss of irritability and predator-evasion behaviour in mass-reared insects, requiring significantly higher overflooding ratios as a result of high mortality due to predation (Hendrichs et al. 2006).

A major advantage of developing a mass-rearing system based on the FRS is that strain replacement becomes a much simpler procedure, and can be done without major disruption of the production process. The size of the population in the initial small colony will, of course, depend on the production level required in the facility, but it will probably not exceed several thousand individuals. Therefore, a new strain can be introduced into the FRS in a sequential manner during one generation, and there will be little interruption in production. This procedure has been used to introduce new Mediterranean fruit fly genetic sexing strains into production facilities.

3.1.3. Diets

All insects that are mass-reared are maintained on an artificial diet. For most species, the larval and adult stages need to be fed. One exception is tsetse flies where larval development takes place in the female, and only adults need to feed. The larval diet of many species is an expensive component of mass-rearing costs, and presents logistical problems if the diet includes components that are poorly defined, with consequent difficulties in verifying their quality. From the perspective of quality control, defined diets would be preferable, but very few are available (Chang et al. 2001), and anyway these would be too costly for mass-rearing purposes. Nevertheless, one can foresee the commercial availability of pre-mixed diets.

Disposal of large quantities of spent larval diet can also be a concern. It not only contains some live larvae but also is rich in organic matter. Recycling of some components of the diet has been considered (the larvae do not actually exploit part of the nutritional value of the diet), but the problem of metabolite accumulation in the diet has yet to be overcome, and thus far no effective practical procedure for any larval diet has been identified.

A major challenge for the future of diets is to include micro-organisms that naturally contribute nutrients (Vijaysegaran et al. 1997). Inoculation of diets with such mutualistic symbionts would not only reduce the need for diet preservatives, but also for costly protein supplements that larvae often only partially use (Chan Jr. et al. 2000) and which are much less abundant in their natural substrate (Lauzon et al. 2004). A recent book (Cohen 2003) highlights many issues related to insect diet development. Research on ways to artificially rear several important pest species otherwise amenable to SIT application is required. The lack of cost-effective mass-rearing procedures for these species has been the major obstacle to the implementation of area-wide control programmes.

3.1.4. Sterile Male Performance

By applying hormonal, nutritional, semiochemical supplements or other bioactive materials to post-teneral sterile males before their release; there is a great potential to improve their subsequent performance or impact in the field. Until recently, research on the critical period between the arrival of pupae at emergence facilities and the release of adults in the field has been largely neglected. Current and future research could result in significant breakthroughs, and it may be possible to recover some of the quality deterioration that has occurred due to colonization, mass-rearing, and irradiation. Thus far, operational programmes have not adopted the findings already made on this subject.

Juvenile hormones (JH) are known to regulate the development of reproductive capacity and sexual signalling in Diptera and other insect orders. Hormonal treatments in the form of JH mimics, applied to emerging sterile males, have been shown to significantly advance sexual maturation. In *Anastrepha* spp. fruit flies, for example, treated males mate 5–7 days earlier than untreated males. This acceleration of reproductive development is crucial for improved SIT application; normally a majority of sterile males are lost to predation and other causes before they reach sexual maturity (Teal et al. 2000).

Nutritional supplements are equally critical for sexual development and signalling in anautogenous species, and adding protein to the diet fed to sterile males prior to release often significantly increases sexual performance (Shelly et al. 2002a, Yuval et al. 2002). The importance of bacteria in the behavioural ecology of some target insects, for example by making required nitrogen and other nutrients available through enzymatic degradation, is still poorly understood. Current mass-rearing practices may even be promoting non-beneficial or harmful bacteria. Thus the inoculation of post-teneral diets with probiotic diets, i.e. a diet that contains beneficial gut micro-organisms, is an area with much potential to improve sterile male performance (Niyazi et al. 2004).

Combining hormone exposure and a protein-enriched diet results in synergistic interactions, thus increasing sterile male competitiveness many-fold when compared with either only protein or only hormone treatments (Teal 2005). Such hormonal and nutritional therapies are affordable, easily incorporated into protocols followed at fly emergence facilities, and earlier release reduces costs associated with holding sterile males.

There is also great potential to use semiochemical supplements to boost sterile male performance. Some species that are attracted to natural attractants sequester these as pheromone precursors into their pheromonal systems, and subsequently release them during courtship. For example, such components fed to sterile males of *Bactrocera* spp. before release can significantly improve their mating competitiveness, and, in addition, such components can act as very potent allomones to deter predators (Tan 2000). Furthermore, pre-release feeding on such attractants can significantly reduce sterile male response to male-annihilation baits in the field (Shelly 1994). On the other hand, non-exposed wild males would still be attracted and killed by male annihilation baits, thus potentially allowing a "male replacement" strategy, consisting of the simultaneous application of male annihilation and sterile male release.

