DIVERSITY, HOST UTILIZATION AND ECOLOGICAL NICHE OF TEPHRITID (DIPTERA: TEPHRITIDAE) FRUIT FLIES IN UGANDA

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DIVERSITY, HOST UTILIZATION AND ECOLOGICAL NICHE OF

TEPHRITID (DIPTERA: TEPHRITIDAE) FRUIT FLIES IN UGANDA

By

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DECLARATION

I, **Brian E. Isabirye** hereby declare that this submission is my original work and contains no material previously published or submitted by any other person to any institution of higher learning for the award of a diploma, degree or any other academic qualification, except where due acknowledgment has been made in the text.

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ABSTRACT

Phytophagous Fruit flies (Diptera: Tephritidae) cause heavy losses on fruits and vegetable crops, and pose a threat to the commercialisation of the horticulture industry in Uganda. In order to develop an effective management strategy against fruit flies, it is important to understand the diversity, patterns of host utilization and ecological niche of the major fruit species, which were the objectives of this study. Major differences in species richness and community structure occurred among the three major mango growing regions. The alien *Bactrocera invadens* was noted to be displacing native fruit fly species. Similarly, fruit infestation was predominated by *B. invadens*, while damage by native fruit flies was negligible. Tropical almonds showed the highest fruit fly infestation incidence (87.9%), and were mainly infested by *B. invadens* (82.1%). *Psidium guajava* and *Mangifera indica* were also favorable hosts. There was significant difference in infestation among mango varieties (p < 0.0001). Among the host fruit species, female *B. invadens* fruit flies frequently oviposited most on fruits that gave better adapted offsprings (support for Preference Performance Hypothesis-PPH), with overall coefficient of determination (R^2) for infestation averaging 75.4%. However, PPH was poorly evident among the various mango varieties, with the trends suggestive of an Optimal Foraging Theory (OFT) (oviposition on readily available fruits). *B. invadens* from different agro ecological zones and fruit hosts were significantly different in morphology (p < 0.0001), which suggested that geographic and host-associated adaptations could produce phenotypic variations that can lead to ecotype and host populations. Precipitation (61.41%) and temperature (29.21%) were the most important determinants of fruit fly distribution in the country. On that basis, the most suitable habitats were central and mid north zones, while the western, north-eastern areas were marginal. Future potential fruit fly habitats were projected to decline by 25.4% on average. *Dacus bivittatus, Bactrocera cucurbitae* and *Ceratitis anonae* were projected to be the least climate change resilient species. *D. cilliatus* (249.3%), *B. invadens* (-1.9%) and *C. cosyra* (-2.2%) were projected to be the most climate change resilient species. Future fruit fly niches were predicted to shift northwards, mainly to the northern moist farmlands. This study has provided knowledge on several aspects of the ecology of fruit and crucial information that can help in the development of adaptative pest management strategies in Uganda.

Key words: Agro ecological zones, *Bactrocera invadens*, Fruit flies, Infestation, Uganda,

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CHAPTER ONE: INTRODUCTION

1.1   General Introduction

Horticulture is one of the fastest growing agricultural sub-sectors in Uganda, with the products listed as strategic exports in the country, with horticulture exports earnings worth US$35 million per year (Uganda Export Promotions Board, 2012). In 2004, Uganda's fruit and vegetable production was equivalent to about 1% of the world's total production (UIA, 2009). Indeed the monetary value of Fresh Fruit and Vegetables (FFV) has been increasing steadily since 2003. Banana, mango, citrus, apple, pineapple, guava, avocado and watermelon dominate the FFV industry in Uganda (Uganda Export Promotions Board, 2012). Among these dominant fruits, the mango (*Mangifera indica* L., Anacardiaceae) industry is recognised as a vibrant and dynamic sub-sector providing income and employment (Agona *et al.,* 2002; UIA, 2009).

Unfortunately, fruit production in Uganda is constrained by a multiplicity of pests and diseases, the most serious of which being the phytophagous fruit flies (Diptera: Tephritidae). These pests constitute one of the major horticultural pest problems in the world, causing heavy losses and thus curtailing expansion of domestic horticultural production and trade of fruits and vegetables. In Africa, it is estimated that about 40% of the 1.9 million tonnes of mangoes produced annually is lost to fruit flies (Ekesi *et al.,* 2006). Fruit flies are also listed as important quarantine insect pests in most countries because of the severe damage they cause to agricultural produce (Lux *et al.,* 2003; Mwatawala *et al.,* 2004; 2006a; Mboyine *et al.,* 2012).

Furthermore, the implementation of quarantine regulations in Africa's most important international markets, the European Union (EU) and the USA, mean that the lucrative export of

fresh mangoes from Africa could be severely limited (Ekesi *et al.,* 2006). The quarantine and Maximum Residue Level restrictions imposed by the Secretariat of the International Plant Protection Convention and the World Trade Organization, bar countries with fruit flies from exporting fruit to countries that are free of fruit fly infestation. Thus fruit flies threaten to erode all the benefits and potential income of the FFV industry.

Fruit flies are very diverse; with close to 4,000 species of fruit flies identified in the world, out of which 450 cause considerable damage to fruits and vegetables (Norrbom *et al.,* 1998). Fruit flies live under different agro-ecological zones (areas with similar combinations of soil, landform, climatic characteristics, constraints, potentials and hence ecological endowment). Several studies conducted under contrasting farming systems, climate and anthropological patterns have provided information on diversity of fruit flies in the East African (EA) region (Mwatawala *et al.*, 2006a; 2010; Rwomushana *et al.,* 2008; Geurts *et al.*, 2012). The preceding studies identified *Ceratitis capitata* (Wiedemann), *Ceratitis rosa (*Karsch), *Dacus cilliatus (*Loew), *Bactrocera cucurbitae* (Coquillett), *Bactrocera invadens (*Drew, Tsuruta and White), *Ceratitis cosyra* (Walker), *C. fasciventris* (Bezzi), *C. anonae* (Graham) as the most important fruit fly species in the region. Other common species identified include *C. catoirii* (Guérin-Meneville), *C. flexuosa* (Walker) and *C. punctata* (Wiedemann). The studies have also provided information on the field infestation, life history and demographic parameters of some species. In addition, there have been attempts to characterise the population genetics of a limited number of species. Further, the biodiversity, host range and distribution, field infestation, life history and demographic parameters of fruit flies in orchards in different agro-ecological zones have also been considered in Kenya (Rwomushana *et al.,* 2008) and Tanzania (Mwatawala *et al.*, 2006a; 2010). On this

basis, provisional control strategies have been formulated. These have mainly been based on combinations of Male Annihilation Technique (MAT), Bait Annihilation Technique (BAT) and Orchard Sanitation (OS) (Ekesi *et al.*, 2006).

Despite the earlier efforts in the EA region to manage fruit flies, the performance of control methods in Sub-Saharan Africa has been largely dismal (Lux *et al.,* 2003). This has been mainly attributed to lack of local knowledge on the ecology of fruit fly populations. A greater understanding of the biology and ecology of fruit flies in Uganda would therefore be beneficial in the following respects: knowledge of the species present and whether or not they include biotypes or ecotypes; knowledge of the fruits and their phenology, the influence of climatic variables on the population dynamics and seasonality of fruit fly populations. Knowledge on the biology and ecology provides clues for development of sustainable integrated pest management strategies.

With the exception of the earlier efforts by Nakasinga (2002) that provided insights into the diversity and mango fruit suitability of pest fruit flies in selected zones, few efforts have been devoted to the assessment of the ecology and biology of the pests at a broader scale across Uganda's agro ecological zones. The differences in agro ecological zones in Uganda with those in the EA region could cause significant differences in the ecology of fruit flies and their damage levels. Ultimately, the differences may lead to variations in faunal composition and hence call for specific control measures. This, therefore, warrants further studies on the diversity, host utilization and ecological niche of fruit flies, as a means to formulation of a knowledge-based surveillance and Integrated Pest Management (IPM) strategy for fruit flies in Uganda.

1.2   Statement of the Problem

Fruit flies remain key pests in horticulture despite efforts to control them. The dismal performance of control efforts in Sub-Saharan Africa (SSA) is partly attributed to a limited understanding of fruit fly ecology and biology. In Uganda, the few studies on fruit flies (Nakasinga, 2002; Nemeye, 2005; Okullokwany, 2006) have yielded some information but too limited to inform pest management. For instance, the diversity of fruit flies in Uganda and how this is shaped by host availability, geographical spread and environmental variability is unknown. It remains unclear whether any of the country's agro ecological zones are unsuitable for pest fruit flies, particularly the invasive *Bactrocera invadens* establishment. Further, it is not clear whether the predicted change in climate (IPCC, 2007) will not alter the suitability and distribution of the fruit fly species in the country.

Due to the cryptic nature of most species in the Bactrocera complex to which *B. invadens* belongs, identification of some species is difficult (Clarke *et al.,* 2005). Identification difficulties of the *Bactrocera* complex species are due to the broad intraspecific morphological variation among members of the *B. dorsalis* complex (Drew *et al.,* 2008). In addition, it is not clear whether allopatric and host associated speciation has taken course among *B. invadens* populations in the different zones in Uganda, yet this would be important in the design of effective management strategies for the invasive species. Circumventing such challenges requires pursuance of new methods and tools with high resolution and discrimination abilities suited for detecting fine-scale differences between species, ecotypes or host races. One such method is geometric morphometrics (Rohlf, 1999).

1.3   Study Objectives

1.3.1   Main Objective

To describe the diversity, host utilization and ecological niche of major tephritid fruit flies in Uganda.

1.3.2   Specific Objective

1. Determine the species diversity of fruit flies in the major mango growing agro ecological zones

2. Assess fruit fly host utilization in the different agro ecological zones.

3. Characterise the morphometric variability of *B. invadens* fruit flies among hosts and mango growing zones

4. Determine the current and potential future spatial distribution of the major tephritid fruit fly species in Uganda.

1.4   Hypotheses

1. There is no significant difference in the diversity of fruit flies in different ecological zones in Uganda.

2. There is no significant difference in fruit fly host utilisation patterns in different ecological zones and among host types in Uganda.

