CHAPTER
13

MICROBIAL APPROACH IN PEST CONTROL

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INTRODUCTION

Synthetic agrochemical insecticides are used against insect pests and contributed to unprecedented effects in nature (Rosas-Garcia, 2009). The growing concern about their ill-effects has resulted in a change in thinking to manage insect pests in an eco-friendly manner. The desire for alternatives that would have no negative effects on the environment has prompted scientists to investigate microbes (Ignacimuthu, 2000; Rosas-Garcia, 2009).

Microbes play various roles in sustaining agricultural and ecological systems through the turnover of organic matter, release of nutrients to plants (Caple, 2004; Helms *et al.,* 2004) and suppression of insect pests (Lacey & Goettel,1995), and bring about a balance of biological populations in ecosystems (Dent, 1995). The microbial world is a highly interconnected one in which microbes interact with living and non-living matter to produce outcomes from symbiosis to pathogenesis, energy acquisition and conversion, climate change, geologic change, food and drug production.

Microbial pesticides are an increasingly important area of biological control. The insecticides are insect pathogens which, unlike chemicals, are target specific (Ignacimuthu, 2000; Rosas-Garcia, 2009). Microbial bio-control agents have several important differences from natural enemies, such as much shorter generation times, vast production of propagules (Van Driesche and Bellows, 2001) and do not seek their prey, but rely on chance contact (Van Emden and Service, 2004). Other requisites of microbial agents are that they must be stable in formulation with a reasonable shelf-life.

Microbial insecticides account for only a 1.6% share of the world insecticide market and 95% of them involve the bacterium, *Bacillus thuringiensis* (Ignacimuthu, 2000; Lacey and Goettel, 1995; Richards and Rogers, 1990). As an alternative to chemical insecticides, it is a highly successful microbial insecticide, currently used in a number of crops, forestry and medicine (Rosas-Garcia, 2009).

ADVANTAGES OF INSECT MICROBIAL INSECTICIDES

Microbial pathogens of insects have several striking advantages:

i. Microbial insecticides are very target specific. They often kill only one or a limited number of orders of insects, and some are even specific within an order (Van Driesche and Bellows, 2001). They can therefore be applied with complete safety to the natural enemies of the target pests (Lacey and Goettel,1995; Rosas-Garcia, 2009).

ii. Unlike a chemical, microbial agents leave no toxic residues (Van Driesche and Bellows, 2001). Microbial pathogens have a limited life outside their host and therefore very short persistence in the environment. By their specificity, microbial pathogens of insect pests are generally non-toxic to humans and domestic or farm animals (Rosas-Garcia, 2009; Van Driesche and Bellows, 2001).

iii. Resistance development is unlikely, or, at least, slow. As far as we use natural microbes, development of resistance towards them in the pest is unlikely. However, this may not be so with pathogens modified by genetic engineering.

iv. Microbial pathogens of insects are compatible with insecticides, and can often be used together.

v. Genetic manipulation of microbial insect pathogens is technically straightforward and can improve their pathogenicity (Van Emden and Service, 2004).

DISADVANTAGES OF INSECT MICROBIAL INSECTICIDES

i. Microbial pathogens, being highly specific, set their own economic limitations, since specificity also means market limitation.

ii. Microbes are living organisms, often with a very short life. So, production of microbial insecticides in commercial scale requires the formulation techniques to ensure a consistent fresh supply with virulence (Van Driesche and Bellows, 2001).

iii. Once applied in the field, they may lose their efficacy if the conditions do not suit their growth. Many are sensitive to UV radiation, therefore chemical radiation shields are required. Many microbial insecticides, particularly fungi, require adequate if not high humidity over some time for survival.

iv. In order infect the normally high insect pest population, a critical threshold population of the microbe(s) is essential (Van Emden and Service, 2004).

v. Research in microbial pathogens is often not as straightforward as with chemicals, since direct toxicity in the laboratory may not mean toxicity in the field as the pathogen(s) may be avoided by certain behavioural and biological properties of the insect pest in the field.

TYPES OF MICROBIAL INSECTICIDES IN PEST CONTROL

Bacteria

Bacterial formulations, such as *Bacillus thuringiensis* contains thousands of heat esistant parasporal bodies - spores and protein crystals - per milligram. Parasporal crystals and spores of *B. thuringiensis* constitute the active ingredients of commercially available products for the control of many lepidopteran pests (Basri *et al.,* 1994; Ramlah Ali and Basri, 1997; Siti Ramlah *et al,* 2003; 2005a; 2007c) in agricultural crops and forestry (Hynes and Boyetchko, 2006; Navon, 2000).

Contact Fungi

Insects can be infected by fungi from spores on the insect cuticle. The germ tube from the germinating spore penetrates the cuticle directly. However, pest control by spraying fungal spores has been unreliable as the spores require moisture to sporulate, which condition cannot be guaranteed in the field. The use of *Metarhizium anisopliae* in soil application against pests has had more success (Van Emden and Service, 2004; Ramle *et al*., 2005a; 2006; Shamsilawani *et al*., 2009).

Ingested Viruses

These pathogens have to be ingested by their host to initiate an infection. They are fairly resistant to adverse conditions, and can survive on relatively dry surfaces, such as leaves, until ingestion by an insect pest (Lacey and Goettel, 1995). The pathogens are less humidity dependent, and the most successful have been the Baculoviruses (in the Baculoviridae family) on arthropods. The subgroups of nuclear polyhedrosis viruses (NPVs) and granulosis (GVs) contain the most important viruses used in pest control. Other viruses include the *Orytces rhinoceros* virus for control of *Oryctes rhinoceros* (Ramle *et al.,* 2005b). It can be applied at very low doses, *e.g*., using naturally diseased caterpillars on cabbage with only two infected larvae a hectare. The development of commercial virus preparations for agriculture is hampered by safety concerns for humans.

Research in MPOB is in several areas - identification and development of new microbial agents for insect pest management, mass production, formulation and application of microbial biopesticides for IPM. This chapter will discus the potential of microbes - bacteria, fungi and viruses - for controlling oil palm insect pests.

ENTOMOPATHOGENIC BACTERIA: *Bacillus thuringiensis*

Bacillus thuringiensis (or Bt) is a soil-dwelling bacterium, gram-positive, spore bearing, rod-shaped and measuring 1-1.2 microns in length. It was first discovered by Shigetane Ishiwata in 1901. Additionally, Bt also occurs in the gut of caterpillars of various moths and butterflies, as well as on the dark surfaces of plants. The first record of its application was in Hungary at end 1920 and in Yugoslavia in the early 1930s against the corn borer (Lord, 2005).

Scientific Classification

Kingdom: Eubacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: Bacillus
Species: *B. thuringiensis*

Bt is classified according to its crystal proteins (ICP), δ-endotoxins or Cry proteins (Schnepf *et al.*, 1998). The bacterium produces a set of powerful protein toxins used widely by farmers to kill over 150 species of noxious insects (Schaechter *et al*., 2006; Lacey and Goettel, 1995). Bt habouring Cry I delta-endotoxins is lepidopteran specific, environmental-friendly and, therefore, an ideal alternative to chemical insecticides. Screening for the *cry* genes in MPOB indigenous isolates found several with potential against palm Lepidopteran pests (Ramlah and Basri, 2002).

Public awareness on the undesirable effects of chemical pesticides on the environment has created a market for safe alternatives like Bt. Palm Lepidopteran defoliators, such as bagworms, nettle caterpillars (Basri *et al.,* 1994; Ramlah and Basri, 1997; Siti Ramlah *et al.,* 2003) and bunch moth, have been effectively controlled with Bt.

Mode of Action of *B. thuringiensis*

A testable model for the mode of action of ICP toxins is the Wolferberger/Ellar, or Receptor/Pore, model (Ellar, 1990; Wolferberger, 1990). The biochemical sequence of activities in the insect gut after ingestion of *B. thuringiensis* crystal depends upon the binding of active toxins to specific molecules called receptors (APN) (Knight *et al*., 1995) on the surface of the mid-gut epithelium cells of susceptible insects before toxin insertion into the apical membrane (Schnepf *et al*., 1998) – just like a key fitting in a lock, with the toxins as key and the receptor the lock.

The Cry action involves two main steps. First, the activated toxin must bind to specific high-affinity receptors in the luminal plasma membrane of the midgut epithelial cells of a susceptible insect (Knowles *et al*., 1991). Second, a toxin must then be inserted into the membrane to form pores of 0.5-1.0 nm (Carrol and Ellar, 1993) and 1-2 nm radius (Knowles and Ellar, 1987) in the susceptible insect. The pores are generated either directly by toxin insertion into the membrane or indirectly perturbing the resident plasma membrane molecules (Knowles and Ellar, 1987). The pores are permeable to both monovalent cations and small neutral solutes, leading to cell swelling, osmotic lysis (Carrol and Ellar, 1993; Knowles and Ellar, 1987; Schwartz *et al.,* 1993) and insect death (Knowles and Ellar, 1987; Chen *et al*., 1995). Both Cry and Cyt are proposed to act by causing small pores in the larval mid-gut epithelial membrane (Knowles and Ellar, 1987).

Electro-physiological and biochemical (Knowles and Ellar, 1987) evidence suggest that toxins cause pores in the cell membrane, upsetting the osmotic balance. Consequently, the cells swell and lyse (Gill, 1995; Knowles and Ellar, 1987). The injected toxins form small non-specific pores, or cation selective channels, in the planar lipid bilayer and kill the insect by disrupting its internal ionic balance (Knowles and Ellar, 1987; Schnepf *et al*., 1998).

Bacillus thuringiensis FOR CONTROLLING BAGWORM, *Metisa plana*

The most common species of bagworm, the oil palm defoliator, in Peninsular Malaysia is *M. plana* Walker (Lepidoptera: Psychidae) (Wood, 1976). Spraying a broad-spectrum long residual contact insecticide has often disrupted the natural balance between the pest and its natural enemies - predators, parasitoids (Wood, 1971) and bacterial pathogens, such as Bt.

Bt is widely used in agriculture as a biological pesticide. In well-managed pest control programmes, Bt has reduced the use of chemical pesticides. It is effective against several lepidopteran pests of a variety of crops, including vegetables, cotton, corn, potato and soyabean (Yang and Wang, 1998).

LABORATORY EFFICACY OF COMMERCIAL *Bacillus thuringiensis* ON OIL PALM INSECT PESTS

The commercial Bt products in the local market were evaluated against bagworm (*Psychidae*) by Basri *et al*. (1994; 1996). Thuricide (*Btb*) was more effective than diflubenzuron, cyfluthrin and endosulfan against the bunch moth (*Pyralidae*), *Tirathaba rufivena*. Unlike XenTari, the older Bt products, using strains such as *kurstaki* (*Btk)*, *Berliner* (*Btb*) and morrisoni (*Btm)*, were ineffective against the bagworms (Basri *et al*., 1994).

Bt is target specific and harmless to natural enemies of oil palm lepidopteran insect pests, man, fish and livestock. Commercial Bt products, such as *Bactospeine*, *Thuricide*, *BCBT*, *Foray*, *Dipel*, *Biobit* and *CGA-BT* with the exception of *Florbac*, were not effective for controlling *Metisa plana* (Basri *et al*., 1994). A subsequent new product - XenTari - containing *B. thuringiensis* subsp. *aizawai* strain 1857 was comparable to trichlorfon in the control of *M. plana* (Basri *et al*., 1996).

RECEPTOR PROTEINS FOR *Bacillus thuringiensis* IN *Metisa plana*

The active ingredients of Bt are δ-endotoxins, gut poisons for Lepidopteran palm defoliators, such as bagworms, nettle caterpillars and the bunch moth. In order for Bt to function against the bagworm, it must first be ingested. Sprayable Bt contains spores which germinate in the gut to produce toxins. In the gut, the Cry proteins, or δ-endotoxin, dissolve in the gut juice, and the dissolved toxin is activated by the gut proteases. The activated toxins bind with receptors in the gut lining called brush border membrane vesicles (bbmv) with sizes of 125, 110, 100 and 80 kDa (Ramlah, 2000; Ramlah and Mahadi, 2001) (*Figure 13.1*). The formation of toxin-receptor complex in the gut membrane manifested as irreversible binding (Ramlah and Mahadi, 2001) which results in cellular osmotic imbalance, cell lysis and death of the insect. MPOB has produced several Bt products based on MPOB Bt1 isolate (Siti Ramlah *et al.,* 2005b, c; 2009a, b).