The mating competitiveness of wild or mass-reared Mediterranean fruit fly males is similarly considerably enhanced by exposure of pre-release sterile males to ginger root oil and citrus peel oils (Papadopoulos et al. 2001, Shelly et al. 2002b). This has great potential in increasing the cost-effectiveness of deploying sterile males, and even exposure to vapour has this effect on emerging males, thus enabling the development of an "aromatherapy" that will facilitate application in fly emergence facilities (Shelly et al. 2004a, b). Another advantage of aromatherapy is that wild females mated initially with wild males are more likely to remate with ginger root oil-treated sterile males, and furthermore, initial matings with ginger root oil-treated sterile males reduce the likelihood of wild female remating with wild males (Shelly et al. 2004a, b). This is important since, in fruit flies, normally the final male partner fertilizes the majority of the eggs in multiple-mated females.

The recent identification of the stimulatory cuticular hydrocarbons in New World screwworm female flies, which elicit mating responses in screwworm males, opens the possibility of applying them to sterile females (Carlson et al. 2005). Commonly, after rearing a newly colonized screwworm strain for a few generations under mass-production conditions, screwworm females experience the loss of these cuticular pheromones, and only sterile males accept them as partners; wild males

consistently reject them and mate only with wild females. Similar pheromonal compounds may exist in the ecological spheres of other pest species, and still await discovery and application to boost sterile insect performance.

Finally, there is a great potential to use sterile males as carriers of various bioactive materials (Knipling 1979). One possibility is autodissemination, in which sterile insects would be inoculated with electrostatically charged powder formulated with entomopathogens (Vickers et al. 2004) or slow-acting insecticides, which would be spread throughout the pest population through intraspecific interactions (Howse 2005). Another is autoconfusion, a type of mating disruption particularly suited for moth pests, in which released sterile males would carry on their bodies pheromone particles that attract wild males. This would transfer particles to them, and subsequently these males would contaminate other males, resulting in increased mating disruption (Knipling 1979, Howse 2005). Since the few released males that would eventually encounter pheromone-calling females are sterile, autoconfusion and the SIT are clearly compatible. This approach may be more cost-effective for area-wide application than conventional mating disruption or even the establishment of a reduced number of dispensers in the field where wild males would get contaminated. A further possibility is simultaneous mating disruption of a moth pest and SIT application against another pest that is much cheaper to mass-rear. The moth pheromone-inoculated sterile males, e.g. Mediterranean fruit flies, would significantly increase the mating disruption of the target moth pest population due to a higher overflooding ratio, and simultaneously suppress the local Mediterranean fruit fly population. There are probably many other possibilities of applying bioactive materials to emerging sterile males, but so far these are only theoretical, and much research is required to explore the various possibilities.

3.1.5. Hybrid Vigour

Hybrid vigour relies on the crossing of two inbred strains to produce an F_1 generation that exhibits increased fitness. It is a general phenomenon, used widely in plant breeding to improve the fitness of plants in the field. To be applied to the SIT, it would require that two inbred strains be reared in a facility, and a simple way exists to select males and females from both strains for directed mating to produce F_1 insects for sterilization and release. At present, in most species, it is not possible to do this automatically. However, it can be tested in the Mediterranean fruit fly, where genetic sexing strains are being used. In facilities using these strains, two separate sexing strains, both based on pupal colour and temperature sensitivity, could be maintained in separate modules. In the generation before release, male pupae (brown) from one strain could be placed with female pupae (white) from the second strain, and vice versa, in adult cages and F_1 eggs collected. These could then be heat-treated, and the surviving males reared, sterilized, and released. This procedure could even be carried out without the need for a pupal colour sorter, as there is a difference in the developmental time of white and brown pupae in the genetic sexing strains (Franz, this volume). A protocol based on this principle is now being evaluated (P. Rendón, personal communication).