3. There is no significant morphometric heterogeneity among *B. invadens* populations infesting different hosts in different ecological zones in Uganda.

4. Fruit fly species'current and future distribution and ecological niches is random across different ecological zones in Uganda

1.5   Justification

Uganda, like any other developing country is looking forward to expanding her economy through increased export trade in fruits and vegetables with new technologies that can secure market access of her produce. Such exports are subjected to sanitary and phytosanitary (SPS) provisions of the World Trade Organization (WTO) agreement and are only permitted at ports of entry when produce is free from quarantine pests and diseases. The single greatest impediment to Ugandan fresh produce exports is fruit flies. The knowledge required to develop an effective control strategy is lacking; and the country does not have the empirical information to prohibit entry of produce infested with fruit flies (new species introductions). Such new entries would form new introductions of the pest flies. Uganda has inadequate information on species composition of fruit flies because no systematic surveys have been made.

A complete list of the fruit fly species on Uganda's proposed fruit and vegetable exports satisfies a fundamental requirement of phytosanitary agreements that govern produce exports between countries. It would also contribute to the scientific evidence that Uganda need to substantiate any claims regarding the presence or absence of pests. Accurate fruit flies records will enable the Uganda National Plant Protection Office (UNPPO) to better negotiate better quarantine requirements by overseas countries seeking to import her produce.

Identification of tephritid species by traditional morphological characters alone has been problematic and there have been several attempts at a generic revision. This is so despite the fact that accurate description of the fruit fly fauna is important for valid pest risk assessments and for deployment of control tactics that exploit chemical ecology of the flies. In addition, it is not clear

whether allopatric and host associated speciation has taken course among *B. invadens* populations in the different zones in Uganda, yet this would be important in the design of efficient and cost effective management strategies. If there is additional knowledge on phenotypic relationships and detailed morphological comparisons, changes to the taxonomic information will be needed to construct a more reliable identification key for the fruit flies.

Risk assessment of fruit fly species in Uganda requires knowledge of regions suitable for establishment of the species and if the effects of climate change are likely to change the species' distribution. There is presently no such knowledge to inform the risk assessment process in Uganda. This study will form a basis for predictions of the incidence of the pests and provide a basis for identifying target areas for intervention. This information will also provide insights into patterns of invasion by invasive Tephritidae species such as *Bactrocera invadens* and hence provide crucial information for their better management.

CHAPTER TWO: LITERATURE REVIEW

2.1 Classification, Taxonomy and Diversity

Fruit flies are dipterans of the family Tephritidae. Tephritid classification began as far back as 1850 by Loew, and has seen several revisions, especially with the advent of biomolecular techniques. In 1850, Loew subdivided the family into two subfamilies: Trypetinae and Dacinae. Later on, Tephritidae was subdivided into four subfamilies: Dacinae, Myopitinae, Trypetinae and Tephritinae. By 2003, there were 4,448 recognized species and subspecies of fruit flies worldwide, grouped in 484 genera, with species diversity greatest in the tropics (Norborm, 2004). The genera *Anastrepha, Bactrocera, Ceratitis, Dacus,* and *Rhagoletis* are the most important fruit pests (White and Elson-Harris, 1992). The Dacini (Trypetinae) tribe consisting of *Bactrocera, Ceratitis,* and *Dacus*, includes 40 genera and 1,000 described species (Norrbom *et al.,* 1998).

The *Ceratitis* genus consists of about 65 predominantly polyphagous species (White and Elson-Harris, 1992). Among these, *C. rosa* (Karsch), *Ceratitis cosyra* (Walker)*, C. flexuosa* (Walker), *C. fasciventris* (Bezzi)*, C. catoirii* (Guérin-Meneville), *C. anonae* (Graham), *C. punctata* (Wiedemann) and *C. capitata* (Wiedemann) are the major *pest* species across Africa (White and Elson-Harris, 1992; Lux *et al*., 2003. *C. rosa* and *Ceratitis cosyra* are broadly distributed across eastern, central, and western Africa, and also in parts of southern Africa while *C. fasciventris*, a close relative to *C. rosa* is distributed throughout central and western Africa (De Meyer *et al.,* 2002; Mwatawala *et al.*, 2006b; Rwomushana *et al.,* 2008). *C. fasciventris* and *C. rosa* are highly polyphagous, and were once considered the same species as they produce viable fertile crosses (De Meyer *et al.,* 2002). *C. rosa* is highly competitive and endures variable temperature ranges,

hence has potential to establish and displace *C. capitata* from cooler areas and hosts, respectively (Ekesi *et al.,* 2006). Another common species in western and central Africa is *Ceratitis anonae*. The latter species has also been frequently detected in Kenya, Uganda and Tanzania (White and Elson-Harris, 1992; De Meyer *et al.,* 2002; Nakasinga, 2002; Mwatawala *et al.*, 2006b; Rwomushana *et al.,* 2008).

Although the majority of the Tephritidae species originate from Africa, *Ceratitis capitata* (Wiedemann), *Ceratitis rosa (*Karsch) and *Dacus cilliatus (*Loew) have invaded other continents. Among these, *Ceratitis capitata* has invaded other tropical regions and has become perpetually established in Latin America, Hawaii, the Mediterranean Basin and Australia. However, recently, *C. cosyra* has become the most frequently intercepted African fruit fly in Europe and is already listed as a potentially invasive fruit fly of quarantine concern in the USA (Ekesi *et al.,* 2006).

Alien invasive species have also invaded Africa, among them the Asian species *Bactrocera cucurbitae* (Coquillett), *Bactrocera zonata* (Saunders) and more recently *Bactrocera invadens* Drew, Tsuruta and White (Lux *et al*., 2003; Drew *et al*., 2005). Since 2003, *Bactrocera invadens*, a fruit fly species attacking mangoes, citrus and other tropical fruits, has been spreading rapidly in East and Central Africa (Ekesi *et al.,* 2006). In Uganda, Nakasinga (2002), Nemeye (2005) and Okullokwany (2006) too reported *Bactrocera invadens* as the most important fruit fly in Uganda because of its effects and distribution.

2.2   Fruit Fly Life History

The life cycle of dacine fruit flies is characterized by high mobility, high fecundity and prolonged life span of adults (Fletcher, 1987). A generalized life cycle of fruit flies is shown in Figure 2.1. Gravid female fruit flies lay their eggs in the flesh of fruit. Larvae hatch from the eggs after about 42 hours (at 25ºC) and feed on the fleshy fruit, reaching the prepupal stage in approximately nine days. Bacterial decay associated with larval utilization of the fruit results in fruit fall. The pre-pupae emerge from the fruit and "hop", burrow and pupate into the top 2-3cm of the soil. Pupal growth occurs in the soil beneath the host tree and is accomplished in approximately 12 days at 25ºC (Fletcher, 1987).
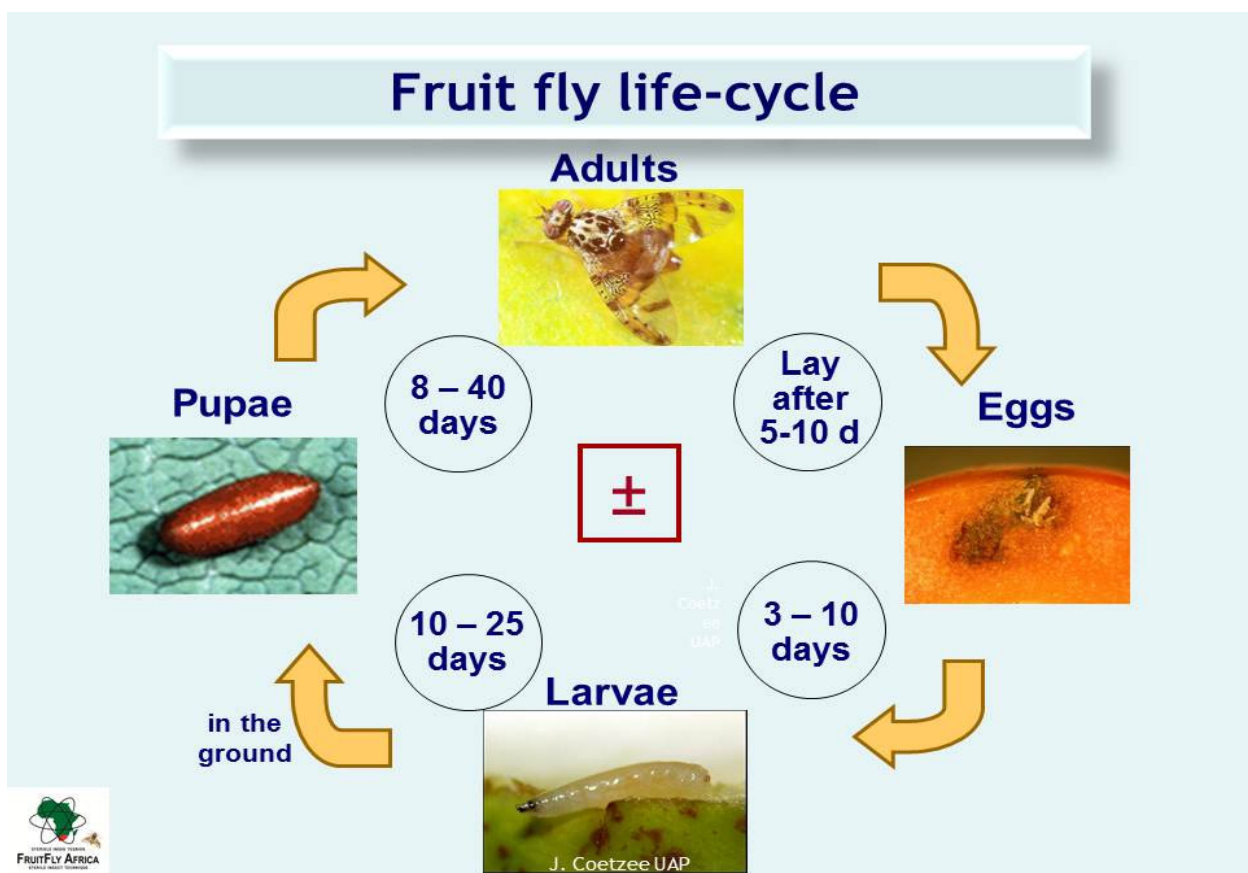
Figure 2. 1: Generalized life cycle of tephritid fruit flies (Adopted from the African Fruit Fly Initiative)

The teneral (emerging) adults emerge from the puparia and tunnel their way out of the soil and fly into foliage. Emerging adults have a natural tendency to disperse away from the emergence site to avoid density-dependent intraspecific competition (Fletcher, 1974). The adults live long, which increases chances of exposure to stressful environmental factors such as moisture, temperature and light (Andrewartha and Birch, 1984). To avoid such stressful conditions, adult flies pursue less stressful microhabitats very hot and moist or light intense environments (Meats, 1981).