USE OF INDIGENOUS *Bacillus thuringiensis*, MPOB Bt1, FOR CONTROLLING *Metisa plana*

A survey on the possible use of MPOB Bt1 for control of palm defoliators was conducted in 2001. Out of 1500 estates contacted, 24% responded. An area of

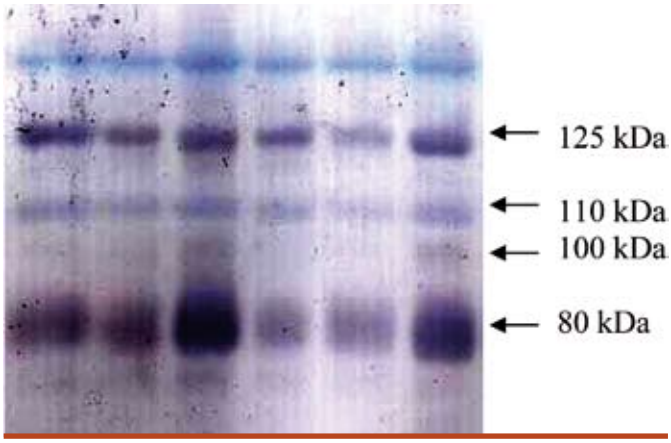

Figure 13.1. Receptors in Metisa plana *for binding with Bt toxins.*

24 929 ha oil palm plantation was infested with palm Lepidopteran defoliators. The highest infestation was in Johor, followed by in Perak and Sabah. For nettle caterpillars, the highest infestation was in Sabah, followed by in Perak. Approximately 48% of the responding estates were interested in testing MPOB-Bt1. Later, in 2000-2005, 49 151.63 ha were infested with bagworms (Norman and Mohd Basri, 2007).

In 2002, field application of MPOB Bt1 by hand-held motorized sprayer on 10-year-old palms at one-sixth (1/6) the rate recommended for commercial Bt reduced the bagworm population in an outbreak by 75% in three days (Ramlah and Basri, 2002). In other words, a once-off application of MPOB Bt1 reduced the bagworm population from 160/frond to 40/frond. The effect was comparable to using cypermethrin. A normal or untreated pupa will subsequently resulted in100-200 first larval instars. The above 40 bagworms/frond after MPOB Bt1 pupated and subsequently produced 25 larvae/frond, implying that many of the survivors could not reproduce. A second application of MPOB Bt1 then brought the population below the economic threshold of five larvae a frond (Ramlah and Basri, 2002).

This local isolate of Bt was propagated in pilot scale as a long-term more effective alternative Bt product. Sufficient bacterium can be propagated using liquid state batch culture in the 500 litre bioreactor at MPOB Microbial Technology and Engineering Centre (*Figure 13.2*). The first two products from MPOB BT1 were tested and found effective for field control of bagworms (Siti Ramlah *et al*., 2005a, b, c). The products are complementary and, hence, can be incorporated with the natural control agents - the predators and parasitoids of bagworm - for IPM of bagworm outbreaks (Siti Ramlah *et al*., 2005a; 2006; 2007a, c).

Figure 13.2. Production of B. thuringiensis *using liquid state culture in 50 litres and 500 litres bioreactors at MPOB Microbial Technology Engineering Centre.*

Figure 13.3. Microbial Technology Engineering Centre at MPOB Head Office, Bandar Baru Bangi, Selangor.

Microbial Technology Engineering Centre (MICROTEC)

The MPOB Microbial Technology Engineering Centre (MICROTEC) (*Figure 13.3*) was formed in 2004 to develop technologies for production of microbial agro-products, such as bioinsecticides for oil palm (Ramlah *et al.*, 2006; Siti Ramlah *et al*., 2005a).

Located at MPOB Head Office in Bandar Baru Bangi, MICROTEC has a microbial laboratory fitted for isolation, culture and identification of microbes. MICROTEC was commissioned on 28 February 2005 and launched by the Minister of Primary Commodities and Plantation Industry on 27 June 2006.

MICROTEC Products

The microbial insecticides produced at MICROTEC are based on *B. thuringiensis* (MPOB Bt1). They are offered as possible alternatives for selective control of target lepidopteran insect pests. Bt produces crystal proteins, toxic to the target insect(s) (Siti Ramlah *et al*., 2005a). Five Bt products based on the first isolate, MPOB Bt1, have been formulated (*Figure 13.4*). A laboratory-prepared medium, MPOB Agro-Nat, was developed. The MPOB Agro-Nat produced at MICROTEC reduced the cost of medium for commercial scale production by 97% and yielded superior products over eight commercial media for propagation of Bt.

Registration of Products

Two brands for products were registered in November 2005. A laboratory bioassay on efficacy of the wettable powder, *Terakil-1*, indicated >80% kill of the

Figure 13.4. Bacillus thuringiensis *products based on MPOB Bt1 from the Microbial Technology Engineering Centre.*

damaging larval instars of bagworms at the recommended dose (Siti Ramlah *et al.*, 2005a,b). The trunk injectible liquid formulation, *Teracon-1*, similiarly controlled >80% of the pests at seven days after treatment with the recommended dose (Siti Ramlah *et al*., 2005c). Field application of *Terakil-1* not only controlled a serious outbreak of bagworms but, unlike chemicals, gave synergistic control with the other biological agents such as the beneficial insects and plants (Siti Ramlah *et al*., 2005a, b, c).

Under the Pesticides Act 1978, these products are considered 'pesticides' and need to be registered before they can be sold to the public. Laboratory tests on the toxicity of both *Terakil-1* and *Teracon-1* against the pollinating weevil, *Elaeidobius kamerunicus*, beneficial insects, indicated both to be relatively harmless (Mohd Najib *et al*., 2007; 2009). The rest of the data on MPOB Bt products' efficacy and toxicity are being documented for registration with the Pesticides Board.

The document for registration of Bt product contains the following information:

i) Draft label of the product. The label should contain the product identity, instructions for use, and cautionary information as stated in the Pesticides (Labelling) Regulation, 1984.

ii) Chemical data for the manufacturing process, material safety data sheet (MSDS) and methods of analysis for the active ingredient.

iii) Toxicology data. The information needed will depend on the class of pesticide, and whether it is a commodity or proprietary pesticide. Generally, informations on acute and sub-acute testing (oral and dermal rates on rats), chronic testing, special studies on wildlife hazards and human toxicology are required.

iv) Efficacy against target organisms, or pests in the registration country.

MICROTEC produces more than 3 t of *Terakil-1* and *Teracon-1* annually (Siti Ramlah *et al.*, 2005b, c). Several companies have shown interest to market the products and two are interested in commercializing the Bt products in cooperation with MPOB.

Apart from oil palm pests, the Bt products can also be used against other pests in field crops, forest, fruits, flowers and vegetables. The use of Bt products from MICROTEC will reduce insect pest attacks without risk to non-target organisms (Ramlah *et al*., 2006; 2005a).

Integrated Pest Management (IPM) of Bagworm by Aerial Spraying *Bacillus thuringiensis*

An IPM system provides farmers, smallholders and oil palm plantations with an economic and appropriate mean for controlling crop pests. The system is achieved through developing a system strategy, often referred to as a delivery system. For successful implementation of IPM under oil palm, it is important to integrate the work of the several experts indifferent disciplines in order to develop IPM programmes relevant to the oil palm plantation. Interdisciplinary research is the most effective way of bringing about integration of pest control measures at the programmes level (Siti Ramlah *et al*., 2007a).

As early as 1962, it was documented that chemical insecticides were causing pest attacks in oil palm by upsetting the natural enemies (Rosas-Garcia, 2009; Wood, 1971). Oil palm is generally free from insect attack and does not need much chemical insecticides. There may be occasional attacks, like bagworms causing sporadic damage, if the natural control agent is disrupted. The attacks, however, may become more frequent and severe if the natural control is disrupted by application of broad spectrum insecticides. Indeed, regular use of these chemicals over several pest generations will result in pest explosions, or outbreaks.

For long-term control of bagworm outbreaks, planters are recommended to implement IPM systems. These systems involve biological control agents and agronomic practices that minimize the pest outbreaks. Non-target specific chemicals are to be avoided, if possible, to reduce the side effects. An IPM system for control of bagworms in oil palm involves three beneficial organisms which are compatible and environmental-friendly. The first is target specific Bt which does not harm the second component - beneficial insects, including the predators and parasitoids of bagworms (Siti Ramlah *et al*., 2007a, b, c; Mohd Najib *et al.*, 2009). The third component is nectar-producing beneficial plants as hosts for the beneficial insects. The beneficial plants are planted along main roads in oil palm estates to attract, shelter and nourish the predators and parasitoids.

Unlike chemicals, Bt reduced the bagworm population without affecting the beneficial insects. Together, Bt and the beneficial insects synergistically reduced the population of bagworms to safely below the economic threshold level (Siti Ramlah *et al*., 2007a, b, c). The parasitoids require the bagworm to complete their life cycle.

Eradication, or complete control, using chemicals, such as methamidophos, that give 100% kill is considered mismanagement in IPM. Without any bagworm, the parasitoids cannot survive, and any future return of the bagworm would likely result in outbreaks in the predator/parasite-free environment.

IPM programme for controlling bagworms outbreak in Southern Perak was led by MPOB since 2007 with the collaborations from members of the industry, Department of Agriculture and Sumitomo Chemical Sdn Bhd. A budget of RM 5 million was allocated by MPOB to subsidize 100% and 50% of the cost of aerial spraying using Bt for the smallholders and estates, respectively.

The aerial spraying of Bt was first done on 1800 ha of *P. pendula* infested area in Estate A on 12 April 2007. The estate had a good establishment of beneficial plants. Thus, the bagworm attack was under control quickly at the start of the trial. One round of Bt brought the infestation down to below the economic threshold level of 10 larvae/frond at 7 days after treatment (DAT). With good establishment of beneficial plants, any outbreak should be easily managed as in Estate A (Siti Ramlah *et al*., 2007a). The use of chemicals, even monocrotophos by trunk injection, should be avoided if possible, or reduced, for the possible havoc they can wreck on the beneficial insects (Basri *et al*., 1990).

A second aerial spray of Bt was done on Estate B over 450 ha of oil palm under *P. pendula* attack, on 12 May 2007. Poor establishment of beneficial plants had led to the bagworm attack which, in some areas, was so severe that monocrotophos had to be resorted to. The census was above 600 larvae/frond at 0 DAT. The timing of the spray was perfect, albeit with the unavoidable presence of some late instars and pupae, due to the multi-staged attack and overlapping bagworm generations. A significant reduction in the bagworm population occurred over time, from 0 to 14 DAT. The pupae not susceptible to Bt resurgenced as first instars at 7 DAT in some sub-blocks. Trunk injection of monocrotophos was done in the sub-blocks to control the overlapping population (Siti Ramlah *et al*., 2007a).

The third aerial spray was on 15 to 19 May 2007 in Estate C over 7500 ha with no establishment of beneficial plants. There was a severe multi-staged attack by *M. plana* and *P. pendula*. Significant reduction in the overall bagworm populations took place from 0 to 14 DAT (*Figure 13.5*) (Siti Ramlah *et al*., 2007a) was noted. In such serious multi-staged bagworm outbreaks, two to three rounds of Bt spray with close monitoring of the situation may be required (Siti Ramlah *et al*., 2007c) .

A total 15 945.04 ha bagworm outbreaks in Southern Perak were controlled using IPM at a total cost of RM 1.355 million.

Aerial Bt Spraying

The first Bt aerial spraying was done in Southern Perak Plantation on 12 April 2007. It covered the whole infested area of 1913 ha. Other sprayings were done in plantations and smallholdings in May, June, August and September 2007. In 2008, aerial sprayings were done in April, May and November, while later ones were

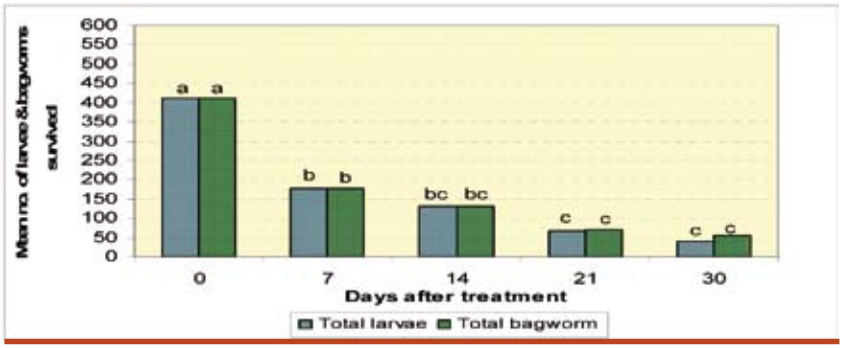

Figure 13.5. Mean survival of larvae and total bagworms in all sub-blocks after aerial spraying with Dipel in Estate C. Bars with the same letters are not significantly different at P<0.05 by Anova. The number of sub-blocks under census was 54. Number of palms under census per sub-block = 3.

complemented by ground spraying. MPOB used its MPOB Bt1 products, *Ecobac-1 (EC)*, in aerial spraying (*Figure 13.6*) and *Bafog-1* (S) by fogging against bagworm outbreaks in smallholdings in Hutan Melintang and FELCRA Sungai Manila from September 2008 (Siti Ramlah *et al*., 2009a, b). By end of 2008, after several rounds of Bt spraying in plantations and smallholdings at Southern Perak, the infested area reduced by 83% to 737 ha.