3.1.6. Radiation Dose

Radiation is one of the many contributing factors to reduced competitiveness of sterile insects; the higher the radiation dose, the more the competitiveness of the insect can be compromised. In most programmes, the radiation dose used produces almost full sterility (as measured by percentage egg hatch from a mating involving either an irradiated male or female insect). Even though this laboratory assay is convenient, it is probably not the only one to use when deciding on the appropriate dose. Measuring egg hatch in the laboratory does not take into account factors such as male competitiveness and sperm competitiveness that, in the end, will combine to determine the hatchability of eggs produced by females of the wild population. These last two parameters will be the major factors in inducing sterility in the wild insects, and they must be taken into account when deciding on the radiation dose to be applied. If the dose is reduced below that which gives full egg sterility, a more competitive insect is released, which in the end induces more sterility in the wild population (Toledo et al. 2004). In other words, an optimum radiation dose for each particular species and strain should be identified (Mehta and Parker 2006). However, better decision-making tools are needed to be able to identify the optimum radiation dose. Of course, the rate of increase of the target population must be considered, since it determines the effectiveness of various combinations of male sterility and release ratios in reducing the target population (Klassen and Creech 1973).

3.2. Improving Technology

As well as improving the quality of the insect, progress will need to be made in all components of SIT implementation, from cage and facility design to programme planning and evaluation. Improved and more reliable air-handling systems in mass-rearing facilities can have a major impact on the efficiency and cost of rearing. Several facilities now have centralized systems where environmental conditions throughout the facility can be monitored and adjusted as necessary. This centralized system brings with it some disadvantages; other facilities have been built using a modular design. Facility design, equipment needs, cost, and space allocation for the different production processes are key components. For the Mediterranean fruit fly, interactive spreadsheets are now available to integrate the relevant data for different production volumes. This facilitates the financing, construction, and equipping of rearing facilities that produce different numbers of sterile males (C. Caceres, personal communication; P. Rendón, personal communication).

3.2.1. Shelf-Life and Shipment

In many areas of the world, pest problems are seasonal, and thus operational programmes need to release sterile insects only during specific times of the year. Nevertheless, insects can be produced year-round in the rearing facility, and it makes economic sense to keep a facility productive for most of the year. In species that have a facultative diapause, this behavioural trait can be used to store insects during the time when they are not required for release. This trait can also be exploited when shipping sterile insects (Bloem et al. 1997). However, many tropical

and subtropical pest species have no diapause, and these insects cannot be stored. Recent developments in the cryopreservation of insect embryos (Leopold et al. 2001) may provide a procedure for stockpiling eggs. If a pest species has an obligate diapause, this prevents the development of mass-rearing technology. Research on developing strains of these species that do not enter diapause is needed so as to overcome this barrier.

Sterile insects are often transported over long distances. This is usually done when the insect is in the late pupal stage, and some form of cooling and/or anoxia is used to prevent adult emergence during transit. This procedure can reduce insect quality, particularly if the transport period exceeds 48 hours. Pupae are usually shipped in cardboard containers containing cool packs to maintain a low temperature, and then the packing materials are discarded, sometimes creating a disposal problem. It would be desirable if durable equipment were designed that would maintain the correct temperature and atmospheric conditions, and increase the safety of insect shipments. There are constraints in transporting biological material, both within and between countries, and anything that can be done to improve the biosafety of shipments is valuable.

There is now another way to ship insects. Fertile eggs are being shipped from egg production facilities to larval rearing facilities that simply rear, sterilize, and release sterile adults. The egg reception facilities do not need to maintain large adult colonies for egg production, and this greatly simplifies their operational protocols. It has been demonstrated that Mediterranean fruit fly eggs can be shipped over long distances for extended periods of time without losing their viability and affecting the quality of the insects that are subsequently mass-reared (C. Caceres, personal communication). This concept permits the egg production facility to concentrate its efforts on the maintenance of a mother colony and a large colony for production purposes (section 3.1.2.). In principle, one large central facility could provide eggs to several satellite insect rearing and sterilizing facilities. This concept has already been put into practice in the Moscamed Programme in Guatemala and Mexico, where heat-treated eggs from a genetic sexing strain maintained at the mass-rearing facility in Guatemala are shipped daily to the male-rearing facility in Tapachula, Mexico (Tween, 2004; P. Rendón, personal communication).

3.2.2. Rearing Systems
The hardware used to produce sterile insects is continually being modified and improved. In countries with high labour costs and good maintenance practices, partial to full automation of mass-rearing systems will become common. Even in less developed countries, some degree of automation for key processes will be required to provide improved consistency in sterile insect quality (IAEA 2003). There are two key biological components, i.e. egg production and larval rearing, where new design components would have a major impact on mass-rearing efficiency. At present, the cages used for maintaining production colonies often allow females to produce only a portion of their full egg potential. This is caused mainly by males harassing the ovipositing females (cages have a high insect density) and a shortening of the female adult lifespan. New cage designs that address these

issues will have an immediate positive impact on rearing efficiency and insect quality (Liedo et al. 2006).