Teneral adults require sugars for energy to sustain their highly mobile habit, protein to attain sexual maturity and lipids for egg production (Fletcher, 1987). Protein obligatory for egg development hence the attraction of female fruit flies to protein food sources (protein and yeasts hydrolysate). Such protein sources are available commercially for fruit fly monitoring and suppression when combined with other management tools. These resources are foraged in the adults' environment, although lipids are possibly synthesized from the beginning (Raghu, 2002). In addition, adults may also actively seek out certain plant-derived chemicals hypothesized to play a role in the mating behavior (Metcalf, 1990; Shelly, 2000; Raghu, 2002). Once sexual maturity is attained, adult flies forage for a mate and copulation ensues. The resultant gravid female then reinitiates the cycle above (Figure 2.1). Unlike females, males mate repeatedly in their lifetime (Mazomenos, 1989).

2.4   Host Utilization

2.4.1   **Host plant use among tephritid fruit flies**

Host use in frugivorous Dacinae is defined on the basis of the host plants female flies lay eggs in and that support larval development (Raghu, 2002). In this respect, most dacines are monophagous (predominantly utilizing a single host plant) or oligophagus (utilizing a group of closely related host plants) and the remaining (<1%) are truly polyphagous (Drew, 1989; Clarke *et al.,* 2002). Of all the species comprising the Tephritidae, about 1,400 species are known to develop in fruits. Out of these, about 250 species already are, or may become, pests by inflicting severe damage to fruits of economic value (White and Elson-Harris, 1992). The host plant is the principle centre for sexual behavior of phytophagous insects; males are reported to purposefully forage for host plants in search for females because females are likely to visit host plants for feeding and oviposition (Landolt and Phillips, 1997). Adult feeding, mating and oviposition of tephritid fruit flies are considered to have close evolutionary and ecological associations with their larval host plants (Metcalf, 1990).

Acquisition of chemicals from plants and their subsequent uses in a sexual role are known for some species of tephritid fly (Shelly and Villalobos, 1995).   For example, males of Mediterranean fruit fly were reported to form tight aggregations and feed on the bark of guava tree which contained high levels of the male attractant *α-copaene*, resulting in a mating advantage for the flies (Shelly and Villalobos, 1995).   The wild tobacco fly, *B. cacuminata* (Hering) is thought to have their primary mating sites on their larval host plants, wild tobacco (Drew et *al.,* 2008).   Many *Bactrocera* species respond to methyl eugenol, a naturally occurring phenylpropanoid found in many plants (Metcalf, 1990; Clarke *et al.,* 2002). Male flies of *B.*

dorsalis, which had eaten methyl eugenol (ME), were more successful in courting and mating females than males that had not eaten ME (Shelly, 2000).

Some fruit fly species show a distinct preference for certain hosts when they are available, but they will infest other hosts when the preferred hosts are unavailable (Fletcher and Prokopy, 1991). For instance, *B. jarvisi* preferred cocky apple, and it infests cocky apple almost exclusively when it is available, despite *B. jarvis* being recorded from many other fruits (Fitt, 1986). Among polyphagous flies of the *B. dorsalis* complex, different host plants may not be utilized equally (Clarke *et al.*, 2005). *Psidium guajava* is the host most utilized by fruit flies of the *B. dorsalis* complex, however, disproportionately large numbers of *B. dorsalis* and *B. papayae* were found to infest *Terminalia catappa* in field collections in Southeast Asia (Clarke *et al.,* 2001).

Among phytophagos insects, foraging for host plants is an active process involving recognition and selection of suitable substrates for food, mates, oviposition, and refuge (Raghu, 2002). Female fruit flies find and assess larval host plants through olfaction, vision and contact. The exercise involves physical cues (colour, size, shape sense of touch) and chemical cues (volatile, nutrition) of host plant (Raghu, 2002; Nufio *et al.,* 2004). In terms of colour, Prokopy and Reynolds (1998) indicated that the color yellow is a supernormal visual equivalent of plant foliage and is attractive to many phytophagous insects. For instance, *B. dorsalis* is attracted to a yellow colour which occurs in almost all their host fruit species (Vargas *et al.,* 1996), while yellow or orange spheres, which resemble the color of ripe wild tobacco host fruit, are most attractive *B. cacuminata* (Raghu, 2002). Host plant volatiles also play an important role in host

fruit orientation by female fruit flies (Raghu, 2002), but the response varies among species (Raghu, 2002). For instance, polyphagous species such *B. tryoni* and *B. dorsalis* may respond to a wide range of fruit volatile combinations, whereas monophagous species are likely to respond to a much more specific chemical group (Fletcher, 1987).

2.4.2 Tephritid Oviposition Choices and Host Quality

The oviposition choice of flies is often related with maximizing the performance of their offspring. In essence, this implies that female flies use information from host fruits to determine host fruit quality to optimize offspring growth and survival (Thompson, 1988). Post-alighting examination of fruit by tephritid female flies involves touching of the fruit surface with the antennae and mouthparts and probing the fruit skin with the ovipositor (Katsoyannos, 2001). Fruit firmness is considered to be a likely indicator of host quality, with harder pericaps generally less attractive for female flies (Rhagu, 2002).

Other than hunger (Prokopy and Reynolds, 1998), mating status influences the response of mature female flies to host fruit, with mated females more attracted to host fruit than unmated females (Prokopy and Reynolds, 1998). This pattern may be caused by the influence of egg load on females (Rhagu, 2002). Female flies carrying a large number of mature eggs in the ovaries tend to accept unsuitable host fruit than female flies carrying few mature eggs in ovaries (Prokopy and Reynolds, 1998). In addition, the prior experience of female flies with host fruit is also an important factor influencing host fruit acceptance (Prokopy and Reynolds, 1998). Female flies can also learn to select suitable host fruit after exposure to that fruit (Hawthorne, 1999).

Gravid female flies in natural settings may not always be able to find suitable host fruit for oviposition, which could be caused by failure to synchronize fruit maturation and time of female adult emergence or resistance of host plants (Aluja *et al.,* 2009). Under limiting conditions, female flies may use different strategies of host utilization (Rhagu, 2002). For instance, when only unripe fruit or fruit with high firmness are presented, females may use soft sites on the fruit, cracks or wounds and existing egg-laying cavities for oviposition (Shelly, 1999), to decrease aculeus wear and save oviposition time (Aluja and Mangan, 2008). Using existing egg-laying cavities or fruit wounds by female flies is considered an evolutionary response to phylogenetic constraints (Aluja and Mangan, 2008). In the same way, super parasitism (use of same fruit by more than one species) has been reported as an adaptive strategy in the field (Nufio and Papaj, 2004).

Although many host plants can sustain full development of tephritid fruit flies, variation in host quality plays a major role in larval survival rate, larval development and adult fecundity. Low fruit fly larval survival in host fruits may indicate the resistance of host plant to larval development, as well as inadequate nutritional composition (Hennessey and Schnell, 2001). Moreover, "unusual" host utilization of female flies such as super parasitism and oviposition into less suitable hosts causes pressure on larvae (Rhagu, 2002). High larval competition and poor nutritional quality of host fruit may often be experienced by fruit fly larvae in nature. In such cases, plasticity of host utilization behaviors found in female fruit flies may also be found in larvae. Little is known, however, about larval biology of most fruit fly species, especially larval behavior.

2.5 Geometric Morphometrics

Shape of structures on an organism is an outcome of genetics, nutritional status and environmental influences (Cardini et al., 2007). Shape not only refers to phenotypic outlines of the structure, but also includes elements of symmetry (i.e. proportionality) and alignment. Geometric morphometrics (GM) is a mathematical technique that measures and compares shape and can be used to study intraspecific variation, development, morphological integration, retro deformation, phylogeography and sexual dimorphism, geographic variation within species (Schutze *et al.,* 2012).

Applications of GM include studies on the ecogeographical and clinal variation in the skulls of the vervet monkey, *Cercopithecus aethiops* (Cardini et al., 2007), identification of hybrid zones and hybrid individuals in a group of genets (*Genetta spp.,* Carnivora, Mammalia) (Gaubert *et al.,* 2006), and shape change in the molar tooth crowns of the Paleocene-Eocene mammal Ectocion (Wood *et al.,* 1983). GM analysis has also been used to analyse uniparental *Aphytis* (Hymenoptera: Aphelinidae) reared from Chaff scale (Woolley *et al.,* 1987) or to compare *Aphytis* species in the lingnanensis group (Woolley *et al.,* 1994). In all these cases, significant discriminant phenotypic variations were recorded among the different species or groups. Among fruit flies, GM techniques have been used to identify *Bactrocera dorsalis* cryptic species (Adsavakulchai *et al.,* 1998; Schutze *et al.,* 2012), and bridging the morphological and biological species concepts to fruit flies (Drew *et al.,* 2008). The technique has also been applied to compare five allopatric fruit fly parasitoid populations (Hymenoptera: Braconidae) from coffee fields (Billah *et al.,* 2008). Geometric morphometrics has also been applied to study fluctuating

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asymmetry (FA): quantification of differences in shape asymmetry (Zelditch *et al.,* 2006). Measures of FA could serve as an indicator of fitness of genetic stress.