Recent data from MPOA indicated that at beginning 2009, the bagworm infested area in estates in Southern Perak had declined greatly to 1290.19 ha. In February and March 2009, during the dry period, estates with IPM, like Kelapa Bali, Southern Plantation and Yew Lian, were not threatened by sudden increase in bagworm outbreak, but had reduced or slight increase in bagworm attacks from 16 ha, 407.24 ha and 117 ha in January 2009 to 17 ha, 393.19 ha and 110.25 ha, respectively. However, Ulu Bernam Estate, which relied on monocrotophos and cypermethrin, recorded a drastic increase in infestation from 150.63 ha to 2665.3 ha during the dry period.

An overall reduction in bagworm infestation from 35 575 ha to 930.41 ha was observed in May 2009 for Perak since the implementation of IPM at Southern Perak in 2007.

Figure 13.6. Pouring of Ecobac-1(EC) *into bucket (a) and pumping of Ecobac-1(EC) from bucket into aircraft tank (b) for aerial spraying.*

Figure 13.7. Aerial spraying of Ecobac-1 (EC) *against bagworms.*

Aerial Spraying of MPOB Bt1, *Ecobac-1(EC)*

MPOB uses MPOB *Ecobac-1(EC)* for controlling the bagworm outbreak in Southern Perak via aerial spray (*Figure 13.7*). *Bafog-1(S)* is used for localized attacks, particularly in peat.

Aerial spraying of Bt in Southern Perak was effective in controlling bagworms. *Ecobac-1(EC)* was used in a resurgence of bagworm attacks in South Perak. It is more convenient and cheaper to aerial spray Bt than to trunk inject cypermethrin for controlling an extensive bagworm outbreak.

Ecobac-1(EC) for Controlling Multi-stage *P. pendula* Outbreaks at Southern Perak

Controlling multi-stage *P. pendula* attacks in peat, where the beneficial plants are not easy to establish, can be difficult. Unlike the chemical insecticides, Bt only kills *P. pendula* which are actively feeding on the leaflets, particularly the first and second larval instars. The third and the fourth larval instars are more resistant, probably because some already pupated at the third instars. Larvae turned to the fourth instars require a higher dose of Bt. Hence, combating multi-staged *P. pendula* attack with Bt requires close monitoring of the different stages of the bagworm, and weekly follow-up aerial sprays. The weekly spray must begin as soon as the first instars are observed until the pupae no longer exist.

The data in controlling a multi-stage *P. pendula* attack using Bt, *Ecobac-1(EC)*, dipel-ES and dipterex are shown in *Figure 13.8*. Three rounds of weekly Bt sprayings were done. As the first instars of *P. pendula* hatched, they were exposed to Bt. At 7 DAT, when almost all the pupae had disappeared (*Figure 13.8b*) and the early instars peaked (*Figure 13.8a*), the second round of Bt spray was conducted. The third spray was done at 14 DAT as a couple of sub-blocks still had larval populations a little above the economic threshold of 10 larvae/frond. The few larvae observed at 21 DAT were at the third or fourth instars (*Figure 13.8a*). The second generation pupae were seen

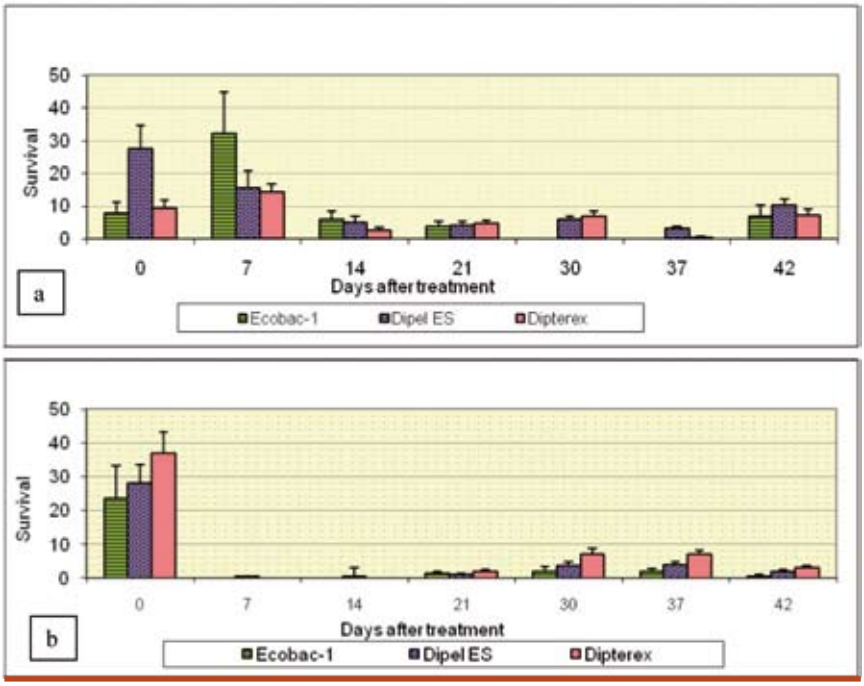

Figure 13.8. Survival of P. pendula *larvae (a) and pupae (b) per frond after aerial spraying with* Ecobac-1 (EC)*, commercial* Dipel ES *and* Dipterex *in Block A, Hutan Melintang. Weekly spraying for first generation of* P. pendula *was done, and follow-up spray against the second generation of* P. pendula *at 42 days after the first spray.*

at 30 and 37 DAT (*Figure 13.8b*). As the second generation larval instars hatched at 42 DAT, another follow-up spray with Bt was done.

Oryctes rhinoceros (Linnaeus) AS A PEST OF OIL PALM

The rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus), is a major pest of coconut and oil palm worldwide (Bedford, 1980). The adult attacks the crown of the palms, feeding on the spear tissues, subsequently killing the palm shoot. The immature stages of the beetle develop in decaying materials, including oil palm tissues such as empty fruit bunches and trunks (Samsudin *et al.,* 1993). The prohibition of burning in replanting therefore provides abundant and conducive breeding sites for it, increasing the risk of infestation (Norman and Mohd Basri, 1997). Surveys in 1995 and 2005 recorded high infestation by the pest, ranging from 33 768 ha to 46 444 ha (Norman and Mohd Basri, 2007). Repeated attacks by *Oryctes* adults can kill young palms and predispose them to other pest attacks (Liau and Ahmad, 1991; Sivapragasam *et al.,* 1990). Damage can also reduce the leaf area by up to 15% (Samsudin *et al.,* 1993) which can result in a 25% crop loss (Liau and Ahmad, 1991).

Control measures include chemical insecticides, such as carbofuran and cypermethrin, placed at the bases of the leaf sheaths. Naphthalene balls in the frond

axils as a repellent provides up to 95% control (Gurmit, 1987). However, they may be ineffective against high pest densities (Chung *et al.,* 1991). Trapping, using a synthetic aggregating pheromone, ethyl 4-methyloctanoate, is also practiced, in particular, for monitoring the beetle population and mass trapping (Norman and Mohd Basri, 1995). Other methods of control are planting a leguminous cover, cultural practices like winkling and applying biological control agents such as the fungus, *Metarhizium anisopliae* (Norman and Mohd Basri, 1995) and *O. rhinoceros* virus.

Metarhizium anisopliae

Taxonomy

The genus *Metarhizium* is in the class Hyphomycetes of the phylum Deuteromycota. The classification by Tulloch (1976) based on conidium size and shape has *Metarhizium* having only two species, *M. anisopliae* and *M. flavoviride.* However, Rombach *et al.* (1987) has *M. album*, isolated mainly from the plant leafhopper (Homoptera: Cicadellidae), as another species. *M. anisopliae*, in turn, has two varieties, var. *anisopliae* and var. *major*. The spore length of var. *anisopliae* is 5-8 μm, var. *major* 9-15 μm (*Figure 13.9*) and *M. album* 3-4 μm. *M. anisopliae* var. *anisopliae* is a cosmopilitan pathogen of innumerable insect pests (Veen, 1968), while var. *major* is largely restricted to *Oryctes* (Scarabaeidae: Dynastinae) (Tulloch, 1976). *M. flavoviride* is mainly isolated from acridids, especially grasshoppers and locusts (Lomer *et al.,* 2001). On the basis of only spore size and shape, *M. flavoviride* cannot be distinguished from other *M. anisopliae*. Based on an internally transcribed spacer ribosomal DNA (rDNA) sequence data, *M. flavoviride* is reclassified as *M. ansiopliae* var. *acridum* (Driver *et al.,* 2000).

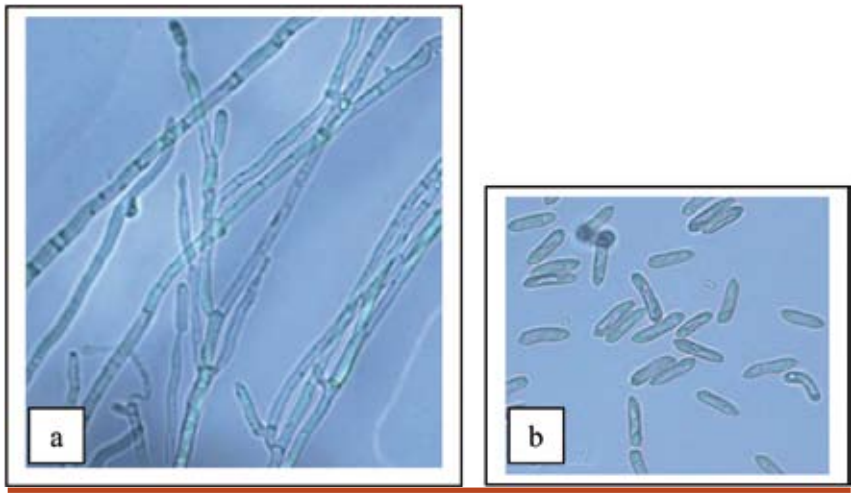

Figure 13.9. Microscopic characteristics of M. anisopliae *var.* major *showing the morphological structures of (a) growing mycelia and (b) spores.*

Identification of indigenous isolates of *M. aniopliae* in Malaysia using RAPD-PCR has shown DNA fragments of var. *anisopliae* and var. *major* (isolates Bp and Mo) to be distinctly different, the differences likely related to the morphological traits, especially spore size (Ramle *et al.,* 1999a). Of four primers tested on isolates var. *major,* three produced identical DNA fragments, suggesting that their genetic bases are closely related. Only primer OPD-06 differentiated var. *major* isolate Bp from isolate Mo, by producing an extra DNA fragment at 396bp from Mo. The RAPD-PCR study also found that the long-spore isolates from the same origin and source have high similarity. For isolate var. *anisopliae,* only one primer produced identical DNA fragments, indicating that var. *anisopliae* has high genomic variability.

Mode of Infection

The disease caused by *M. anisopliae* is commonly called green muscardine disease because of the green colour of its spores. The fungus produces spores which come into contact with the insect cuticle. With a conducive microclimate, the spores germinate, producing young germ tubes which penetrate through the cuticle, physically as well as with the aid of enzymes produced, such as proteases, esterases, chitinases and lipases (Charnley, 1992). Once in the haemocoel, the fungus grows and produce insecticidal cyclic peptides, or destruxins, to overcome the defense mechanism of the insect. The fungus multiplies in all the internal organs and eventually kills the insect. After the insect death, the fungus proliferates until the insect body is 'hairy' with mycelium which then form new spores (*Figure 13.10*).

Figure 13.10. Dead larvae of O. rhinoceros *infected by* M. anisopliae.

Pathogenicity

Natural infection of *M. anisopliae* on *O. rhinoceros* was reported by Friederichs (1920) in 1912 in Western Samoa. Since then, attempts have been made to control *O. rhinoceros* with *M. anisopliae* with some success in the Pacific region (Bedford, 1980). Latch (1976) found that three of 27 isolates of *M. anisopliae* killed 100% of the larvae of *O. rhinoceros* 7-16 days after treatment (DAT). All the isolates were *M. anisopliae* var. *major* with spore length 9-15 μm. Other laboratory studies also showed *O. rhinoceros* larvae to be susceptible to isolates var. *major* but not to isolates var. *anisopliae* (Ferron *et al.,* 1975; Latch and Fallon, 1976; Sivapragasam and Tey, 1995). Initial bioassays in MPOB on two isolates of var. *anisopliae* (Ma and Ml) and two isolates of var. *major* (Bp and Mo) showed that the latter isolates caused 100% mortality at 12-14 DAT. The LT_{50} for the isolates var. *major* was 9 days, shorter than that of the isolates of var. *anisopliae* of 14 days (Ramle *et al.,* 1999a). Four isolates of var. *major* were isolated from natural infected larvae from different sites in Malaysia (*Table 13.1*). Bioassay found that all killed 100% of *O. rhinoceros* larvae at 14 DAT (Ramle *et al.,* 2007).

Toxicity

Oral administration of *M. anisopliae* var. *major* at 5000 mg kg^{-1} did not kill male and female rats. Similarly, male and female rats treated dermally at 2000 mg kg^{-1} survived the two weeks study period. Post mortem at two weeks after treatment did not show any significant changes in the rat organs which were similar to the organs of the control animals. It was concluded that the fungus is safe for rat with oral LD_{50} >5000 mg kg^{-1} and dermal LD_{50} >2000 mg kg^{-1} (Ramle *et al.,* 2004). Acute tests on the freshwater fish, *Tilapia nilotica*, found that after 24 hr exposure, none of the fish had died at all the concentrations tested (0.1, 1.0, 10.0 and 100.0 mg $litre^{-1}$ spores). Mortality was only recorded at very high spore concentrations of 1000 mg $litre^{-1}$ (25% mortality) and 2000 mg $litre^{-1}$ (40% mortality). In conclusion, the spores of *M. a*. var. *major* were not toxic to *T. nilotica* at recommended dose when tested at 25°C-27°C for four days (Ramle *et al.,* 2004).