Larvae are usually reared on an artificial diet held in a container. When the larval period is complete, larvae are separated from the diet and allowed to pupate. Normally this process is costly, wasteful, and in some cases unpredictable. In some species, the larvae pupate in the diet, and special procedures are required to obtain adult insects. The development of a defined liquid diet held in a type of fermenter, in which the larvae grow to maturation before being separated from the diet, would be a major change in rearing philosophy. Unless unexpected problems in rearing systems arise, the status quo tends to be maintained. Facility managers must ensure that innovations and new concepts are continually evaluated.

3.2.3. Sterilization

Insects are usually sterilized by ionizing radiation produced from ^{60}Co or ^{137}Cs sources. The half-lives of ^{60}Co and ^{137}Cs are 5.27 and 30.07 years, respectively (Bakri et al., this volume). These irradiators are effective, but have some disadvantages. They require a considerable amount of regulatory support. The radioactive source declines with time, and thus radiation times get longer; eventually the source has to be recharged, a complicated and expensive procedure. It is becoming increasingly difficult to arrange for the transport of this type of irradiator to some parts of the world. At present, exposure to ionizing radiation is the best way to sterilize insects. However, the logistical difficulties associated with using conventional irradiators suggest that other options should be explored (Bakri et al., this volume). Electron beam technology to generate electrons, or photons such as X-rays, is an option. These systems can be switched on and off as needed, and require no complicated regulatory framework, but at present they are very expensive to purchase and operate, and require substantial maintenance expertise. Very little information on using this type of equipment to sterilize insects is available.

Sterility can also be generated biologically through cytoplasmic incompatibility, when different insect populations of the same species are mated that carry endosymbionts of the genus *Wolbachia* (Townson 2002). The development of molecular methods of sterilizing insects is discussed in section 4.3.

3.2.4. Release Technology

The area-wide approach to pest management requires that sterile insects be distributed as adults over large areas; this is usually done by aircraft. The current practice is to release sterile insects via an auger from a chilled container in the aircraft, removing the need to hold and release insects in bulky biodegradable containers, saving airplane space and thus greatly reducing flying time and hence costs. This procedure requires that adult insects, after emergence from pupae, be collected and placed in the chilled container. The logistics of this procedure for very large numbers of insects are daunting, given that insect quality must be maintained at as high a level as possible. The technology to hold insects at a low temperature for several hours in an aircraft, and to achieve the required distribution over a release area, has greatly improved. Nevertheless, there is still a major concern about the

negative impact on insect quality from the stress of low temperature and high insect density in the release machine. The low temperature creates a high humidity, and the resulting condensed water makes the insects wet. In the dense holding columns, such wet insects become stuck together, exiting the release machine in clumps instead of a continuous stream. Also, the moving augers of the mechanical delivery system damage a small proportion of the insects.

One indication of the future is a new system that is based on cryogenics (liquid CO_2 to make dry ice pellets) as the cooling component. It simplifies maintenance, eliminates the high electric load on the aircraft, and allows greater control over temperature and humidity, thus minimizing damage to the sterile insects (Tween 2005). Computer software linked to a satellite-guided aerial navigation system is programmed to deliver an adjustable number of sterile insects (as needed in each release block) and to turn off the release machine when outside the target blocks. The performances of the pilot, aircraft, and machine are recorded, and can be analysed after each flight (Dowell et al., this volume). This system can deliver up to 50 million sterile insects per flight, and enables the simultaneous release of several species of sterile insects and parasitoids (Tween 2005).

3.2.5. Field Monitoring

The widespread use of the Global Positioning System (GPS) and geographic information systems (GIS) technologies has dramatically increased the accuracy and ease with which insect populations can be monitored before, during, and after the implementation of a programme. This increase in precision will enable much better use of programme resources, and rapid decisions to modify programme activities are now possible. The use of bar-coded traps, and the ability to enter field data directly into hand-held computers for rapid downloading at the field centre, will increasingly make a major contribution to the accuracy and accessibility of data, and enable managers to monitor the efficiency of trapping personnel (Cox and Vreysen, this volume).