2.6   Fruit Fly Ecological Niche

Species distribution models relate recorded species occurrences to variables describing the environment to predict distributions over an entire area of interest (Guisan and Thuiller, 2005). Species distribution models have been used to assess the potential threat of pests or invasive species (Ungerer *et al.,* 1999), to obtain insights into the biology and biogeography of species (Steinbauer *et al.,* 2002), to identify hotspots of endangered species (Godown and Peterson, 2000) or predict biodiversity (Maes *et al.,* 2003), to prioritize areas for conservation (Chen and Peterson, 2002) and to establish suitable locations for species translocations or cultivation (Cunningham *et al.,* 2002). Notably, species distribution models are currently the only means to assess the potential magnitude of changes in the distributions of multiple species in response to climate change (Meynecke, 2004).

There are three broad categories of variables (factors) that determine the distribution of species: abiotic and biotic, and those that affect the ability of species to disperse or move (Soberon, 2007). Since the choice of independent variables for modelling is often driven by the availability of variables in a format suitable for modeling, several distribution-modelling exercises have considered only variables describing the abiotic environment, such as climate, edaphic factors and topography, or non-specific biotic variables, such as land cover, habitat and plant productivity. Nonetheless, climate and habitat variables have repeatedly been shown to be very good correlates of species distributions, especially among insects (Elith *et al.,* 2006) and many

have been hypothesized to have direct effects on species occurrence (Hawkins *et al.,* 2003). A meta-analysis of studies of species richness patterns found that climate variables were the strongest correlates of biodiversity in the vast majority of cases (Hawkins *et al.*, 2003), hence justifying the selection of climate variates in species niche modelling.

While criticisms have been leveled at bioclimatic models due to their exclusion of biotic interactions and dispersal scenarios, these models play a vital role in assessing potential distributions of species and are useful 'first filters' for identifying locations and species that may be most at risk from a changing climate (Pearson and Dawson, 2003). Ecological Modelling models often represent the most feasible method of examining potential distributions of species for a number of reasons. First, the cost of field surveys to assess species distributions can be prohibitive, especially if a large number of species is involved: bioclimatic models can be used to extrapolate habitat-specific information from one region to another to assess the likelihood of the presence of a species or multiple species.

Further, when little is known about the ecology and biology of a species, such models provide the only method of estimating current and future potential distributions. Most of the historical occurrence data derived from museums and herbaria records consists of presence-only data in form of GPS records. Such data cannot be easily examined using usual statistics techniques. However, they offer insightful outputs with bioclimatic models (Kadmon *et al.,* 2003).

Species distribution models can be used to predict how the distributions of species will change in the future as a result of climate and land-use changes. Such a model is built for the current time,

using contemporary species occurrence and climate data, and then projected to reflect predicted changes in the climate or land use in the future (Randin *et al.,* 2009). Model future projections can be used, for example, to predict where invasive species like *B. invadens* will be able to establish viable populations (Herborg *et al.*, 2009). This particular application is important in informing risk assessment due to fruit flies as it helps in assessment of regions suitable for establishment of fruit fly species and the potential effects of climate change on species' distribution. This also provides a basis for identifying target areas for intervention and offers timely insights into the invasion biology of the invasive Tephritidae species such as *Bactrocera invadens*.

The Bioclim model (Nix, 1986), which assumes that climate ultimately, restricts species distributions has found many applications in predicting the current or future distribution of species (Hijmans and Graham, 2006). The output of the model is a set of variables with biological significance to the species niche (Busby, 1988). These variables are interpolated across the geographical surface (at various scales) on the basis of longitude, latitude and elevation. The model thus generating a 'bioclimatic envelope' of the species can be used to identify the current potential distribution (all areas with climatic values within the species bioclimatic envelope) and assess whether these areas will remain climatically suitable under future climate scenarios (Chapter 7). Consequently, studies on potential responses of species to climate change have often employed bioclimatic model (Guisan and Zimmermann, 2000; Pearson and Dawson, 2003). A recent compilation of such studies on the effect of projected climate change indicated that an alarming number of species might lose a large part of their distribution range, hence the basis for Chapter 8.

CHAPTER THREE: SPECIES COMPOSITION AND COMMUNITY STRUCTURE OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) ACROSS MAJOR MANGO-GROWING REGIONS IN UGANDA*

ABSTRACT

This study assessed the species diversity and community structure of tephritid fruit flies across three major mango-growing regions in Uganda. Data were collected for two years (2010-2012) using fruit-bait and lure traps. A total of 368,332 specimens belonging to 10 species in four genera - *Bactrocera*, *Ceratitis, Trirhithrum* and *Dacus*- were collected. Of these, 98.9% belonged to *Bactrocera invadens*, while the second and third most common species (*Dacus bivittatus* and *Ceratitis anonae*) represented 0.4% and 0.3%, respectively. Significant differences in species richness and abundance of fruit flies occurred among the three regions but there was no significant difference in evenness and diversity of fruit flies across zones. Although generally similar (98.0%) in composition, there was significant discrimination (P < 0.000) by the fruit flies among the three regions. Analysis of community structure showed significant difference across the three zones (ANOSIM R = 0.095, P = 0.01). The Lake Victoria Crescent (LVC) was significantly different from Northern Moist Farmlands (NMF) (P = 0.003) in community structure, but not with Western Mid-altitude High Farmlands (WMHF). *C.rosa* contributed the highest difference in zonal structure, followed by *C. fasciventris* and *C. cosyra*. Rank-Abundance curves showed a geometric series distribution of the species composition of fruit flies in the three zones, depicting a scenario of competitive displacement of native fruit fly species by *Bactrocera invadens*. A comprehensive and sustainable response strategy to *B. invadens* and other fruit flies needs to be devised urgently to guard the benefits of the horticulture industry.

3.1   Introduction

Fruit flies of the dipteran family Tephritidae are key pests of several fruit crops including mango worldwide. The majority of species are highly polyphagous and their feeding results in substantial yield and quality losses (Ekesi *et al.,* 2006). In much of Africa, potential income from the production, processing and export of fruit is not realized because of production losses due to fruit fly infestation. Additionally, fruit flies are quarantine pests and thus constrain the contribution of the horticulture subsector to regional and international trade volumes (Ekesi *et al.,* 2006). Mango is a key horticultural crop that is either consumed directly or processed into juices, fruit concentrates and other value-added products (UIA, 2009). About 2 million tonnes of mango are produced annually in Africa but between 30-50% of this is lost due to fruit flies (Ekesi *et al.,* 2006). Where control measures are poorly or not implemented, like in much of sub-Saharan Africa, yield losses can exceed 80% (Ekesi *et al.,* 2006). It is thus imperative that fruit fly management strategies are implemented to realize potential incomes from trade in horticultural produce/products.

A cardinal requirement in the sustainable management of insect pests is correct identification of the pestiferous species, their distribution and seasonality (Jang *et al.,* 2003). Such information then guides the selection and implementation of appropriate pest control tactics. There are a number of tactics for management of fruit flies (McInnis, 2003), but the male annihilation technique (MAT) using species-specific lures is one of the most widely applied and most effective (Jang *et al.,* 2003). To support the development of a robust fruit fly management programme in Uganda to complement efforts in other countries of East Africa, it is prudent that the fruit fly fauna in Uganda is described.

In East Africa, specifically Kenya and Tanzania, the fruit fly fauna has been well described (Mwatawala *et al.,* 2006a, 2006b; Rwomushana *et al.*, 2008; Mwatawala *et al.,* 2009a; Geurts *et al.,* 2012). However, for Uganda only two studies (Nakasinga, 2002, Okullokwany, 2006) attempted to describe the fruit fly fauna in the country. The genus *Ceratitis* (Macleay) is endemic and *Bactrocera* species are known to occur, having invaded Uganda following their introduction into East Africa from Asia sometime in the 1990s (Drew, Tsuruta, and White, 2005).

This study thus set out to:

(i)   Identify the fruit fly species present in Uganda, and

(ii)   Assess the fruit fly community structure across three agro ecological zones that are key mango production areas.

3.2   Materials and Methods

3.2.1   Study area

The study was conducted in three agro ecological zones: Western Medium High Altitude Farmlands (WMHF), Lake Victoria Crescent (LVC) and Northern Moist Farmlands (NMF) (Wortman and Eledu, 1993). Selection of these regions was purposive to target sites that have fruit flies and areas of relatively higher fruit productivity. The three zones represent the major fruit growing regions in Uganda (Figure 3.1).

Figure 3. 1: Location of the three Agro ecological zones and sampling sites in Uganda

3.2.2 Description of the AEZs

Lake Victoria Crescent: Encompasses areas along Lake Victoria that range in altitude from 1,100 to 1,400 meters above sea-level (masl). Temperatures are relatively stable and range between 18 to 28 ^{o}C. Rainfall is bimodal (rainy seasons in March-May and October -December), is evenly distributed and ranges between 700-2100mm annually. Banana and coffee are main crops that are often intercropped with annual and biennial root crops (sweet potato, cassava), vegetables and fruits. Crops are commonly grown as polycultures on plots less than one hectare.

Northern Moist Farmlands: This area is in northern Uganda between latitudes 32.082 and 33.353. It ranges in altitude between 1,000 -1,524 masl, with a temperature range of 15°C – 32.5^{o}C. Rainfall is largely unimodal (rainy seasons in April to September) and ranges between

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700-1700mm annually. Annual crops, mainly cotton, cereals, dominate the area and cassava that are usually grown as monocultures. Many of the domesticated fruit fly host species present in the other two zones are not cultivated or are less frequent in the NMF. Where orchards and areas of substantial fruit growing is done, the areas are not surrounded by reasonable fruit habitats that would serve as important refuges and source areas for alternative hosts for fruit flies during fruit off season.