Toxicity tests on the oil palm pollinating weevil, *Elaeidobius kamerunicus*, were conducted by directly spraying the adult weevils and post-anthesised male spikelets with spore suspensions of 10^7 spores/ml concentration. Direct spraying caused <40% mortality of the adult weevils at 14 DAT. Spraying the post-anthesised male spikelets

TABLE 13.1. ORIGINS OF ISOLATES *M. anisopliae* VAR. *major*

No	Name	Origin
1	BP	Bagan Pasir, Perak, Malaysia
2	CI	Carey Island, Selangor, Malaysia
3	SE	Sedenak, Johor, Malaysia
4	ST	Sg Tekam, Pahang, Malaysia

did not affect the emergence of new adults from the spikelets. Furthermore, none of the adult weevils were infected by *M. anisopliae* after emergence. The stag beetle, *Aegus chelifer*, is commonly found in the same breeding habitats as *O. rhinoceros*. This beetle is generally a feeder on fruits, flowers or leaves (Wood, 1968). Laboratory tests were done on the third instar larvae of *A. chelifer* placed in plastic containers filled with rotting oil palm trunk on which had been sprayed spore solutions. At 12 DAT, the fungus had caused 83.3% mortality to the larvae (Ramle *et al.,* 2004).

Preliminary Assessment in Field

A preliminary field trial to evaluate the effectiveness of *M. anisopliae* to control the rhinoceros beetle was conducted by applying the spores as wet and dry inoculum on rotting chipped oil palm trunk heaps at seven months after felling (Ramle *et al.,* 1999b). For the wet application, each plot was drenched separately with 10 litres water containing spores at the rates of 10^8, 10^9 and 10^{10} spores/plot. For the dry spores, the plots were broadcast with sporulated maize at the rates of 3 kg and 6 kg/plot.

Application of both inoculum at the highest rates significantly ($P<0.05$) reduced the third larval instars (L3) population. At three months after treatment (MAT), the number of live larvae in the plots drenched with 10^{10} spores were about the same as in the plots broadcast with 6 kg at ~5 larvae/plot. This was significantly lower than the control with 34.8 larvae/plot. The effects of the fungus on the adult beetles were varied and inconsistent, possibly due to their migrating behaviour. Infection of the fungus on pupae was hardly observed – only one from 87 samples.

The density of viable spores in the soil was significantly higher ($P<0.05$) in the plots treated at the highest rate of both treatments, and this was possibly the cause of the large number of dead or diseased insects. The density of viable spores was significantly positively correlated ($r^2 = 0.650$, $P<0.001$, $N = 69$) to the mortality of beetles. A converse negative correlation ($r^2 = 0.437$, $P<0.001$, $N = 69$) was found between spore density and *Oryctes* population.

Initial *in vitro* Mass Production of Spores

Mass production of *M. anisopliae* for its spores was attempted in a double storey cabin. The production processes involved a two-phase process: 1) propagation of mycelia in liquid media, and 2) production of spores on solid media.

A series of experiments were done to improve the yield and quality of spores (Ramle *et al*., 2005a). *M. anisopliae* grew well on grains, such as rice, barley and broken maize. Broken maize produced the highest yield of 3.28 g/bag, followed by barley (2.65 g/bag) and rice (1.31 g/bag). The spores were harvested by the wet method. The mature spores in the bags were washed by adding water plus wetting agent, filtered and then dried at 5°C for 6-9 hr. The method yielded 4.01g spores/bag with better spore viability over dry harvesting which only yielded 2.89 g spores/bag.

Subsequent trial with the addition of palm oil in maize indicated that the yield of spores from maize supplemented with oil was 5.56 g/bag, significantly higher (P<0.05) than from maize without oil (4.05 g/bag). Harvesting the spores by washing the maize with water containing detergent produced 6.36 g spores per bag, significantly higher (P<0.05) than from washing with only water of 3.8 g. The spores of *M. anioplae* are sensitive to high temperature. Incubating them at 25°C and 30°C did not affect their germination, but incubation at 35°C reduced the germination of the spore to <20%.

Viability of the spores decreased with their moisture content in storage. After drying for 30 hr, the spore moisture content was down to 7.5%, and it was maintained at this level. Age had an effect on the spore viability with the mature spores germinating better (87.85%) than the young spores (66.21%).

The effects of fungal maturity on the yield and viability of the spores were also studied. Sporulated fungi in the bags had their fungus grown for different periods – 20-25, 26-30, 31-35, 36-40, 41-45 and 46-50 days after inoculation (DAI) – and then harvested by the wet method. The yield of spores gradually increased from 6.5 g at 20-25 days to 12.38 g at 46-50 days. The spore viability also gradually increased but peaked at 73.95% at 31-35 days, then fell significantly to 65.97% at 46-50 days. Therefore, the recommended age of mycelium for spore harvesting is 30-40 days (*Figure 13.11*).

Mass Production of *M.anisopliae* in Pilot Plant

A pilot plant to mass produce *M. anisopliae* var. *major* spores was set up jointly between MPOB and FELDA Agricultural Services Sdn Bhd (FASSB) in Pusat Perkhidmatan Pertanian Tun Razak (PPPTR) Jerantut as the Metarhizium Technology Centre (METEC) (*Figure 13.12*). Full operation commenced in mid 2005. The

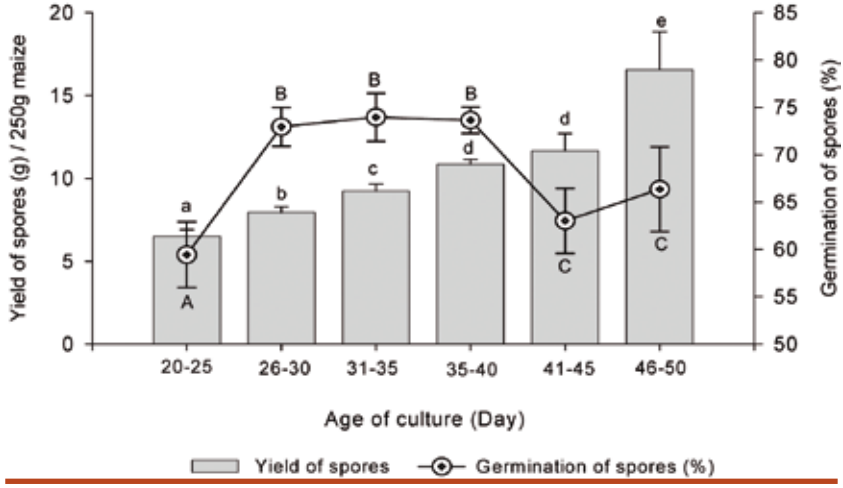

Figure 13.11. The effects of age of cultures on production and viability of spores of M. anisopliae *var.* major. *Bar and line graphs with same letters are no different (P>0.05) by Duncan Multiple Range Test.*

Figure 13.12. Metarhizium Technology Centre at Jerantut, Pahang.

projected production capacity was 3000 kg and 4000 kg for the first and second years, increasing to 7000 kg for next 3 years and finally 12 000 kg in the sixth to the tenth years.

Each step in the process was done in a specific room – one for medium preparation and sterilization, inoculation, fermentation, harvesting, drying and packaging. The harvesting, drying and packaging were to be in a specially designed room – the Negative Pressure Room (NPR). The movement of air in/out from the NPR was through a HEPA filter to prevent spore escape. The temperature will be maintained ≤28°C. General microbial work, such as preparation of media, isolation, subculturing, inoculum quality inspection, spore yield estimation, maintenance of pure cultures and final product quality inspection will be done in the laboratory.

Production and Optimization Process

The mass production of *M. anisopliae* spores and powder formulation of the spores were reported by Ramle *et al.* (2006). The production of spores was in three phases, each comprising several methods. The flow of each process and the quality control measures are shown in *Figure 13.13*. A two-stage incubation was used. First, the fungus was multiplied in liquid medium, then transferred to solid medium – broken maize - for sporulation. The substrate was placed in high density plastic bags, then sterilized at 121°C for 20 min, inoculated with mycelia and finally incubated at 28°C for 30 days. The spores were harvested by washing, filtered off and dried in a drying cabinet for several hours (*Figure 13.14*).

A series of experiments on the various factors affecting the yield and quality of spores was done by Ramle *et al.* (2006). For maximizing the capacity of the incubation room, a tray system was introduced. The trays were stacked on the floor (*Figure 13.15*).

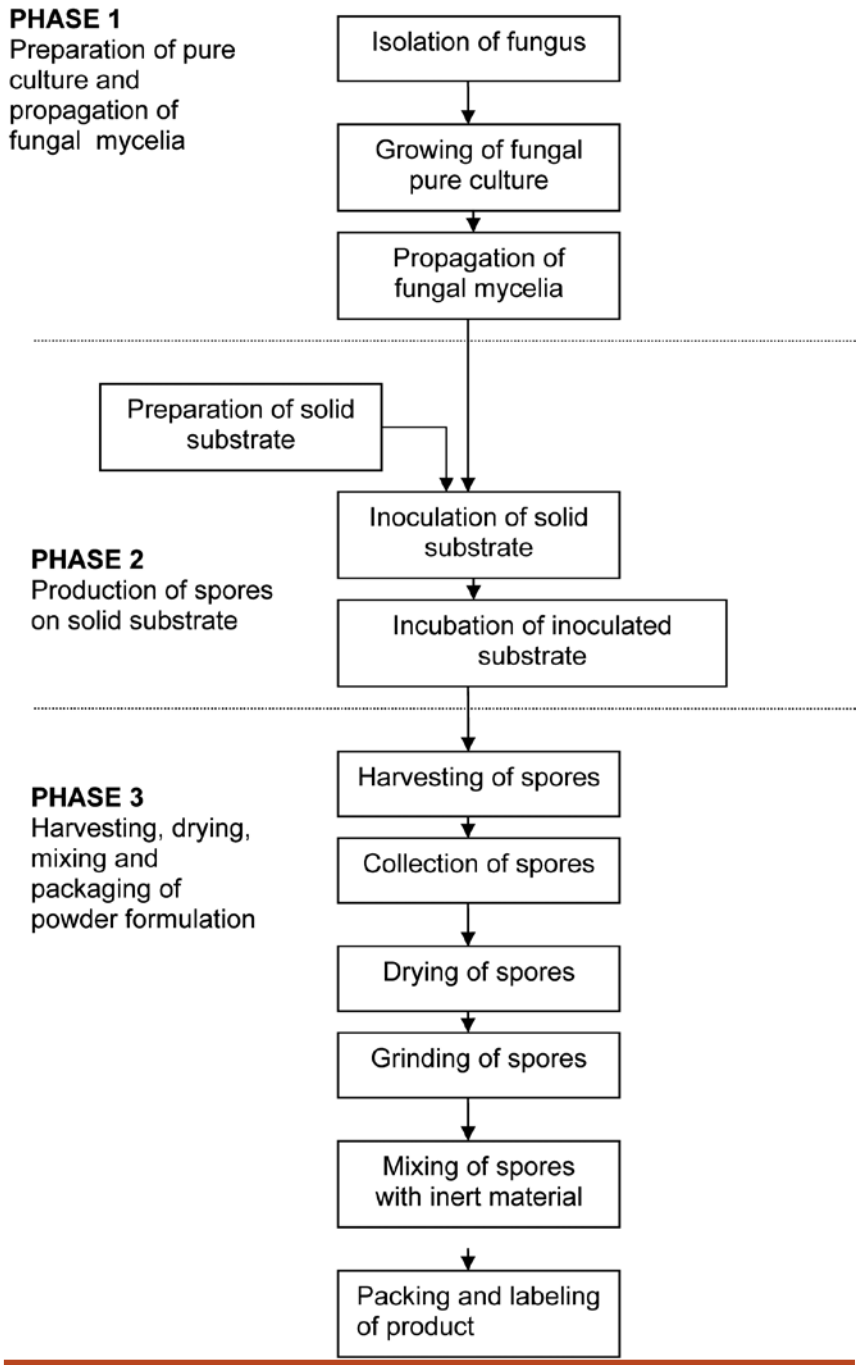

Figure 13.13. Flow chart of process for producing powder formulation of M. anisopliae *spores in pilot plant.*

Figure 13.14. Harvesting of spores of M. anisopliae *by filtration in a pilot plant.*

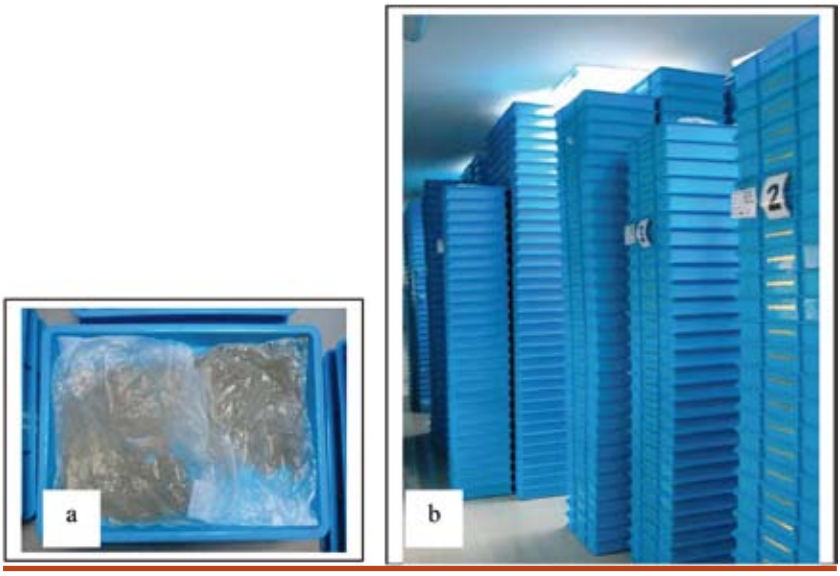

Figure 13.15. Incubation of inoculated bags in trays. (a) Two bags are placed in a tray. (b) The trays stacked on the floor.