Field monitoring also requires effective methods to attract and trap insects of both sexes, and in many species there is active research on trapping methods. Data from traps are used to calculate the ratio of released to wild insects, and to directly monitor the relative size of the wild population (Vreysen, this volume). Traditionally, since females are responsible for population growth and hence damage levels, most emphasis has been placed on developing efficient female traps. However, when sterile insects are released, it is important to monitor males in the field. Nevertheless, when all-male releases of Mediterranean fruit flies are made, traps biased to preferentially collect females are useful to minimize the recapture of sterile males, and to facilitate discrimination between wild and recaptured sterile flies.

During monitoring it is useful that released insects be easily and unequivocally differentiated from wild insects trapped in the field. (However, it is acknowledged that the New World screwworm eradication programme in Central America was implemented without marking the released sterile insects.) To accomplish this, usually larvae or pupae destined for release as sterile insects are marked with a dye (Parker, this volume). When trapped insects are brought in from the field, their

origin is determined by examining them for the presence of the dye. This process is very labour intensive, expensive, and subject to significant errors of interpretation. Hagler and Jackson (2001) have described several new technologies that may be improvements on these dyes. Morphological markers have often been suggested as possible ways to monitor insects in the field, but usually they are associated with reduced competitiveness and therefore cannot be used effectively in the field. In the Mediterranean fruit fly, a phenotypic mutation has been isolated; it may be useful in the field since it does not appear to be associated with reduced competitiveness both during mass-rearing and in field cage competition tests (Niyazi et al. 2005). Molecular methods offer a solution to marking for special situations, but they are not practical to screen large numbers of trapped insects. As indicated below, markers involving the use of transgenic insects would bring along regulatory problems.

3.2.6. Suppression

Before releasing sterile insects, it is essential to suppress the field pest population. Since the SIT must be carried out on an area-wide basis, suppression techniques are included in this same requirement (Mangan, this volume). The area-wide approach will often require that pest populations have to be suppressed in inaccessible areas and also in areas where there is no human population. Although sterile insects can be successfully distributed by aircraft, the same is not always possible with a suppression "technology", e.g. in many countries, it is not permitted to conduct aerial spraying with traditional insecticides, especially over organic crops or sensitive areas. This puts severe restrictions on the type of suppression technology that is appropriate for area-wide application. Another difficulty involves suppression activities in urban areas, where many host plants can be located. The human populations in these areas need to be persuaded that what can be a very intrusive intervention will eventually benefit the economy of their community, however, better suppression methods are needed. There is considerable interest in developing, for use in such sensitive areas, bait stations that both attract and kill insect populations (Katsoyannos and Papadopoulos 2004).

A suppression technology that can be distributed by aircraft is highly desirable, and this has led to the identification of a new insecticide-bait formulation for fruit fly suppression. Spinosad is an insecticide developed from the bacterium *Saccharopolyspora spinosad* Mertz and Yao, and it has been incorporated into a protein bait spray that has been organically certified (Peck and McQuate 2000; USDA/APHIS/PPQ 2000; Mangan, this volume; Nagel and Peveling, this volume). However, this insecticide is not immune to the resistance problem; resistant strains to spinosad were rapidly selected in the house fly *Musca domestica* L. (Shono and Scott 2003).

Effective tsetse fly suppression is now possible using the sequential aerosol technique (SAT), where extremely low amounts of insecticide are delivered by low-flying aircraft at certain times when climatic conditions are optimal (Allsopp and Phillemon-Motsu 2002). Effective suppression technology, and an "environment" in which it can be used, will always be an absolutely essential component of AW-IPM programmes integrating the SIT.

4. TRANSGENESIS

It is now possible to routinely introduce genes into the germ-line of many pest species, and much of this development has been predicated on using transgenic insects in new types of control programmes, including the SIT (Hoy 1992, Robinson and Franz 2000, Robinson et al. 2004). There has been much speculation in this area, but as yet no transgenic strains of pest insects have been produced that could be used in a programme with an SIT component. Current thinking suggests that transgenic technology may eventually benefit operational programmes in the four areas outlined below (Handler 2002). The current public perception of the use of transgenic organisms in general (NAS 2002) does not encourage their use in the SIT, although paradoxically, the release of sterile transgenic insects is probably one of the lowest risk strategies for transgenic organisms (Hoy 1995, 2000; Alphey 2002).

4.1. Genetic Sexing

As described by Franz (this volume), the SIT can often be made much more effective if only males are mass-reared and released (Rendón et al. 2004; Lance and McInnis, this volume). Traditionally, genetic sexing strains have been based on Mendelian genetics, and use a combination of selectable markers and male-linked translocations to achieve sex-linkage. These strains have disadvantages — the mass-reared colony is only 50% fertile, females in the colony are homozygous for the marker, and they show reduced viability. Most importantly, the systems are not transferable to other species. Considering that the Mendelian systems can take many years to develop for a single species, this is probably the major disadvantage.