Western Medium Altitude High Farmlands: This zone, located in western Uganda, along the Congo border has an average altitude of 1,235 masl with a range of 600-4,500 masl. Rainfall is bimodal (rainy seasons in March-May and August–November) with some parts of the area, especially in the mountain areas, receiving more than 2,250 mm as mean annual rainfall while the low lands receive about 1,200 mm as mean annual rainfall. Mean temperature is 22.5^{o} C. The main crops grown are bananas, coffee, beans, maize and a variety of vegetables, fruit and root crops. Bananas, coffee and cotton are the main commercial crops.

3.2.3   Sampling Procedure

In each agro ecological zone (AEZ), three orchards or areas with substantial mango growing and other potential and actual hosts were selected as sampling sites. A trapping programme using species-specific lures was conducted over a period of 24 months (September 2010-September 2012) across the three AEZs. The annual trapping period was divided into six cycles, each with two months corresponding to lure changing dates. The set up of the experiment followed a split-split-plot design where the cycles were the main plot, attractants the sub-plot and AEZ the sub-sub plot (Mwatawala *et al.,* 2009a).

Trapping focused on fruit-infesting species, mainly Ceratitidinae fruit flies (such as *Ceratitis* and *Trirhithrum*) and the Dacinae fauna (such as *Bactrocera* spp). Since specific species are attracted to different lures, a variety of lures – Torula yeast, Trimed lure, Methyl Eugenol, Cue lure, and Terpenyl acetate – were used at different sites so as to obtain a more comprehensive indication of the fruit fly diversity in the three-agro ecological zones. Trimedlure was used to attract members of the genus *Ceratitis*, sub-genera *Ceratitis* and *Pterandus*. Methyl eugenol was used to attract members of the genus *Ceratitis* subgenus *Pardalaspis*, as well as the invasive *B. invadens*. Cue Lure was used to attract members of *Dacus* and *Bactrocera cucurbitae*. Torula yeast protein bait, which is less specific than the other lures, was also used to attract other fruit flies. In each orchard three trees were randomly selected. On each tree, four traps, each fitted with one lure type, were mounted. An insecticide strip of dichlorovos was placed in each trap to kill the adult flies. All traps were suspended at a height of approximately 2 m. The traps were emptied on a fortnight basis and the lures renewed bi-monthly. The protein bait was replaced once a week. Trapped fruit flies were placed into vials containing 90% alcohol for preservation, labelled and transported to the National Agricultural Research Laboratories for identification and counting.

3.2.4   Fruit Fly Species Identification

Different types of keys were used: recent systematic revisions for *Ceratitis* and *Trirhithrum* that were also incorporated in an unpublished digital multi-entry key. For *Dacus* taxa, confirmation of the identification was done at the Royal Museum of Central Africa under the guidance of Dr. De Meyer. Voucher specimens were kept in collections at the National Agricultural Research Laboratories, Uganda and Makerere University Zoology Museum and the Royal Museum for Central Africa, Belgium.

3.2.5 Data Analysis

Data from the four lure traps and the protein bait were pooled to obtain total fruit fly diversity per study site per month. For each agro ecological zone, species richness and abundance were calculated using the pooled data. All variables were tested for normality using Shapiro-Wilk test and the strongly skewed variables were transformed prior to analyses if necessary to meet the assumption of normality and homogeneity of variances. Variables expressed as percentages (%) were arcsine-square-root (+0.5) transformed, while the number of species or counts of individuals were log ($\log(x+1)$) transformed. Where transformation was not sufficient to improve data shape, an appropriate non-parametric test was applied. Biodiversity R (Biodiversity R, 2009) and PAST (Hammer *et al.,* 2002) were used to calculate diversity indices: species richness, information indices, evenness and dominance. Species accumulation curves were obtained for each zone. The Bray-Curtis similarity index (Hammer *et al.,* 2002) was used to compare similarities among zones and to construct a species composition similarity dendrogram for the three zones. Beta diversity for the three zones was computed using Whittaker, Cody and Wilson-Shmida's beta diversity estimators. The differences in fruit fly individuals and species, evenness and diversity among the three zones were tested with General Linear Model (GLM) analysis of variance (ANOVA) in R. Where the GLM test indicated significant differences, post-hoc Tukey (HSD) test was used.

Discriminant Analysis (DA) using ADE4 software (Thioulouse *et al.,* 1997) was used to determine whether zones differed significantly in species patterns and to visualize the zones on a 2-dimensional map (Thioulouse *et al.,* 1997). Subsequently, community structure was assessed using Analysis of similarities (ANOSIM) for test of significant difference between the three

zones. Pair-wise post-hoc ANOSIMs between all pairs of zones and overall multi-group were computed using Bray-Curtis Similarity Percentage (SIMPER) (Hammer *et al.,* 2002). To assess species displacement levels, the abundance (Log_{10}) was plotted against species ranks and tested for conformity to the theoretical distribution curves using the Chi-Square test.

3.3   Results

3.3.1   Fruit Fly community composition across the AEZs

The flat species accumulation curves (Figure 3.2 A and B) show that sampling effort in the three zones adequately recovered most fruit fly species. Over the 24 months sampling period, 368,332 individuals were collected. For all three zones, mean monthly trap captures were very high (> 5,000 individuals). The individuals belonged to 10 species in four genera: *Bactrocera*, *Ceratitis, Trirhithrum* and *Dacus.* The maximum species estimated by Chao, Jacknife and Bootstrap richness estimators for the whole study area was 11 species. *B. invadens* accounted for 98.9% of the collection, while the second and third most common species (*Dacus bivittatus* and *Ceratitis anonae*) represented 0.4% and 0.3%, respectively. The remaining fruit flies constituted less than 0.4% of the total catch.
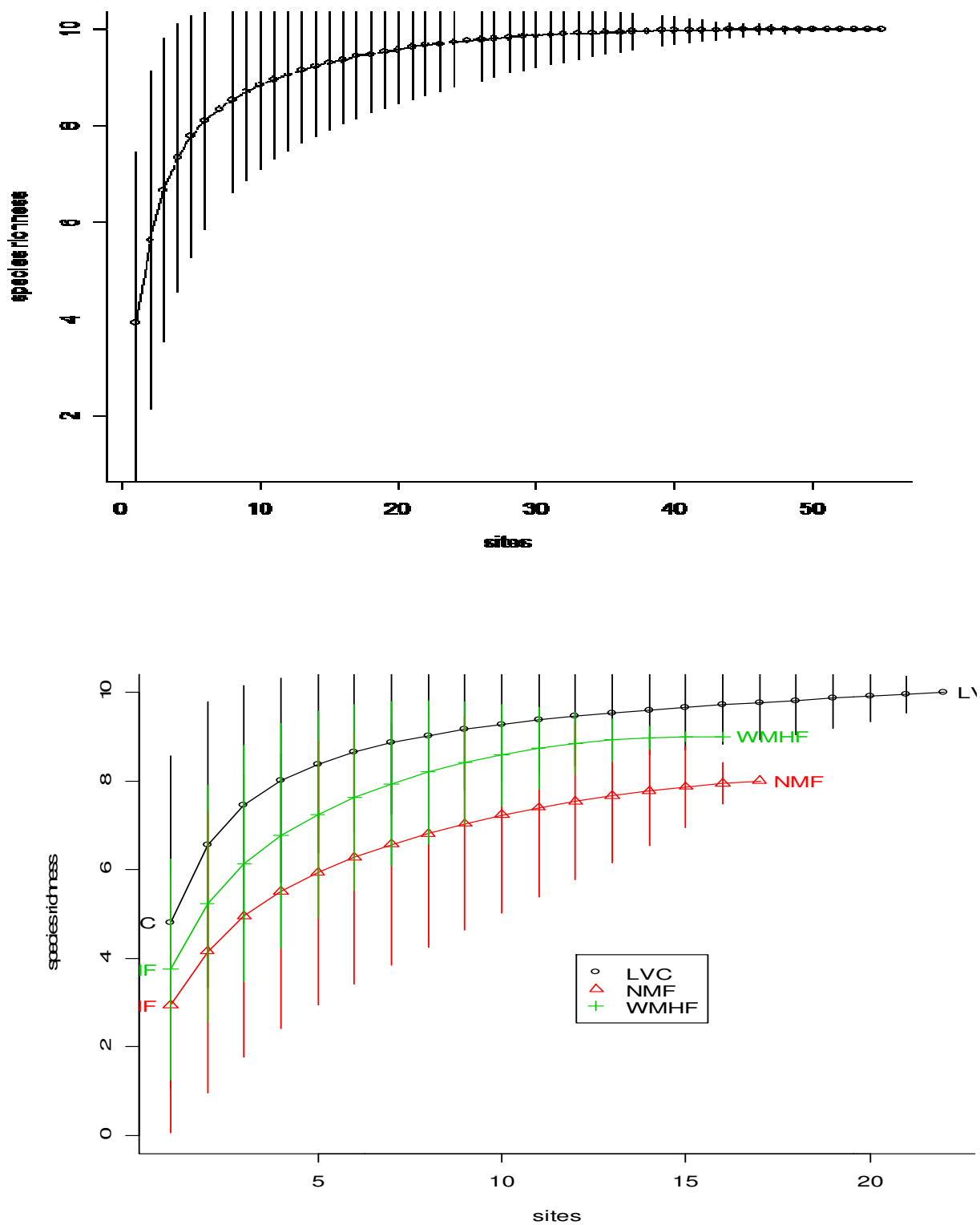
Figure 3. 2: Species accumulation for all samples (A) and for the three Agro ecological zones (B). The bars on the accumulation curves indicate +2 and -2 standard deviations.

The Rank-Abundance curve indicated that *B. invadens* was the most abundant species in each of the three zones (Figure 3.3). The next most abundant was *D. bivittatus* followed by *C. anonae* for LVC and WMHF, while for the NMF, *C. anonae* and *C. capitata* followed in that order. Beta diversity between zones (between-habitats diversity) was relatively high: 1.5463, 80.5 and 20.498, respectively. However, within a given zone, there was low beta diversity as fruit fly diversity was less differentiated among orchards or places of sampling.