The number of bags with inoculated maize increased to 7392 bags a month. The spore harvest was 43-62 kg a month. Maize inoculated with liquid medium at pH6, pH7, pH8 and pH9 yielded 8.82-10.25 g spores a bag (*Figure 13.16*).

Germination of the spores produced was 82.20% to 90.29% (*Figure 13.17*). Mycelium produced in liquid medium at pH6, pH7, pH8 and pH9 yielded more and better quality spores.

The effects of different amounts of maize and sizes of the autoclavable plastic bag on the yield of spores and contamination level were determined. The results indicated that the size of plastic bags and amount of maize did not matter. The yield

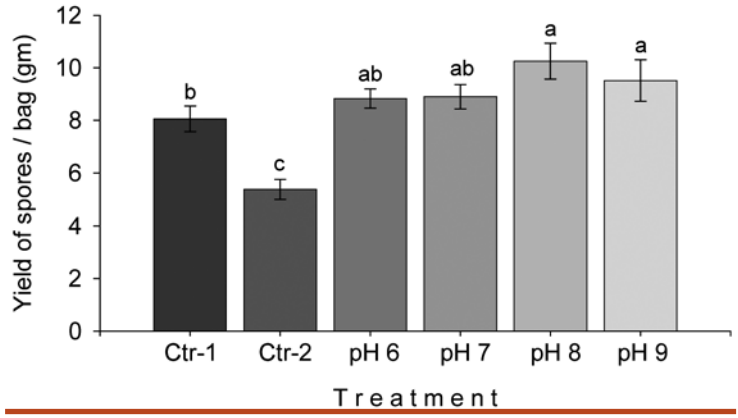

Figure 13.16. Effects of pH of liquid medium on production of M. anisopliae *spores in bags of 300 g maize. Bars with the same letters are not significantly different by the LSD test at P<0.05.*

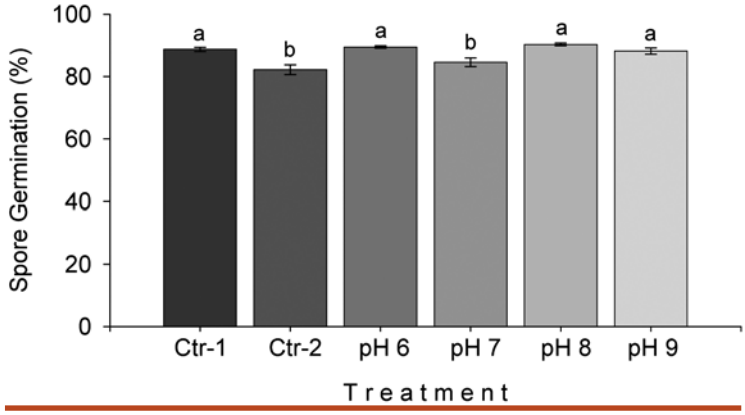

Figure 13.17. Effect of pH of liquid medium on germination of M. anisopliae *spores. Bars with the same letters are not significantly different by the LSD test at P<0.05.*

of spores ranged from 8.32 g/bag to 9.99 g/bag. However, the size of bags influenced the level of contamination - the bigger the bag, the higher the possibility of getting contaminated. Based on the study, small plastic bags (12" × 18") and 300 g maize were routinely used for production in the plant. The optimized supplementary materials and conditions for mass production of *M. anisopliae* spores in the pilot plant are:

- 300 g (green weight) broken maize (grade B);
- size of autoclavable plastic bag 12" × 18";
- sterilization conditions: 121°C for 20 min;
- supplementary materials - antibacterial solution 0.001% at 40 ml kg^{-1} maize, 10 ml palm oil/kg maize;
- use high quality 3-month-old pure culture from single spore isolation techniques;
- incubate inoculated bags at 28°C; and
- dry spores at 15°C-20°C overnight, and another hour at 28°C.

Powder Formulation

The powder formulation contains pure dry spores as active ingredient and clay (hydrated alluminium silicate) as inert material. The formulated product improved the stability and viability of the fungus.

The stability of the formulation stored at 5°C, 15°C and 28°C were determined at 3, 7, 9, 12 and 15 months after storage (MAS). Up to seven months, the spore viability at all storage temperatures was high at >60%. At 9 MAS, the viability at storage temperature 5°C and 15°C was 62.8% and 66.1%, significantly higher ($P<0.05$) than that stored at 28°C which was 52.2%. At 12 and 15 MAS, the viability at 5°C and 15°C remained high at 62% and 51%, respectively. But the viability of spore stored at 28°C decreased from 52.2% at 9 MAS to 28.5% at 12 MAS and 1.65% at 15 MAS. The effectiveness of the stored product at 7 and 15 MAS was tested against the third instar larvae of *O. rhinoceros*, and both produced 100% kill at 13 DAT.

Registration of Powder Formulation

Under the Pesticide Act 1978, the powder formulation is a pesticide and needs to be registered before public sale. The product was registered by FASSB. The registration documents were submitted to the Malaysian Pesticide Board in 2005 under the brand name *Ory-X* (*Figure 13.18*) and approved on December 2007 (Reg. No: LRMP. R1/7765). The product is now widely used in FELDA plantations undergoing replanting since 2006.

Figure 13.18. Ory-X ®, wettable powder formulation of M. anisopliae *for biocontrol of rhinoceros beetle,* O. rhinoceros.

Evaluation of Product *in vitro*

The powder formulation of *M. anisopliae* spores from the pilot plant was tested in the laboratory against field-collected third instar larvae of *O. rhinoceros*. The treatments were 10 ml spore solutions prepared by adding 0.010 g (T2), 0.020 g (T3), 0.031 g (T4), 0.041 g (T5) and 0.051 g (T6) of the powder formulation. The larvae were monitored daily for 12 days. At 8 DAT, the mortality in T4, T5 and T6 were 83.3%, 73.3% and 100%, respectively, significantly higher ($P<0.05$) than in the other treatments. By 10 DAT, all the treatments, except T3 (0.01 g spore/tray) had caused 100% mortality, and at 12 DAT, even T3 did so (Noor Hisham *et al.,* 2005). The larval cadavers were covered by the green spores of *M. anisopliae* at two to three days after death.

Evaluation of Product in Small Field Trials

The product was then evaluated in small field trials in oil palm to oil palm replanting at FASSB Jerantut, Pahang (Noor Hisham *et al*., 2005). The trials used artificial rotting heaps of 2 m × 2 m, or 4 m^2. A total of 50 third instar larvae were placed in each plot (= heap). Six rates - 0.5 g, 1.0 g, 1.5 g, 2.0 g and 2.5 g m^{-2}, - of powder formulation were tested by mixing with water and applied at 0.75 liter m^{-2} breeding material. The solutions were applied using two sprayers - a high volume sprayer and mist blower. The results showed both the sprayer and blower to be equally effective. As early as two weeks after treatment (WAT), all treatments had caused >80% mortality, except for T4 of 1.5 g m^{-2} applied by mist blower, which only caused 72.2% mortality. At three WAT, application of powder by mist blower at T2 (0.5 g m^{-2}) and T5 (2.0 g m^{-2}) had caused 100% mortality. At five WAT, all the treatments had total kills (*Table 13.2*).

TABLE 13.2. MORTALITY OF ORYCTES LARVAE TREATED WITH DIFFERENT RATES OF POWDER FORMULATION OF *Metarhizium* SPORES AND APPLIED BY MIST BLOWER OR POWER SPRAYER

Sprayer	Treatment (rates, g spores m^{-2})	Cumulative mortality (%) over time after treatment (weeks)				
		2	3	4	5	6
Mist blower	T1 (Ctr)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
	T2 (0.5 g)	90.8 a	100.0 a	100.0 a	100.0 a	100.0 a
	T3 (1.0 g)	83.8 a	94.6 a	99.3 a	100.0 a	100.0 a
	T4 (1.5 g)	72.2 a	91.1 a	97.3 a	100.0 a	100.0 a
	T5 (2.0 g)	94.3 a	100.0 a	100.0 a	100.0 a	100.0 a
	T6 (2.5 g)	86.5 a	95.4 a	99.3 a	100.0 a	100.0 a
Power sprayer	T1 (Ctr)	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
	T2 (0.5 g)	92.3 a	98.6 a	99.3 a	100.0 a	100.0 a
	T3 (1.0 g)	88.7 a	97.0 a	98.5 a	100.0 a	100.0 a
	T4 (1.5 g)	93.7 a	97.9 a	99.3 a	100.0 a	100.0 a
	T5 (2.0 g)	95.5 a	96.2 a	98.5 a	100.0 a	100.0 a
	T6 (2.5 g)	97.2 a	98.5 a	98.5 a	100.0 a	100.0 a

Note: Means in the same columns with the same letter are not significantly different by Duncan's multiple range test ($P<0.05$). After Noor Hisham *et al*. (2005).

Evaluation of Product in a Large Field Trial

The trial was in FELDA Jengka 18 and 19, in an area with decaying oil palm residues about one year after chipping. Three rates of application were tested - 0.5 g (T2), 1.0 g (T3) and 1.5 g (T4) - per chipping point (about 12 m^2). At 2 and 4 months after treatment (MAT), there was still no difference in the *O. rhinoceros* polulations in all the plots ($P<0.05$). At 2 MAT, the highest infection by *M. anisopliae* was in T4 at 16.5%, significantly higher ($P<0.05$) than in the Control at 4.4%. At 4 MAT, infection in T2 (17.1%) also exceeded ($P<0.05$) that in the Control (6.2%).

Evaluation of Product in a Commercial Oil Palm Field

The experiment was done in Paloh Estate, Johor which had recently undergone standard replanting (Ramle *et al.,* 2007). The area is undulating with the old oil palm chipped in heaps about 24 months old, and the new palms the same age. The rotting heaps of old oil palm biomass were already covered by the leguminous cover. Two rates of the product were tested - 0.2 g (2.2×10^7 spores) and 0.4 g (4.4×10^7 spores) per m^2 of rotting heap. The product was mixed with water to a solution and applied by a high volume sprayer attached to a tractor. At 8 MAT, both T1 and T2 had significantly reduced ($P<0.05$) the L2 and L3 larvae, pre-pupae and pupae. Reduction of the four stages reduced the overall *O. rhinoceros* population in T1 to 30.8 individuals per plot (ISP) and T2 to 41.0 ISP, significantly lower ($P<0.05$) than in the Control of 132.1 ISP (*Figure 13.19*).