Genetic sexing using molecular approaches, although they have to overcome similar problems such as strain stability and competitiveness, are expected to be more generic, and thus transferable between different pest species. They can either be targeted towards killing females or transforming putative female zygotes into males, and both systems require conditionality to ensure maintenance of colonies. As in conventional sexing strains, if females are targeted for killing, then lethality should be induced at an early stage, ideally in the egg, as very large numbers of zygotes can be treated together. This requires that early-acting sex-specific promoters are identified and placed under conditional control. In *Drosophila*, a tetracycline repression system has been used to construct female killing systems (Heinrich and Scott 2000, Thomas et al. 2000), but as yet such a system has not been demonstrated in a pest insect. There are many assumptions that have been made in extrapolating these small-scale laboratory experiments on *Drosophila* to pest species being reared in very large numbers in mass-rearing facilities, and verification is urgently needed.

Transforming females into males requires a detailed knowledge of sex determination in the species under study, but fortunately in Diptera, there is quite a lot of conservation at the molecular level of many of the genes involved in this process, even if there is considerable variation in their functional relationships (Shearman 2002). In most dipteran pest species studied so far, the primary signal for sex resides on the Y chromosome in the form of a male-determining factor. Using

Drosophila DNA sequences as probes, various sex-determining genes have been cloned and studied in different species. In the Mediterranean fruit fly, one of these genes *transformer (tra)* has been the target for transforming females into males by injecting double-stranded RNA (dsRNA) for part of the *tra* gene into embryos (Pane et al. 2002). The dsRNA acts to prevent expression of the *tra* gene. From 272 adults treated with dsRNA, 231 showed the normal male phenotype (including normal male sexual behaviour), 37 were intersexes, and 4 were normal females. All the males were XX and carried no Y chromosome, and when crossed with normal females only female progeny were produced (Pane et al. 2002). This is remarkable, even if it is based on quite high levels of dsRNA in the injected embryos. The same sex transformation effects have now been demonstrated following transgenesis (Saccone et al. 2005).

4.2. Marking

As indicated above, sterile insects for release are usually marked with a fluorescent powder; transgenic techniques could enable them to be marked with a fluorescent protein. Using a genetic marker for released insects requires that the marker be dominant, and that it can be monitored even in dead adults, as insects are usually dead when removed from traps. There are currently two fluorescent protein markers available to accomplish this, i.e. green fluorescent protein (GFP) (Prasher et al. 1992) and red fluorescent protein (DsRed) (Matz et al. 1999). Generalized expression of the proteins can be obtained by using polyubiquitin and actin promoters (Peloquin et al. 2000, Handler and Harrell 2001), and the protein can be observed in dead insects (A. M. Handler, personal communication; G. Franz, personal communication). Particular strains can show very strong levels of expression, but the fitness cost to the insect for the production of this exogenous protein is unknown. Also, no data are available on the effect of this marker on the behaviour of the insect in the field, or indeed on the response of conspecifics or predators to fluorescent insects. This is especially relevant since insects use the UV spectrum for vision.

4.3. Sterilization

The use of ionizing radiation has proven to be an extremely effective way to sterilize insects for release in the field. To reduce somatic damage, the procedure is done at as late a developmental stage as possible. This physical process is a fail-safe procedure when the correct protocols are followed. It is also not subject to the development of resistance, can be used on any strain, and does not interfere with the mass-rearing process. As with all the procedures to which released insects have to be subjected, radiation does have some negative effect on the competitiveness of treated insects. However, the detrimental effects of radiation have sometimes been exaggerated. The overall competitiveness of a released insect is determined by a whole combination of different factors related to factory adaptation, selection, transport, and handling and release procedures, etc. Itô and Yamamura (this volume)

strongly make the case that reductions in competitiveness in the field are much more likely to be due to colonization and long-term mass-rearing effects than to radiation.