The LVC was the most species rich (10 species) while the NMF was the least species rich (8) (Table 3.1).The Bootstrap estimation of richness per zone estimated 9-10 species for the NMF and WMHF and for the LVC, 10 species. *B. invadens* was the most dominant spp. in each one of the three zones while the second and third most dominant species was variable among the zones (Table 3.1). In the LVC, the second and third most abundant species were *D. bivittatus* and *D. ciliates*, in the NMF they were *C. anonae* and *C. capitata*, and in the WMHF, *D. bivittatus* and *C. anonae*, respectively. Species richness for the LVC was significantly different (P<0.01) from the other two whose difference in richness was not statistically significant. Except for *C. anonae,* all other *Ceratitis* species are comparatively very low in numbers. *Dacus* species were nearly non-existent in the NMF.
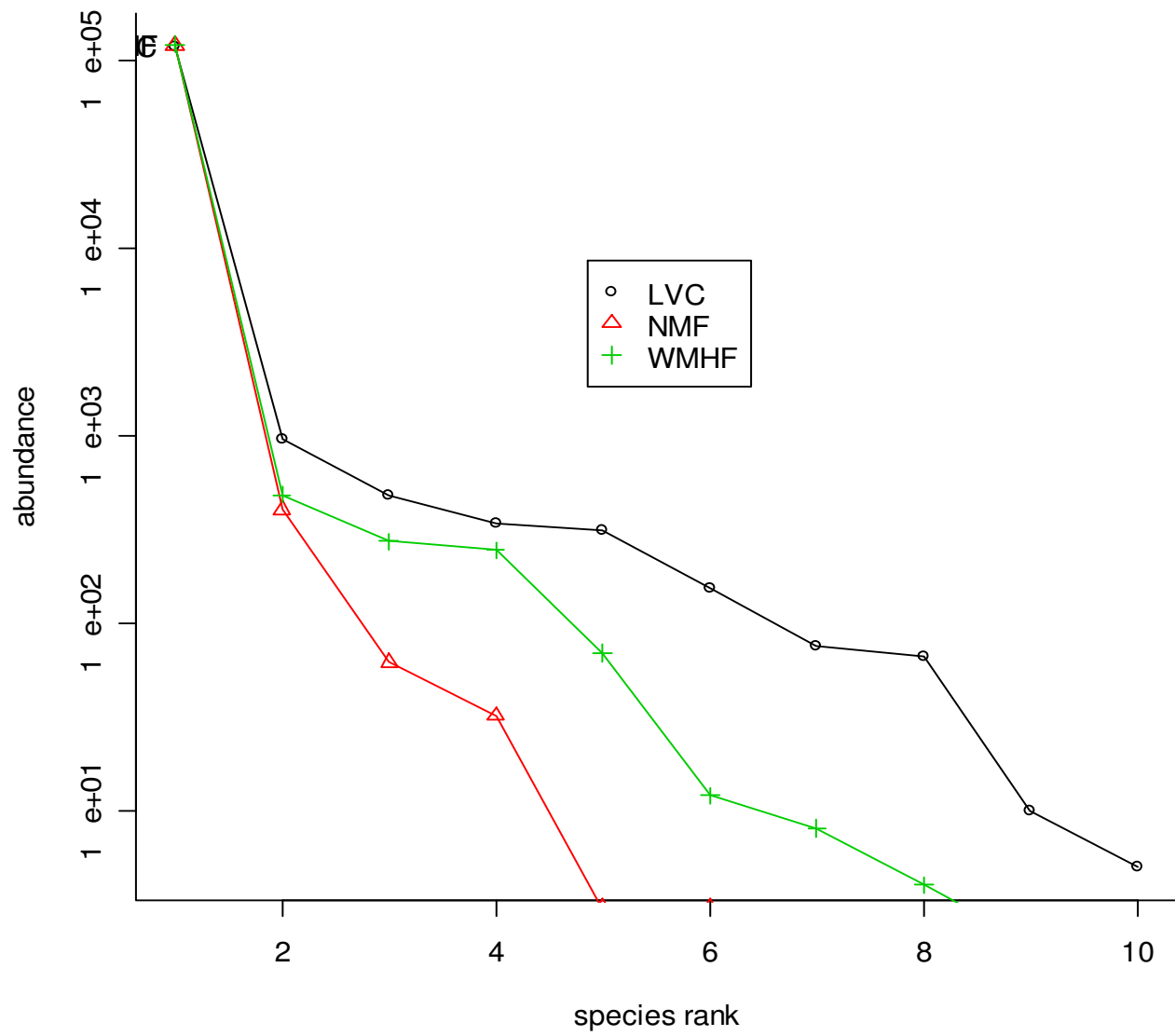
Figure 3. 3: Rank-Abundance (Loglinear Scale) curves for fruit fly species across in each zone: Lake Victoria Crescent (LVC), Northern Moist Farmlands (NMF) and Western Mid Altitude High Farmlands (WMHF).

Table 3. 1: Numbers of fruit flies species collected at the different sites in three different agro ecological zones in Uganda.

	Total individuals trapped Per					
Fruit fly species	**Zone**			**Mean Monthly Capture Per Zone**		
	LVC	NMF	WMHF	LVC	NMF	WMHF
Bactrocera invadens	119,245	122,112	122,982	5,420	7,183	7,686
B. cucurbitae	152	3	4	7	0	0
C. anonae	339	401	272	15	24	17
C. cosyra	75	3	2	3	0	0
C. capitata	66	62	69	3	4	4
C. fasciventris	5	0	12	0	0	1
C. rosa	10	0	0	0	0	0
Trirhithrum coffeae	312	32	8	14	2	1
Dacus bivittatus	956	2	485	43	0	30
D. cilliatus	478	1	244	22	0	15
Total individuals	**121,638**	**122,616**	**124,078**			
Number of Species	**10**	**8**	**9**			

Fruit fly catches per month varied significantly across the three zones (P = 0.0001), with the highest being in WMHF (7,755 individuals per month) and the least recorded in LVC (5,529 individuals per month). Lake Victoria Crescent zone had the most diverse fruit fly population (H' = 0.129), followed by WMHF (H' = 0.062), and the least was in the NMF (H' = 0.030) ($X^2_{3,}$ = 0.828, P > 0.05). The NMF had the highest evenness of fruit flies (12.9%) > WMHF (11.8%)

>LVC (11.4%), although the difference was not significant. Equitability was low (Fig 3.4) across the zones because of the dominance of *B. invadens* in all of them.
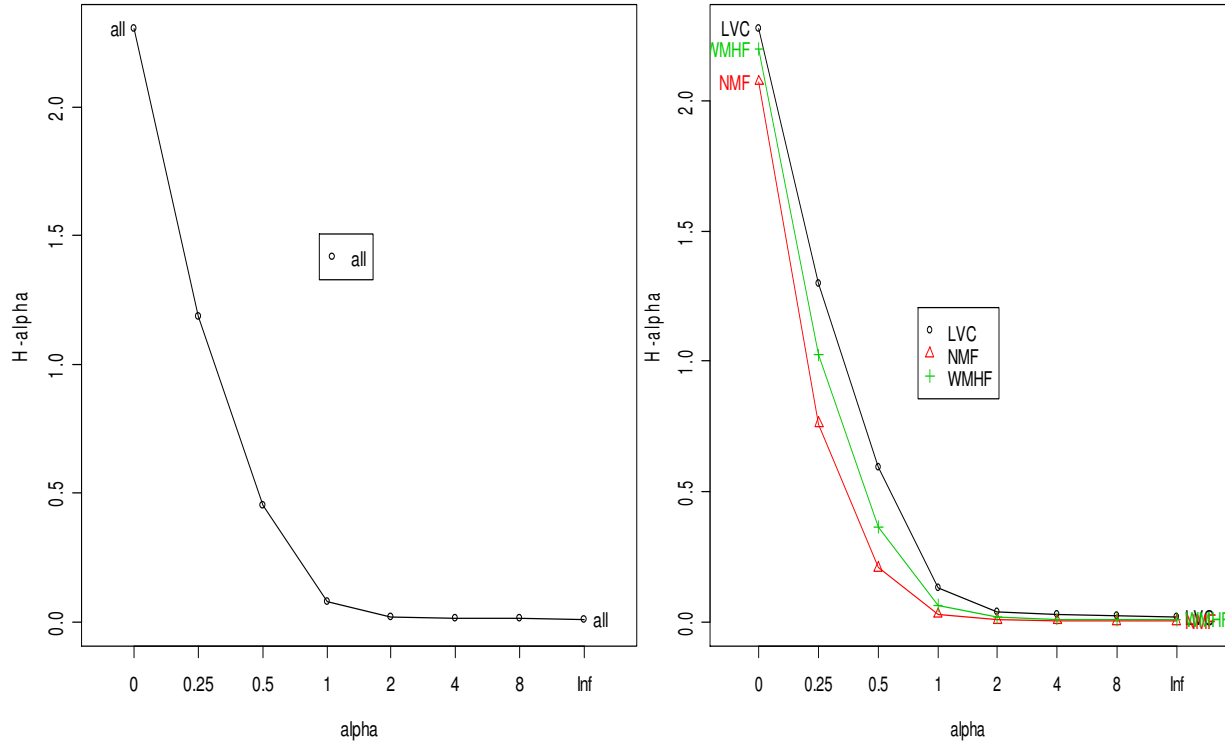
Figure 3. 4 Species Renyi's Equitability Curves for all, and individual zones: Lake Victoria Crescent (LVC), Northern Moist Farmlands (NMF) and Western Mid Altitude High Farmlands (WMHF).