The cover crop delayed the impact of the fungus, but also protected it from adverse factors and provided it the conducive conditions to germinate and initiate infection. Therefore, at 8 MAT, the beetle population was significantly reduced by

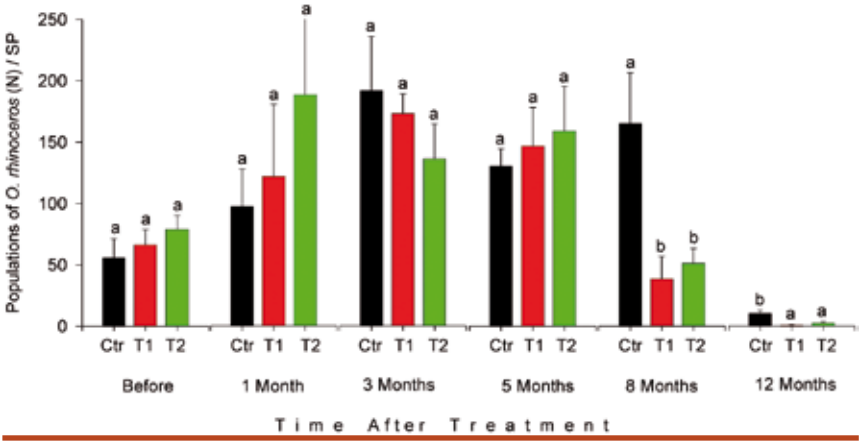

Figure 13.19. Effects of M. anisopliae *spores applied as powder formulation on the overall populations of* O. rhinoceros*. Ctr: Untreated control, T1: 0.2 g m^{-2} rotting heap, T2: 0.4 g m^{-2} rotting. The numbers in each months after treatment (MAT) with the same letters are not significantly different ($P>0.05$) at the Least Significant Difference.*

TABLE 13.3. INFECTION LEVELS OF EACH STAGE OF *O. rhinoceros* AFTER TREATMENT WITH POWDER FORMULATION OF *M. anisopliae* SPORES

Month after treatment (MAT)	Treatment	Infection by *M. anisopliae* (%) on each stage of *O. rhinoceros*						
		L1	L2	L3	Pre-Pupae	Pupae	Adult	Total*
1	Ctr	0	0	0.71	0	0	0.32	1.03 a
	T1	0	0.03	2.14	0	0	0.50	2.67 a
	T2	0	0	1.70	0.05	0.02	0.31	2.08 a
3	Ctr	0	0	1.45	0.02	0.02	0.66	2.15 a
	T1	0	0.06	2.93	0.10	0.02	0.64	3.75 ab
	T2	0	0.07	5.25	0.24	0.14	1.16	6.86 b
5	Ctr	0	0.03	2.76	0.05	0.03	1.77	4.64 a
	T1	0	0.99	11.1	0.11	0.03	2.66	14.90 ab
	T2	0	1.17	8.70	0.17	0.13	2.20	12.40 b
8	Ctr	0	0.69	8.09	0.15	0.06	3.63	12.60 a
	T1	0	3.39	20.00	0.28	0.28	9.38	33.30 b
	T2	0.13	3.63	17.70	0.50	0.39	8.05	30.40 b
12	Ctr	0	5.83	14.60	0.40	0.40	30.80	52.00 a
	T1	0	3.47	6.94	0.69	0	56.70	67.80 a
	T2	0	2.15	8.60	0.27	0	61.90	72.90 a

Note: Ctr: Untreated control, T1: 0.2 g m^{-2}; rotting heap, T2: 0.4 g m^{-2} rotting heap.
*The numbers at each MAT with the same letters are not significantly different at ($P<0.05$) by the Least Significant Difference

33.3% in T1 and 30.4% in T2, significantly higher ($P<0.05$) than in the Control (12.9%). The infection further increased to 52.0% (control), 67.8% (T1) and 72.9% (T2) at 12 MAT (*Table 13.3*). The product at both rates did not affect the non-target insects. The oil palm pollinating weevil, *E. kamerunicus*, was not affected as no difference could be detected in their population density in the treated and control plots. The stag beetle, *A. chelifer*, was also not affected. Although infection could have occurred on both the larvae and adults, the percentage was low, ranging from 0%-0.6% at 5 MAT and 1.7 and 2.5% at 8 MAT.

Oryctes rhinoceros VIRUS

Taxanomy

Basic research on identification of the causative agent of the diseased grub showed that, it was caused by a rod-shaped non-occluded insect virus named *Rhabdionvirus oryctes* or *Oryctes* virus (OrV) (Huger, 1966). Later, the virus was re-placed in a new genus, under Baculoviridae, Sub-group C, and became commonly cited as *Baculovirus oryctes*. In 1997, the virus was assigned to a new *Oryctes* virus family (Evans and Shapiro, 1997). Recently, OrV was proposed to be assigned in a new genus, Nudivirus, together with the *Heliothis zae* virus 1 (HzV-1) (Wang *et al.,* 2007).

History of Virus Release Programme

The fascinating history of discovery of the *O. rhinoceros* virus has been reported by Huger (2005). The devastating damage on coconut by *O. rhinoceros* in the South Pacific in the 1950s and 1960s intensified the search for possible pathogens to control it. An extensive survey around the tropics identified numerous diseases caused by microbes in insect pests, but none against the beetle (Surany, 1960). In 1963, the search concentrated on Southeast Asia, especially Malaysia. First instar grub with infection symptoms caused by *Oryctes* virus were then discovered.

The first release of *Oryctes* virus in Western Samoa in 1970 was proven successful (Marschall, 1970). The virus was first investigated on the larvae and adults, life span, fecundity of the females and mortality of *O. rhinoceros* (Zelazny, 1972; 1973; Monty, 1974). The promising results led to release of the virus in several coconut growing countries in the South Pacific Islands (Hammes, 1978; Gorick, 1980; Young and Longworth, 1981). Remarkable reduction in palm damage and the pest population were observed in one to two years after the virus introduction (Bedford, 1986). In oil palm growing countries, such as Philippines, Indonesia, Maldives and India, the released virus reduced the population of *O. rhinoceros* (Zelazny and Alfiler, 1991; Zelazny *et al.,* 1992; Dhileepan, 1994). MPOB initiated the *Oryctes* virus project for biocontrol of *O. rhinoceros* in 1996, jointly with AgResearch New Zealand and with several oil palm plantations in Malaysia. The findings have been reported and discussed by Ramle *et al.* (2005b).

Diagnosis

The virus infects the larvae and adults of *O. rhinoceros*. The guts of infected adults are commonly swollen and filled with a milky fluid (*Figure 13.20a*), while infected grub becomes translucent when viewed against light (*Figure 13.20b*). A simple, repeatable, sensitive and specific method to diagnose *Oryctes* infection was developed based on the polymerase chain reaction (PCR) (Richards *et al.,* 1999). Using a pair of DNA primers that specifically amplify the OrV DNA, OrV infection is confirmed if a single DNA band at 945 bp appears in the agarose gel (*Figure 13.21*). A series of tests has optimized the PCR method (Ramle *et al.,* 2010). Addition of bovine serum albumin (BSA) in the reaction increased the PCR sensitivity. The method is capable of detecting *Oryctes* virus from 1 million times diluted DNA, the equivalent of the virus DNA concentration as low as 2.23 pg μl^{-1}.

A test to compare the sensitivity of the developed PCR method against the visual symptoms of infection was conducted. Out of 839 swollen guts with milky fluid, 97.6% were diagnosed infected. The PCR method was also able to detect early stage infection. From 307 healthy-looking adults, 36.1% were found infected. The PCR method detected 83.2% of adult beetles caught in a pheromone trap to be infected by OrV, 13.6% higher ($P<0.05$) than based on the gut morphology (69.6%).

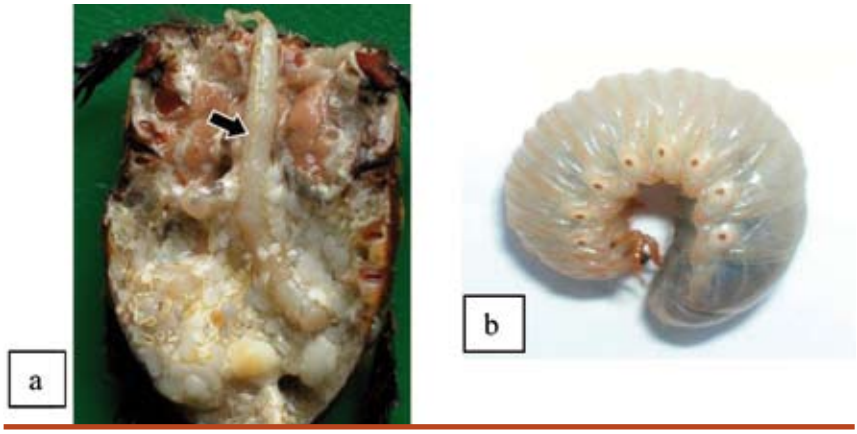

Figure 13.20. Symptoms of Oryctes *virus infection of rhinoceros beetle. (a) Swollen gut full of milky fluid from infected adult (arrow). (b) Translucent infected grub.*

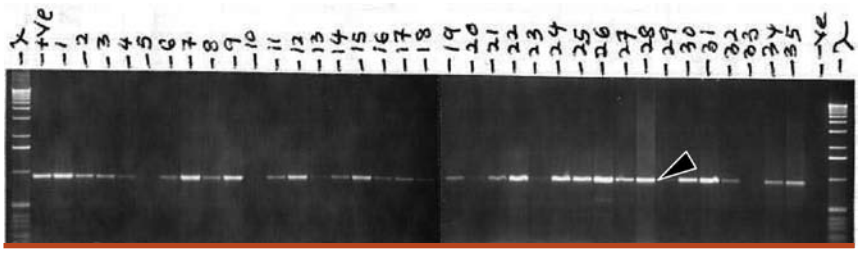

Figure 13.21. Examples PCR products for diagnosing Oryctes *virus in rhinoceros beetle. Adult confirmed infected by single DNA band of 945 bp (arrows) appearing on agaroe gel. λ, DNA Marker: +ve, positive control; -ve, negative control. Numbers against the lines indicate the number of samples used in the test.*

Virus incidence

The incidence of *Oryctes* virus in the natural population was determined from adults sampled in pheromone traps and breeding sites, and from the immature stages. The adult beetles were commonly infected, but with the infection level dependent on the collection and storage methods. Adults caught in pheromone traps in the same container have higher virus incidence than adults captured individually. Thus, some transmission of the virus must have occurred in the traps. In traps, the virus incidence can reach as high as 98.1%, as compared to only about 65% in individually-captured adults. Adults from breeding sites have much lower incidence (21.8%), possibly as they are young and some may not be exposed to the virus.

In the larvae, the virus presence was detected higher in the gut tissue (20.9%) than the haemolymph (13.7%). The incidence of virus increased with the age of the grubs. The incidence in larvae L1 was 0%-3%, in L2 0%-25% and in L3 30%-35% (*Table 13.4*). The pre-pupae and pupae were largely free of the virus. Of the 20 pre-pupae and 17 pupae tested, none were infected (*Table 13.4*). The pre-pupae normally burrow deep into the soil to form a cocoon which minimized contact with anything,

TABLE 13.4. INCIDENCE OF *O. rhinoceros* VIRUS IN INMATURE STAGES OF RHINOCEROS BEETLE

Developmental stage	Number of samples (N)	Percentage of virus infection (%)
L1	37	1.35 (0-3)
L2	81	12.5 (0-25)
L3	81	32.1 (29-35)
Pre-pupa	20	0
Pupa	17	0

Note: Samples collected from Sing Mah and Jendarata Estates in Perak.

including the virus. As the pupae mature, the new adults would be mostly free of the virus.

Genetic Variation and Distribution

The extracted DNA from *Oryctes* virus following the method of Ramle *et al.* (2005b) was characterized by endonuclease enzyme, HindIII. This enzyme had been used before to characterize the *Oryctes* virus from various regions in Asia (Crawford *et al.,* 1986). Four DNA profiles were identified and designated as OrV types A, B, C and D (*Figure 13.22*). The type A virus was identical with strain PV505 as characterized by Crawford *et al.* (1985). It was commonly isolated from many locations in Malaysia (*Figure 13.4*). Insertion of a single band at 15 471 bp differentiated the type B virus from the other types (Wang *et al.,* 2008). The virus was previously isolated from two sites - Carey Island in Selangor and Bagan Datuk in Perak (Ramle *et al.,* 2005b). Now, it is detected in Johor (Wang *et al.,* 2008). The type C virus has a single band inserted at 2.0 kbp, and had been only isolated in Sabah previously. The type D virus has two bands inserted at 15 471 kb and between 2.0 kbp and 1.5 kpb, and was only isolated before from a plantation in Kelantan.

Virulence

The virulence of *Oryctes* virus types A, B and C was tested against the third instar larvae, neonates and field-collected adults. A virus solution prepared in 10% sucrose was placed on the beetle mouthparts. *Table 13.5* gives the mortality, LT_{50} values and infection of *O. rhinoceros* from the treatment. On the L3 larvae, the B virus caused the highest mortality (86.9%), significantly higher ($P<0.05$) than by types A (26.7%) and C (13.3%). The type B virus was more prevalent in the larval cadavers than OrV types A and C. The LT_{50} for type B was 33.9 days, while for types A and C >100 days. The mortality of neonates gradually increased with time. Type B again caused the highest mortality (86.7%) and infection (57.9%). The LT_{50} for *Oryctes* virus type B was 36.8 days, the shortest of all.