The transgenic strains that have been proposed will induce embryonic lethality in eggs fertilized by released fertile transgenic males carrying a dominant lethal gene, the so-called RIDL system (Thomas et al. 2000); this would eliminate the need for irradiation. Any system to induce sterility in the field through dominant lethality must be conditional in some way so that efficient mass-rearing can be carried out. This conditionality can be achieved by using transcriptional activation or suppression systems based on the presence or absence of antibiotics in the larval diet (Heinrich and Scott 2000, Thomas et al. 2000). The permissive condition in the facility will require the presence of an antibiotic or its analogue during the mass-rearing of the colony, although not the male production for release. Following release of the transgenic males, the female progeny of a wild female mated with such a male would die in the absence of the antibiotic. However, more recent experiments have shown that it is possible to engineer a *Drosophila* strain so that both male and female progeny would die as embryos (Horn and Wimmer 2003). Recently, in the Mediterranean fruit fly, transgenic strains have been produced that also lead to lethality following mating of transgenic males with wild-type females (Gong et al. 2005). However, these strains will require further refinement because they are not completely sterile in matings with wild females, and the majority of the lethality occurs in the late larval stage.

A specific concern regarding these types of systems, that require the addition of a bioactive compound to very large volumes of larval diet, is the disposal of the diet (e.g. the Mediterranean fruit fly facility in Guatemala produces about 25 tons of diet per day). This is a real issue since mass-rearing facilities often sell spent larval diet as cattle feed. In addition, maintaining the appropriate concentration throughout the diet will not be easy. A third concern is the effect of the diet on the level of antibiotic. As the diet is a microcosm of bacterial and fungal growth, it will not be easy to standardize the exposure of the larvae to the antibiotic. It will also be important to choose the appropriate type of dominant lethality with which to kill the progeny in the field. Any genes involved in cell death mechanisms, or which show general cell toxicity, would probably not be suitable and/or would raise environmental concerns. As effective sterilization of the wild females remains the key to a successful programme that includes the release of sterile insects, there is no room for error in the sterilization procedure, and any proposed biological system must be extremely robust, controllable, and accurate.

There has been much argument about the relative merits of using either biological or physical methods to induce lethality in field populations (Alphey and Andreasen 2002). While it may appear that the molecular approach has some potential advantages, it is important to distinguish the characteristics of these two methods in producing sterility in a field population. Both approaches, to be effective, must act in a dominant manner and induce lethality in eggs produced by wild females following mating with released males. The key difference, and the one that will finally determine whether the molecular approach has any practical value, is that molecular sterility is based on a single dominant factor. Any variation in the expression of the sterility factor following interaction with the large and

heterogeneous genome in the field population will very quickly lead to selection for non-sensitive females and loss of sterility induction. This is a major weakness of any molecular approach; there is no practical way to evaluate the possibility that this might happen without compromising the eventual use of the technology. One solution that has been proposed is to introduce several constructs into the same individual, but the fitness costs of such multiple insertions still has to be determined. In contrast, sterility produced by radiation is based on the action of an almost infinite number of dominant lethal mutations, and every released male carries a different set of sterilizing factors (Robinson 2002); thus it is very difficult for a field population to develop any sort of resistance (Whitten and Mahon, this volume). Radiation-induced sterility has built-in redundancy, and in spite of some disadvantages it remains the only proven and environment-friendly technique for the introduction of sterility into field populations of pest insects.

4.4. Paratransgenesis

A fourth area in which transgenic technology may benefit future programmes that include the release of sterile insects is manipulating their symbiotic organisms. Such mutualists are often required by some important vectors for disease transmission, and paratransgenesis of symbionts offers major possibilities to develop vector strains that are refractory to transmission. One example is tsetse fly species, where the technology to impede trypanosome transmission by blood-feeding sterile males may soon be available (Aksoy et al. 2005). Another possibility is to harness, for paratransgenesis purposes, the *Wolbachia*-induced cytoplasmic incompatibility phenomenon that can be used to induce sterilization to suppress or modify natural populations (Zabalou et al. 2004).

4.5. Technical and Regulatory Constraints

Any transgenic strain proposed for sterile insect release will have to meet stringent criteria regarding the expression of the specific trait that it carries and the overall quality of the strain itself. These are not inconsiderable technical constraints, as has been demonstrated for the introduction of sexing strains based on classical approaches (Franz, this volume). The impact of large-scale rearing under extremely stressful conditions, and the number of individuals reared (often exceeding millions/week), can uncover significant genetic and biological events that can never be induced and studied under typical laboratory conditions, and for which the consequences are at present unknown. It is safe to say that extensive evaluation of a transgenic strain will be required before it can be used in the field.

A major constraint in using transgenic strains for field release, even when sterilized, is the regulatory aspect. Whereas there are no real regulatory constraints for strains based on classical approaches (section 2.6.), for transgenic strains there are a number of not yet developed regulatory hurdles that will have to be overcome before transgenic insects can be released. An elegant way to overcome such constraints has been proposed in Lepidoptera, where females are the heterogametic sex. Transformation to develop sexing strains using dominant lethal conditionals

only expressed in females would result in only colony females carrying the transgenic constructs, with the resulting sterile males being non-transgenic (Marec et al. 2005).