3.3.2   Community Structure across Agro Ecological Zones

The agro ecological zones were generally similar in composition with respect to fruit fly species. The Bray-Curtis similarity index generated two clusters — one for the LVC and the second cluster comprised both the NMF and WMHF, with an overall cophenation correlation or cluster accuracy of 99.99%.   The clustering, based on relative abundance and similarity in monthly incidence, consisted of group one: *T. coffeae*, *C. cosyra, B. cucurbitae, C. rosa, D. bivittatus* and *D. cilliatus,* and two: *C. capitata, B. invadens*, *C. anonae* and *C. fasciventris*, with the latter in an individual cluster (Figure 3.5).
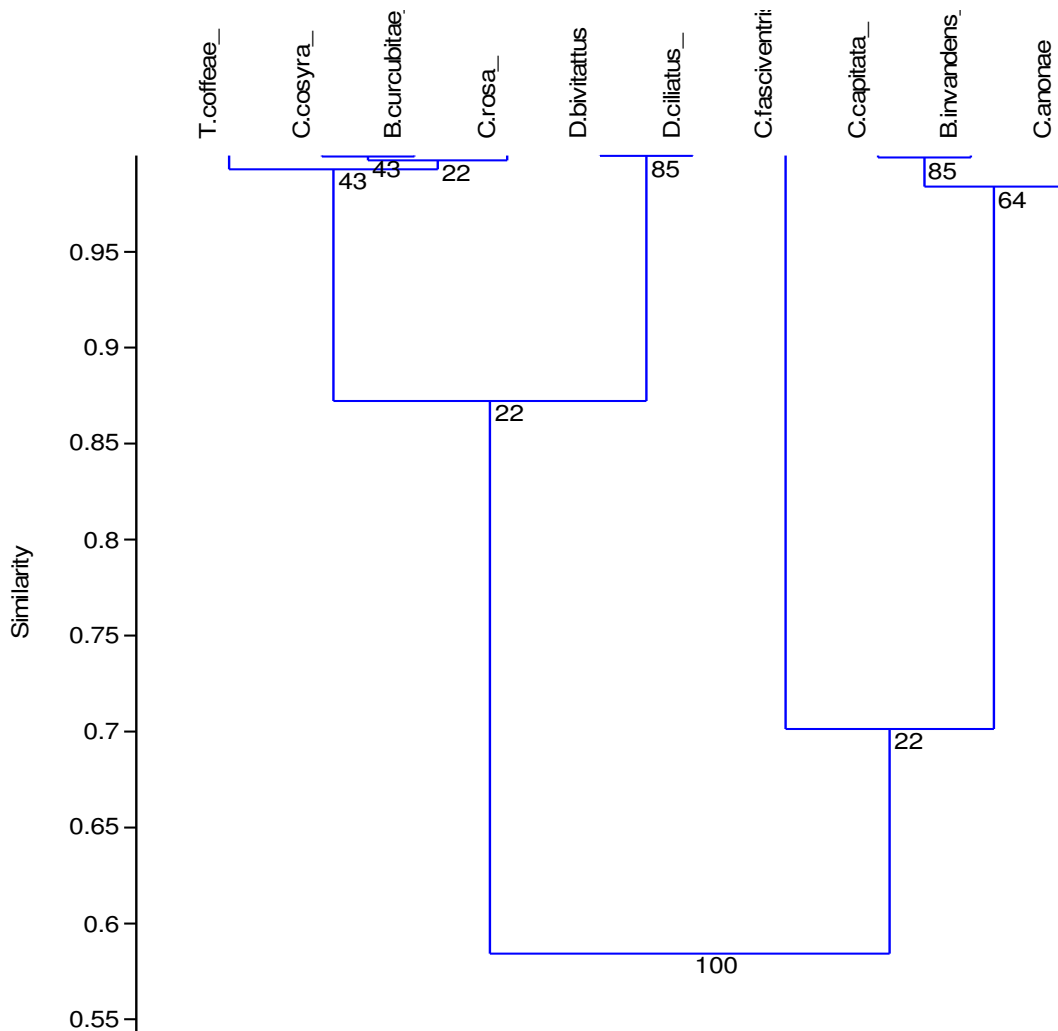
Figure 3. 5 Bray-Curtis Similarities in Fruit fly composition among the three agro ecological zones and Species Communities in the study.

On the basis of relative dominance and catches (composition), there was significant discrimination of the 3 AEZs (discrimination sensitivity = 69.15, P < 0.001). There was a significant association (P < 0.001) between fruit fly species and AEZs (Figure 3.6).
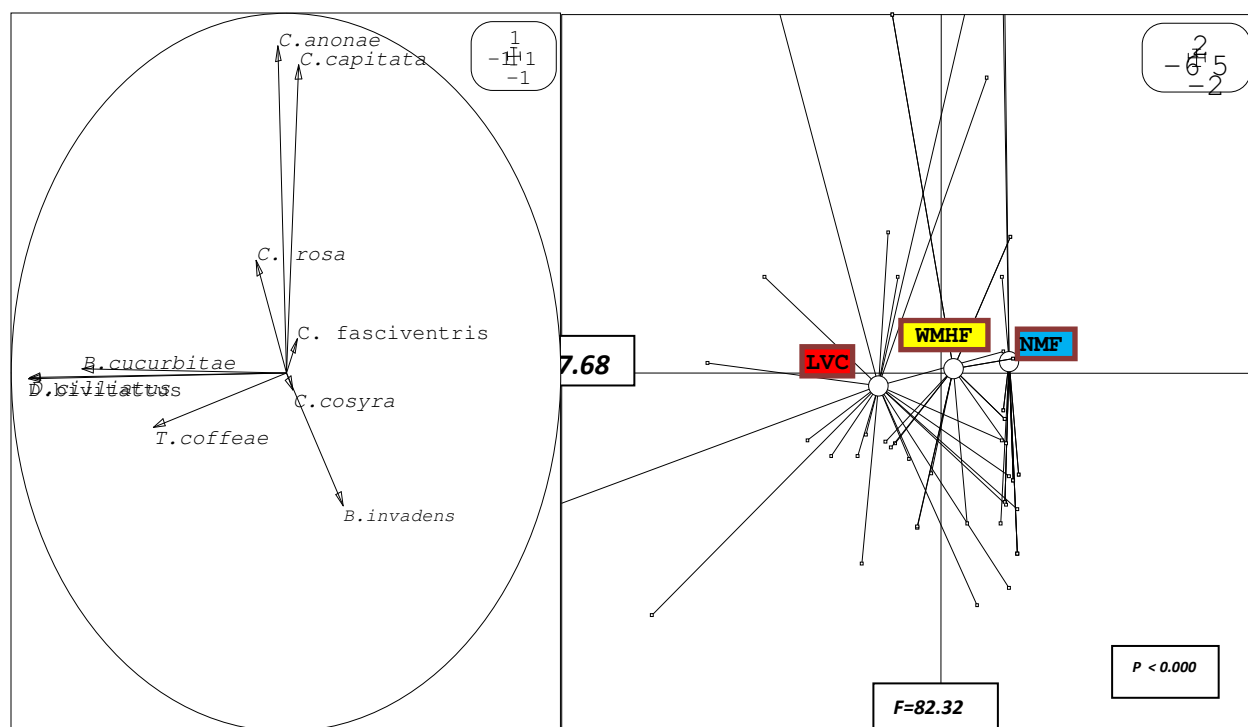
Figure 3. 6: Discriminant Analysis (DA) ordination graphs for the agro ecological zones and fruit fly diversity. The zones are: Lake Victoria Crescent (LVC), Northern Moist Farmlands (NMF) and Western Mid-Altitude High Farmlands (WMHF) based on Fruit fly abundance.

The Factor loadings and average means of associations between AEZs and the various fruit fly species are shown in Table 3.2. The species *D. ciliates*, *T. coffeae*, *D. bivittatus* and *B. cucurbitae* were more associated with the LVC than with the other two zones. The WMHF and NMF zones were not particularly affiliated with any of the rest of the species. *B. invadens* was also not particularly affiliated with any AEZ or any of the other fruit fly species. Although the WMHF recorded relatively more FAR complex species (*C. fasciventris, C. anonae and C. rosa*) and *C. capitata*, there was no significant association of these species with a particular zone. The squared cosines score showed that Factor 1 was significantly loaded with LVC and NMF with coefficients of determination of 66.7 and 60.7%, respectively. Similarly Factor 1 differentiated LVC and NMF mainly on the basis of *T. coffeae* (67.6%), *B. cucurbitae* (27.6%), *D. bivittatus*

(59.4%), and *D. cilliatus* (59.3%). On the contrary, Factor 2 was significantly loaded with WMHF with a coefficient of determination of 23.2%. Though relatively less, the component also was associated with *D. bivittatus* (40.6%), and *D. cilliatus* (40.7%), confirming that these two are generalist species just like *B. invadens*. *C. anonae* and *C. Capitata* always occurred in similar proportions in zone but appeared to be less in sites with higher *B. invadens* composition.

Multivariate analysis of community structure among the three zones showed significant zonal differences (ANOSIM, R = 0.095, P = 0.01). Pair-wise post-hoc comparisons between all pairs of zones showed that LVC was significantly different from NMF (P = 0.003), but not with WMHF. An overall multi-group Bray-Curtis Similarity Percentage (SIMPER) assessment of the species primarily responsible for the observed difference among zones, showed a 54.7% average dissimilarity among zones. *C. rosa* contributed the highest difference in zonal structure, followed by *C. fasciventris* and *C. cosyra* (Table 3.2).

Table 3. 2: Discriminant Component Loadings showing barycenter (weighted average) of the three zones, Fruit fly distribution (weighted averages of site scores) and Squared Cosines of the zones and Site Scores in the two most important components.