On the adults, the LT_{50} of type A was 33.1 days, the shortest of all (*Table 13.5*). The adults responded differently to the virus from the larvae and neonates, with the

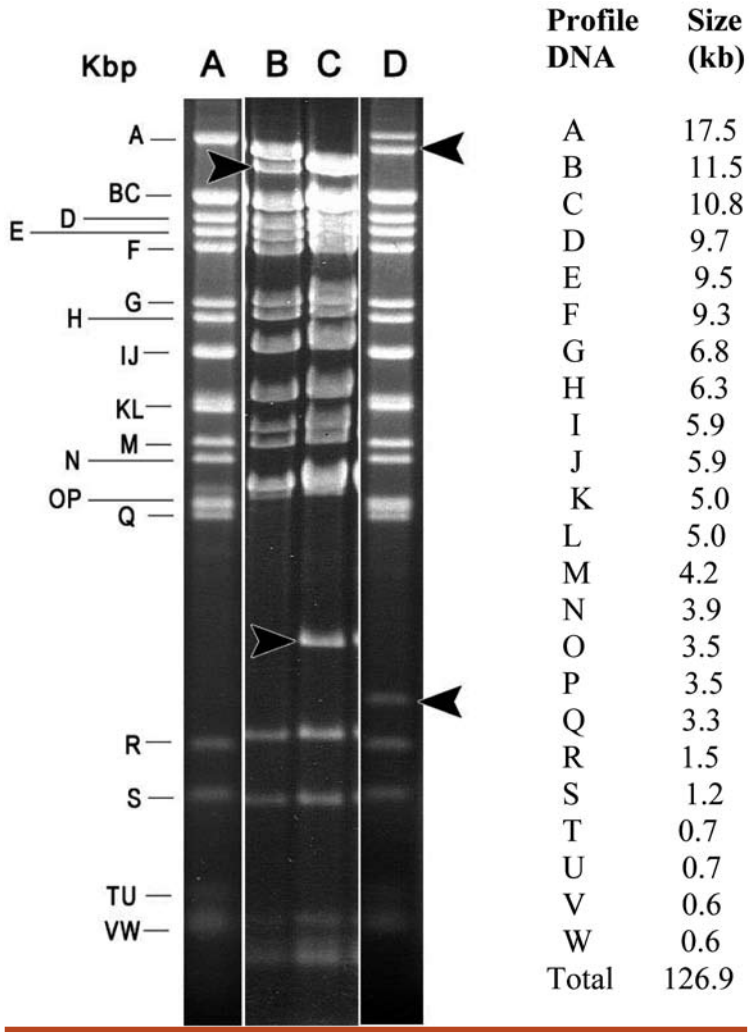

Figure 13.22. DNA profiles of Oryctes *virus types A, B, C and D. DNA size was estimated based on Crawford et al.(1985). Arrows show DNA fragments to identify Oryctes virus types B, C and D from* Oryctes *virus type A.*

type A virus the most effective. At 30 DAT, mortality was 70.8%, significantly higher ($P<0.05$) than types B (10.4%) and C (9.4%). This high rate of mortality, however, gradually decreased with time then hovered at ~50.0%, similar with types B and C. This finding showed that introduction of the same virus already present in the population can increase the mortality. However, the impact seems transient, as its effectiveness reduced with time until finally to the same level as by the other virus types. Marschall and Ioane (1982), who did repeated virus introductions to infected adult populations, found the same thing.

TABLE 13.5. MORTALITY, INFECTION AND LT_{50} OF *O. rhinoceros* VIRUS AGAINST THE LARVAE, NEONATES AND ADULTS OF RHINOCEROS BEETLE

Virus type	Larvae *			Neonates *			Adults **		
	Mortality (%)	Infection (%)	LT50 (day)	Mortality (%)	Infection (%)	LT50 (day)	Mortality (%)	Infection (%)	LT50 (day)
A	26.7 a	18.7 b	> 100	63.7 a	28.8a	48.58	50.0 a	83.3 a	33.12
B	86.7 b	40.7 a	33.95	83.3 a	57.9 b	36.89	45.2 a	64.6 a	62.38
C	13.3 a	8.0 b	> 100	10.7 b	3.2 c	> 100	29.9 a	62.5 a	86.35

Note: Data from *56 and **50 day after treatment (DAT).
Mortality is 'corrected mortality' following the formula of Abbot.
Means in the same columns with the same letters are not significantly different at $P<0.05$ by the Least Significant Difference (LSD).

Production Method

Bulk production of *Oryctes* virus in the 1970s was commonly *in vivo*, using the larvae as inoculum (Bedford, 1980). The amount of haemolymph increased in infected larvae, making them translucent when viewed against light (*Figure 13.20b*). In our study, *Oryctes* virus types B and C were inoculated on healthy field-collected L3 larvae. For type B, of 28 larvae inoculated, 17 became infected, but for type C, only 4 out of 25. The average amount of haemolymph produced from each cadaver was 1.46 ml (N = 4), equivalent to 370 500 Infective Units (IU). At the recommended rate of inoculation of 1500 IU/adult, the amount can inoculate 245 adults. The virus solution was also freshly prepared from the midgut of adults collected from the field. The method to prepare the pure virus solution from either the gut tissues or haemolymph was similar. Basically, the whole gut, or haemolymph, was transferred into a 1.5 ml vial and homogenized using a motorized micropastle until a cloudy solution was formed. The homogenate was filtered through a 0.45 μm cellulosic membrane, and the filtrate (virus solution) stored at -30°C.

Field Introduction

Field introduction of the virus required a series of steps - pre-release site assessment, introduction of virus, post-release monitoring and impact assessment.

Pre-release site assessment. This involves the background work to identify the existing natural existing virus type(s), the infection level in adults and larvae, estimating the age of the adult population and the insect density. Sample adults were caught in pheromone traps and individually placed in cylindrical plastic vials. The insects were dissected for their guts which morphological appearances were recorded. The gut tissues were tested for virus by PCR (Ramle *et al.*, 2010) and the virus(es) characterized by restriction endonuclease enzyme HindIII (Ramle *et al*., 2005b). Infection was determined on the females, males and larvae for use as pre-release data.

Many of the earlier virus introductions had monitored the level of palm damage before/after the introduction (Purrini, 1989; Dhileepan, 1994). New and old damage on the palms by *Oryctes* attack was inspected. Damage on the first three fronds, number of holes or cut leaves on old fronds and number of little leaves were estimated. The total number of frond produced by each palm was also counted. Other activities, such as identifying the release block and placement of pheromone traps in the release area had to be done before introduction of the virus. It was recommended that the release site for the virus introduction program be >100 ha.

Introduction of virus. The virus was released by the capture-inoculate-release technique of Zelazny (1978). Young healthy adults were collected from pheromone traps, or from breeding sites in the prospective release site. The young adults were

commonly free from soil, having a shiny elytra free from scratches. The collection should be done one to two days before the virus introduction. Prior to OrV inoculation, the adults were placed in a container without food for 24 hr to facilitate the inoculation as the starved adults will then consume more of the virus solution. The virus inoculation was done on site by dripping the *Oryctes* virus solution on the mouth parts of the beetles. The adults were released by just placing them on the ground near a palm base. Trapping and pesticide application were stopped for at least a month to give the inoculated adults maximum laxity to transmit the virus.

Post-monitoring and impact assessment. After the one month or so grace period, adults were sampled from pheromone traps and larvae from the breeding sites. All the samples were placed individually in plastic vials to avoid cross contamination during transport and storage. For the first six months, the sampling was done monthly, then every three months until 24 months. In each sampling, the adults were collected for three to four consecutive days in the morning. Of the adults caught in traps, only 20% were collected, the remainder released. The capture was sorted into sexes and the following parameters estimated.

a) Population density of adults, male:female ratio;
b) Virus infection in the grubs and in males and females as determined by the PCR technique;
c) Determination of the virus spread by DNA analysis using endonulease enzyme HindIII; and
d) Palm damage every 6 months using the same method for the pre-assessment.

Impact of Virus Introduction on Young Palms

Oryctes virus type B was introduced in an estate with 6-month-old oil palm by the release of 150 inoculated adults in the centre of a field. The subsequent trends in virus infection and population density are shown in *Figure 13.23*. The infection gradually increased from 37.4% pre-introduction to 100% at 3-4 MAR. Thereafter, the infection was slightly reduced but remained >90% until the end of the experiment. Infection in the control plot, ~3 km from the released site, also increased but slowly. It peaked at 100% at 11 MAR. Census of larvae at 5 MAR found that of 62 collected, only seven were infected (11.3%), while at 6 MAR none of the 6 larvae collected were infected. This finding supports previous results from many countries in which the disease is already established (Zelazny and Alfiler, 1991; Zelazny *et al.,* 1992; Dhileepan, 1994). It was found that the virus infection was common among the adult beetles, but much less so in the larvae.

The increased virus infection was maintained high in the adult population until 15 MAR (*Figure 13.23*). While the highest level of 2.62 adults/trap/night (a/t/n) at the beginning reduced to 1.6 a/t/n at 4 MAR and 0.93 a/t/n at 6 MAR, then slightly increased to 1.48 a/t/n at 11 and 15 MAR. There was a negative correlation between

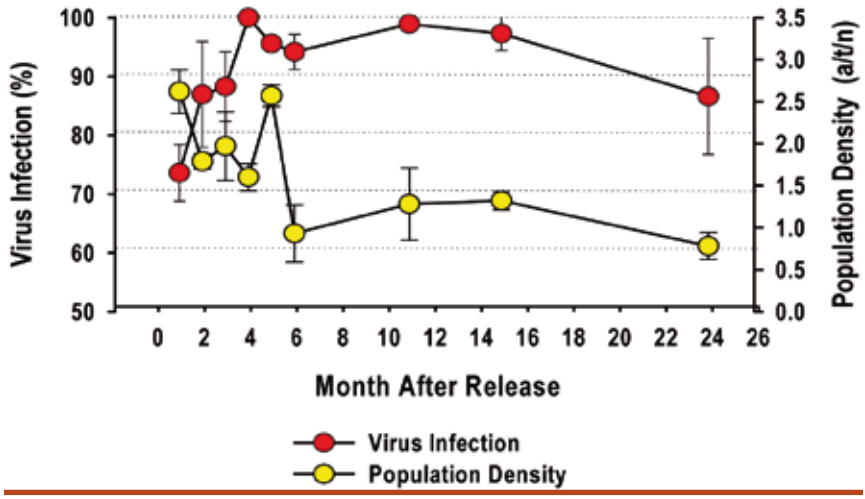

Figure 13.23. Percentage of Oryctes *infected by* Oryctes *virus and density of adult beetles in young oil palm area.*

virus infection and population density (Y=100.60 – 5.65x, R^2=39.74%, F=2.72, P<0.05); however, it was not significant. The number of males was also affected – their proportion falling from 43.5% at 2 MAR to the lowest 28.7% at 4 MAR before recovering to hover at 30%-37%. A similar reducing trend in the males was also recorded in the Maldives by Zelazny *et al.* (1992) - from 63.2% (6 MAT) to 50.9% (12 MAT), then rising to 58.4% at 24 MAR.

The type B virus started to establish as early as 3 MAR (*Figure 13.24*). Its DNA profile was detected in the inner field (where it was released) at 3 MAR, then spread to the middle and outer plots, finally even in the control plot at 11 MAR. It then persisted at high levels as its profile was even observed at 15 MAR. This suggested that the rapid increase in virus infection at 3-4 MAR was due to the released *Oryctes* virus type B.

The average palm damage was significantly reduced (P<0.05) from 11.2% to 4.0% at 15 MAR and then 3.4% at 24 MAR. This level of control was considered satisfactory, being below the threshold to apply insecticides.

Impact Introduction of Type B Virus in Immature Palm Area

The type B virus was then introduced in Pekan Estate, Kluang, Johor to palms of 3 years old replanted with zero burning. A total 125 adult beetles (90 females and 35 males) were released. The existing *Oryctes* virus was type A. The area was divided into 3 release regions - inner (IR), middle (MR) and outer (OR) regions. Pheromone traps were placed at the boundary of each region. At the time of release, most of the rotting oil palm residues (of the old crop) were fully covered by the leguminous cover.