5. CONCLUSIONS

Global trends towards a cleaner agriculture and less aggressive pest control will increase the demand for more target-specific and biologically based methods such as the use of sterile insects. Furthermore, international trade is creating an environment in which area-wide approaches to the management of insect pests have a comparative advantage. Increasingly, overcoming technical and scientific constraints, and introducing new technological innovations, will allow sterile insects to be used against new target pests, and improve cost-effectiveness against current targets.

Nevertheless, these innovations and improvements are no guarantee for the successful implementation of the SIT as part of AW-IPM programmes. These programmes are management-intensive compared with most other control operations. Close coordination among many simultaneously occurring activities is required; their execution requires precision in time and space. Since the area-wide approach also depends on the cooperation and participation of all stakeholders in the target area, excellent management of communications with all stakeholders is indispensable, paying close attention to political, socio-economic, and environmental sensitivities. Therefore, effective programme implementation depends on the establishment of an efficient management structure that is semi-independent of normal government bureaucracy and corruption. A common denominator of failed programmes has been the lack of such dynamic structures and dedicated leadership to respond flexibly to changing situations. Equally important is transparency, and an effective external oversight structure to maximize limited resources and to attract stakeholder funding.

A major unexploited opportunity is the integration of sterile insects and augmentative biological control. As long as key pests are treated with insecticides, natural and augmentative biocontrol is disrupted, and hence there is only a limited potential for growth in the application of pollinators or natural enemies to deal with secondary pests. On the other hand, when a key pest is managed using biologically based tools (e.g. host resistance, mating disruption, sterile insects, etc.), natural and mass-reared biological control agents can complement these methods, and also play an important role in controlling less important pests. Thus, the existing augmentative biocontrol industry (ANBP 2005, IBMA 2005) and the SIT appear to be natural allies, and the expected gradual progress in commercializing the SIT will probably advance within this context. The private sector biocontrol industry already has the technical "know how" to manage the mass-rearing, quality control, handling, and shipping of beneficials, and therefore could make a significant contribution to improve the SIT technology. Adding sterile insects to their products could provide a complete "biological package". A pioneer in this approach is Bio-Bee in Israel, which is expanding from greenhouse to field pest management (Bassi 2005).

There is also much unexploited potential in applying sterility to natural enemy production and facilitation (Greany and Carpenter 2000). This involves using sterile insects not to transfer sterile sperm but as sterile hosts/prey/vectors for parasitoids/predators/pathogens to facilitate the use, and enhance the efficacy, of biological control agents. At present considerable research is being conducted to exploit the many potential possibilities and applications to facilitate natural enemy production, handling, and shipment, and also to provide sterile hosts for the establishment and maintenance over critical periods, or early season build-up (or in border or trap crops), of natural enemy populations in the field.

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Sterile Insect Technique

Principles and Practice in Area-Wide Integrated Pest Management

Edited by V.A. Dyck, J. Hendrichs and A.S. Robinson

The sterile insect technique (SIT) is an environment-friendly method of pest control that integrates well into area-wide integrated pest management (AW-IPM) programmes. The first of its kind, this book takes a generic, comprehensive, and global approach in describing the principles and practice of the SIT. The strengths and weaknesses, and successes and failures, of the SIT are evaluated openly and fairly from a scientific perspective. The SIT is applicable to some major pests of plant, animal and human health importance, and criteria are provided to guide in the selection of pests appropriate for the SIT.

A great variety of subjects are covered, from the history of the SIT to improved prospects for its future application. The major chapters discuss the principles, technical components, and application of sterile insects. The four main strategic options in using the SIT — suppression, containment, prevention, and eradication — with examples of each option, are described in detail. Other chapters deal with supportive technologies, economic, environmental, and management considerations, and the socio-economic impact of AW-IPM programmes that integrate the SIT.

This book provides a wealth of information and reference material never before available in one volume. It will be a standard reference on the subject for many years. The authors, from 19 countries, are highly experienced in the subject, and reflect the international character of SIT activities.

Since no university offers courses on the SIT, the book's audience will be mainly students in general animal and plant health courses, but the in-depth reviews of all aspects of the SIT and its integration into AW-IPM programmes will be of great value to researchers, teachers, animal and plant health practitioners, and policy makers.

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