Infection by the virus slowly increased and peaked at 6 MAR, then fell and plateaued at 60%-70% for eight months, before falling further to <40% (*Figure 13.25*). An increase in virus infection after its introduction was commonly reported in many

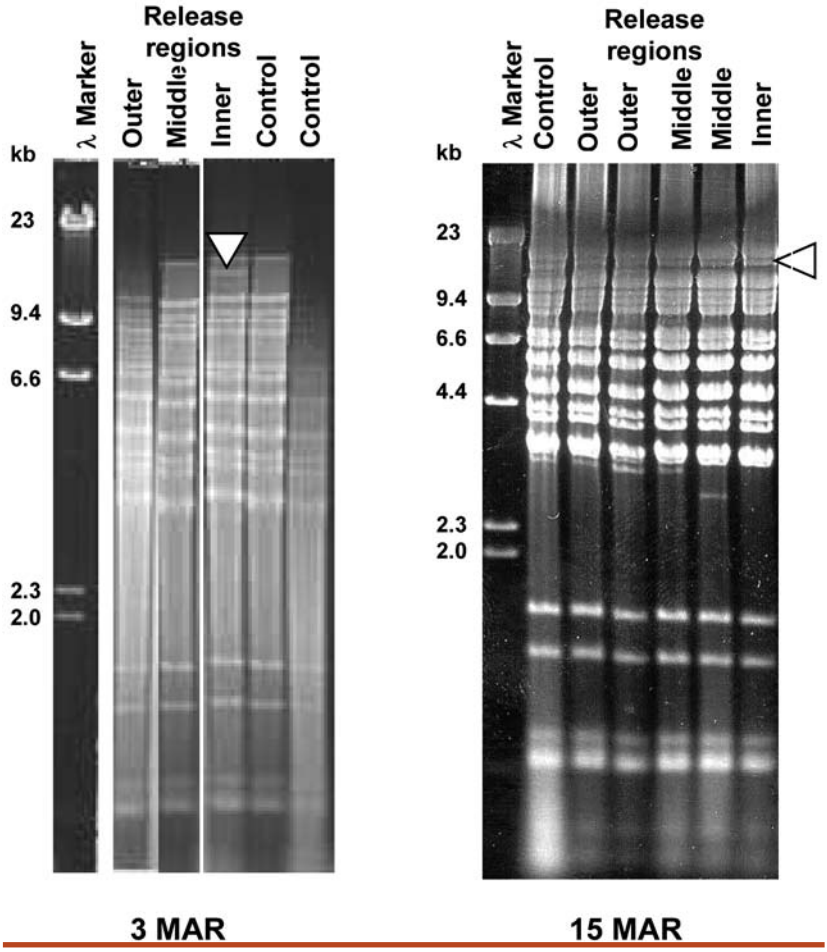

Figure 13.24. Evidence of establishment of released Oryctes *virus type B in young palm area at 3 and 15 months after release. Arrows show band used to differentiate type B virus from the existing type A.*

previous studies, especially in the Pacific Islands (Zelazny, 1973; 1977; Marschall and Ioane, 1982). A study in the Philippines (Zelazny and Alfiler, 1991) and Maldives (Zelazny *et al.,* 1992) showed the highest virus infection of 30%-32% as early as 3-9 MAR, then gradually reducing to 10%-15% at 20 MAR. The beetle population fell from its highest at 2 MAR, to its lowest at 4-6 MAR, and maintained at the low level for some time before inching up again to a second peak at 16 MAR. From the 16 MAR onwards , the population remained low (0.71 to 1.33 a/t/n) for 10 months (*Figure 13.25*). This was also found by Marschall and Ioane (1982) whose beetle population fell as early as 2-3 MAR, then gradually increased to a much higher level at 4-5 MAR. The virus infection and population density had a weak negative correlation, suggesting that the reduction in beetle population in the release sites had other causes beside the virus.

The proportion of males to female is a key parameter used to estimate the impact of a virus release program. Males are normally more easily infected than females (Zelazny *et al.,* 1992). The natural behaviour of the males to stay longer in the breeding

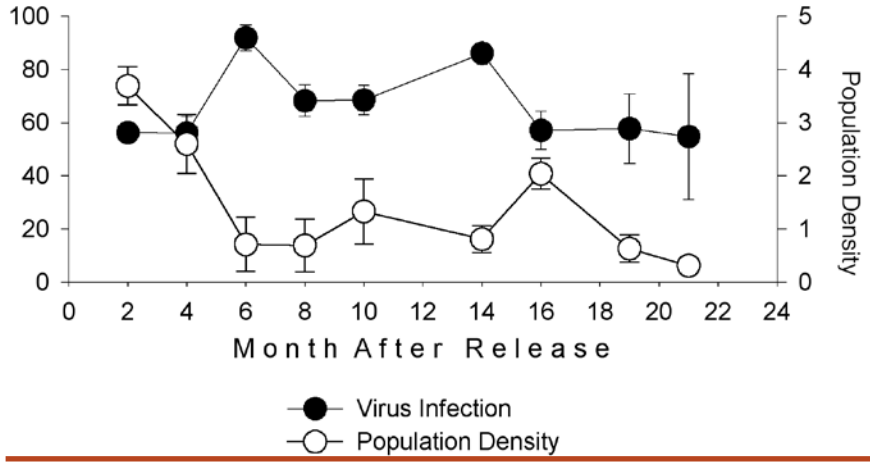

Figure 13.25. Impact of released Oryctes *virus type B virus on infection and population density of rhinoceros beetle in Pekan Estate.*

sites to prepare a 'nest' for the eggs exposes them to more contamination. Females spend more time foraging for food in the palms before mating and laying their eggs. Here, the male population only began to fall in the later stages at three to four months after the virus introduction (*Figure 13.26*). This delay is possibly because the infected adults needed some time to transmit their infection to other healthy adults. In the field, infected adults needed at least 2 months to transmit the virus to other healthy partners (Gorick, 1980). The slower fall in beetle population may possibly be due to slower virus transmission among the adults, as they already had some adaptation/immunity to the virus. This phenomenon has been recorded in Philippines where the virus naturally occurs (Zelazny *et al.,* 1989). The sex ratio of male/female has a significant positive correlation with the number of males and, therefore, both parameters can be used to monitor the impact of the virus introduction.

The *Oryctes* virus mainly affected the adults rather than the larvae. The infected adults had a reduced life span by four to five weeks (Zelazny and Alfiler, 1991). In this study, the virus infection of the males and females were not significantly different in all

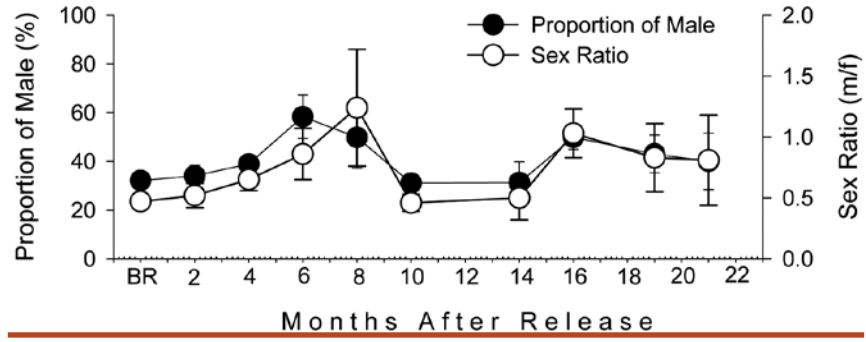

Figure 13.26. Proportion of males, and sex ratio of rhinoceros beetle in Pekan Estate. BR - before release.

the experimental sites, although slightly higher in the males up to16 MAR. The infected males were possibly the older ones, as male adults are reportedly more frequently infected than the females (Zelazny, 1977; Zelazny *et al.,* 1992). The females were mostly newly emerged, and still free from the virus. However, the females are more susceptible to the *Oryctes* virus once infected (Zelazny, 1973), and the infected ones produced fewer eggs and died earlier than the infected males. The reduced number of females increased the proportion of males and the sex ratio. This is possibly a factor contributing to a slower reduction of both parameters as well as the population density in the early 3 to 6 MAR. The reason why females are more susceptible is still unknown and needs further investigation.

The introduction of Oryctes virus type B into a population of adults already infected with Oryctes virus type A virus successfully reduced the adult population and also palm damage to below the economic threshold level of 5% (*Figure 13.27*). Reduced palm damage was also reported in previous virus introductions, such as in Western Samoa, Willis Islands and

India (Bedford, 1980; Dhileepan, 1994). The DNA profile of the released type B virus was only recorded in the inner release region at 4 MAR (*Figure 13.28*). Ramle *et al.* (2005b) introduced the *Oryctes* virus type B virus in a high population density area, found that the virus spread was detected as early as 3 MAR, and the virus then established in the whole experimental area at 11 MAR.

Future Research

Screening for the effective types of virus should be continued, not only from the beetles in oil palm areas but also from other crop areas. Efforts have been made to obtain more Oryctes virus from various research institutions, such as those in

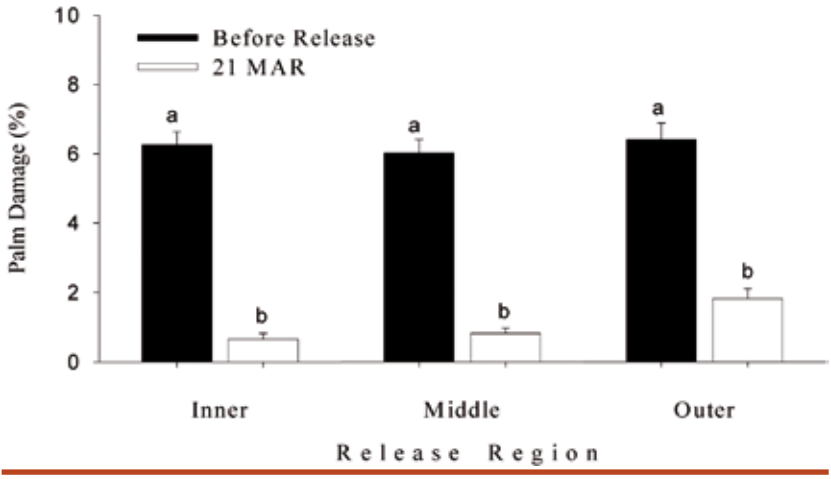

Figure 13.27. Percentage palm damage in the release region in Pekan Estate. Bars with the same letters were not significantly different at P<0.05 by the Least Significantly Difference (LSD), MAR; Months After Release

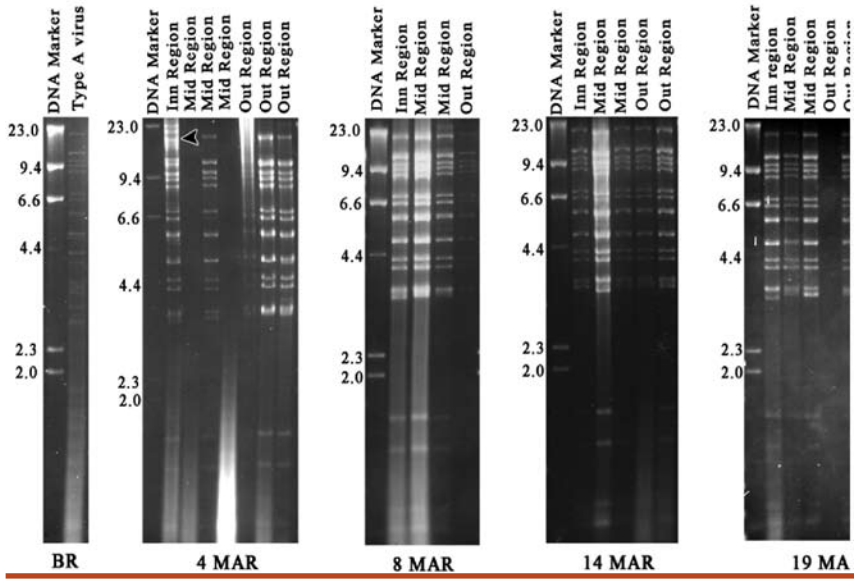

Figure 13.28. Genomic analysis of Oryctes virus DNA from Pekan Estate, Kluang, Johor before (BR) and at 4, 8, 14 and 19 months after release. A single band (arrow) at 15,471 bp confirms the released virus DNA obtained from a beetle caught at 4 MAR in the inner release region.

India, Philippines, Indonesia, South Pacific Islands and African countries. A standard bioassay

should be established to assess the virulence of the *Oryctes* viruses. The use of insect cell culture, such the cell line DSIR-HA-1176, has proven suitable for replicating the *O. rhinoceros* virus, and, therefore, been used to study the *Oryctes* virus mophorgenesis, infectivity and protein make-up (Crawford, 1982; Crawford and Sheehan, 1985). Besides being used to produce high quality Orycets virus, DSIR-HA-1179 has also enabled the genetic study of *Oryctes* virus, such as construction of the virus physical map for 12 geographical origins (Crawford *et al.,* 1985; 1986). The *Oryctes* virus genetic variation can be helpful in the taxonomy and identification of the effective *Oryctes* viruses (Jackson *et al.,* 2005).

Specific DNA primers for each Malaysian *Oryctes* virus and other types of *Oryctes* viruses are required and need to be developed. By using PCR, the primers can be used in post monitoring study to trace the *Oryctes* virus transmission, and simultaneously differentiate the released *Oryctes* virus from the existing viruses in the population. This rapid and accurate method only requires a low yield of DNA to detect the virus presence (Ramle *et al.,* In press). This new diagnosing method can perhaps measure the role the released *Oryctes* virus, especially when incorporated in a control programme with other pathogens.

Generally, in areas where presence of the *Oryctes* virus did not affect the adult population, it is recommended to use Metarhizium to kill off the larvae population (Ramle *et al.,* 2007). Importantly, the findings of this study will be used to develop an effective integrated bio-management system for oil palm against the rhinoceros beetle.

CONCLUSION

IPM of bagworms outbreaks using selective Bt products, beneficial insects and plants can reduce the need for chemicals. Unlike chemicals, microbial products, like Bt, are safe to the environment and non-toxic to beneficial insects, palm pollinators, natural enemies of bagworms, and can contribute to sustainable agriculture. Acceptance by the industry of microbial agents, such as Bt, is reflected by the number of agencies showing interest in the products. The powder formulation of *M. anisopliae* has proven effective in reducing the larvae population of *O. rhinoceros*. However, the adult population is better suppressed by introducing the *O. rhinoceros virus*. The microbial products will give oil palm planters a choice in control measures with greater safety to the operators and non- target organisms, such as the pollinating weevil, mammals, fish and other coleopteran insects. The application of these products is virtually harmless to humans and the environment. It is envisaged that microbial insecticides will contribute greatly to sustainability of the oil palm industry in Malaysia by reducing its chemical use and creating a cleaner oil palm ecosystem.

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