Zone/ Species	Component loadings F1	Component loadings F2	Cosines (R2) (F1)	Cosines (R2) (F2)
LVC	-0.817	0.106	0.667	0.011
NMF	0.779	0.316	0.607	0.1
WMHF	0.296	0.482	0.088	0.232
B. invadens	-0.042	-0.097	0.002	0.009
C. anonae	-0.016	0.006	0	0
C. cosyra	0.046	0.02	0.002	0
C. capitata	0.114	-0.084	0.013	0.007
C. fasciventris	0.084	0.128	0.007	0.016
C .rosa	-0.05	-0.032	0.003	0.001
T. coffeae	-0.822	0.569	0.676	0.324
B. cucurbitae	-0.525	-0.181	0.276	0.033
D. bivittatus	-0.771	-0.637	0.594	0.406
D. cilliatus	-0.77	-0.638	0.593	0.407

3.3.3   Speculated displacement of indigenous Fruit fly Diversity by *B. invadens*

Figure 3.7 below shows the Rank-Abundance curves depicting the geometric series distribution of the species composition of fruit flies in the three zones. *B. invadens* had a dominance of 98.9% over the rest of the fruit fly fauna.
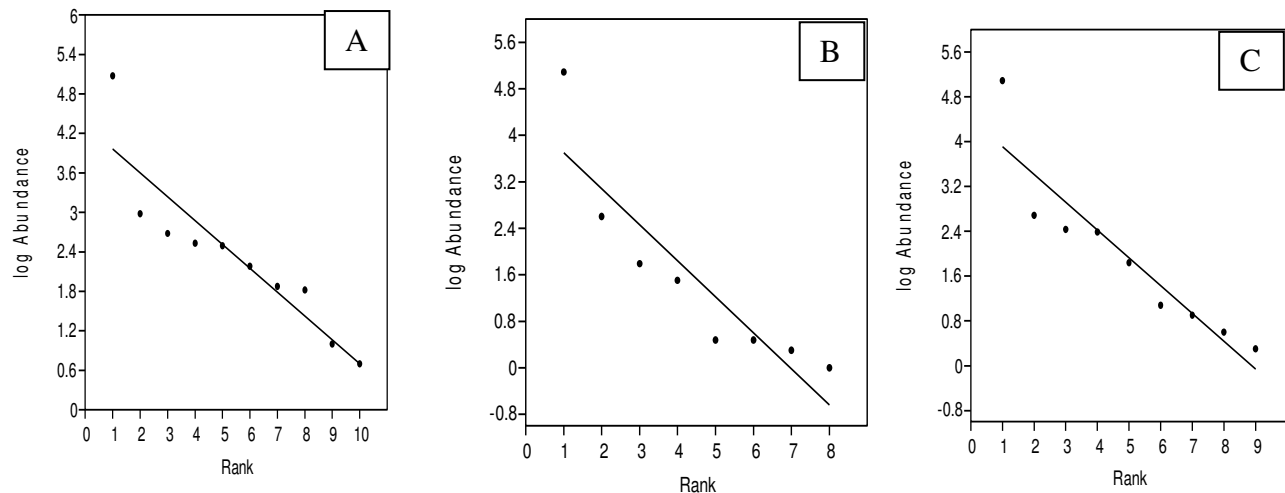
Figure 3. 7 Rank-Log Abundance graphs showing the conformity to the Geometric Series Distribution of Fruit Flies in the three zones: A: Lake Victoria Crescent (LVC), B: Northern Moist Farmlands (NMF) and C: Western Mid Altitude High Farmlands (WMHF).

3.4 Discussion

3.4.1 Community composition and structure of Fruit fly Species in Uganda

This study presents the first comprehensive record of the tephritid fauna for Uganda. Similar to Kenya and Tanzania, the fruit flies in Uganda belong to four genera: *Bactrocera*, *Ceratitis* *Trirhithrum* and *Dacus*. Except for *Bactrocera* species, all the other eight species are believed to be native to the country. Previous studies (Nakansinga, 2002) indicated *Ceratitis* spp. as the most important fruit fly in mango but it is evident that other genera are also important. The study also reveals that all regions have very high tephritid infestation levels, largely dominated by *B. invadens*. If this species is excluded from the counts then the NMF zone has lower infestation levels than the other two zones. These differences may be linked to the agro ecological characteristics of the zones, specifically the climate and farming systems.

The NMF has fewer alternative hosts for fruit flies, which explains why it also had lower species richness and up to four other species were represented by less than 10 individuals in the year-long trapping programme. The results on community structure suggest that the pest status of each genus may differ across geographical regions. This may perhaps be explained by differences in host preference for each genus although this study was unable to investigate this during the course of the study. For instance, the majority of the species of the genus *Bactrocera, Ceratitis* and *Dacus* encountered in this study are well-known economic pests attacking a broad diversity of cucurbit fruits (Mwatawala *et al*., 2006a; Rwomushana *et al*., 2008). Therefore, the dominant representation by genus *Dacus* in LVC and WMHF is likely due to abundance of cucurbit hosts in these zones. The conspicuously low incidence of *B. cucurbitae* in the WMHF could be due to the high altitudes in the zone, which elsewhere have been demonstrated to inhibit *Bactrocera*

colonization (Geurts *et al.,* 2012). Cucurbits are not a common crop in the NMF. Given the dominance of coffee growing in the area, it explains the higher association of the zone with *T. coffeae* species, whose main host is coffee (Mwatawala *et al*., 2006a). The infrequent encounter of most *Ceratitis* species (save for *C. capitata*) that have been hitherto reported (Nakasinga, 2002), is likely due to the narrow nature of their host range among indigenous fruits (Ekesi *et al.,* 2006) or due to continued displacement by *B. invadens* (Mwatawala *et al*., 2006a; Rwomushana *et al*., 2008).

The relatively high between-habitats diversity (beta (β) diversity) observed in this study was probably a result of both environmental heterogeneity, species niche differentiation and dispersal limitation processes (Nekola and White, 1999). Mobility and dispersal processes are important determinants for beta-diversity at small and intermediate scales (Soberon, 2007). Longer distances between agro ecological zones than between respective orchards might increase dispersal limitation and, therefore, lead to different species pool adapted to local conditions (Chen and Peterson, 2002). The distance between orchards and sampling points within an orchard was more or less constant within a zone, although it increased between agro ecological zones. The large β-diversity at the macro scale compared with the meso- and micro scale concurs with the distance decay of similarity hypothesis (Nekola and White, 1999). This study has added to the evidence that insect community composition varies most significantly over broader spatial scales, even when total species richness does not (Summerville *et al.,* 2003, 2006).

Zones differed in farming systems, which could ultimately affect β-diversity and relative abundance of species. The intensive banana-coffee farming system characteristic of the LVC

harbors a range of food and cash crops (Munyuli, 2012). In addition, the bimodal nature of rainfall favors all year fruiting of alternative hosts. Due to the polyphagous nature of most of the species recorded, it is not surprising that the LVC recorded significantly higher (10 species) diversity, compared to NMF and WMHF. Munyuli (2012) too recorded higher diversities of butter flies under heterogeneous landscapes and farming systems in Central Uganda. NMF has lower and less evenly distributed rainfall in the region and infrequent fruit hosts; hence low diversity (Tscharntke *et al*., 2005).

3.4.2 *B. invadens* dominance over indigenous fruit flies

Results in this study showed conformity of the curves to geometric series, indicative of communities of highly uneven species-abundance distribution and low diversity characterized by a few dominant species (*B. invadens*). The dominance by *Bactrocera* genera observed here indicates displacement of other species by *B. invadens,* already reported in Kenya and Tanzania (Mwatawala *et al.,* 2004; Mwatawala *et al.,* 2006a, 2006b; Ekesi *et al*., 2006). *B. invadens* dominance in horticulture monocultures has also been demonstrated in West Africa (Mboyine *et al.,* 2012). *B. invadens* was reported to be displacing indigenous *Ceratitis* species of mango, constituting up to 98 and 97% of the total population in Kenya (Ekesi *et al*, 2006) and Tanzania (Mwatawala *et al*., 2006a), respectively. Indeed the species showed no significant community discrimination for any particular zone, although the relative abundance varied. In particular, results have shown that the distribution pattern fits the theoretical geometric distribution series as proposed by Motomura (1932). The polyphagous nature, dominance in certain hosts, and rapid spread make *B. invadens* a devastating pest in Africa (Drew *et al.,* 2005). *B. invadens* displacement of other species has been attributed to competition (Ekesi *et al*., 2006), and annual

and within-habitat spatial distribution activity (Mwatawala *et al.,* 2004; Drew *et al.,* 2005; Mwatawala *et al.,* 2006a, b; Rwomushana *et al*., 2008; Mboyine *et al.,* 2012; Geurts *et al.,* 2012).

Displacement of native fruit fly species by *B. invadens* has also been attributed to a resource clumping effect, in which increased interspecific competition and displacement increases with increased resource clamping (Kiesecker *et al.,* 2001). In this study, *B. invadens* dominance and pressure was highest (99.6%) in the NMF, followed by WMHF (99.1%) and least in LVC (98.1%). Although the difference is not significant, the trend in displacement can be attributed to differences in resource clumping. The LVC farming system offers scattered and diverse fruit opportunities that avoids resources clumping, unlike in the NMF where fruits only occur in isolated orchards surrounded by expansive savanna grasslands. More work will be needed to assess the general importance of landscape level distribution of resources on the effect of the African invader on native species.

3.5.0   Conclusions

There are at least ten tephritid fruit fly species in Uganda but *B. invadens* is the most widely distributed. This species is also apparently displacing the native fruit fly fauna. The agro ecological zones were significantly differentied by their faunal relative composition, which may be in turn attributed to the inherent environmental and host plant composition. There were significant differences in species richness and abundance of fruit flies among the three zones but not in evenness and diversity of fruit flies across zones. Since *B. invadens* has devastating economic impacts on the horticulture industry, and particularly mango (Lux *et al.,* 2003; Ekesi *et al.,* 2006) the study proposes that an integrated fruit fly management programme is implemented

urgently to contain the very high infestation levels. Such a programme should also contain infestations by the other fruit fly species and thereby guard the benefits of the horticulture industry.

CHAPTER FOUR: FRUIT FLY (DIPTERA: TEPHRITIDAE) HOST RANGE AND RELATIVE INFESTATION OF SELECTED MANGO VARIETIES IN THREE AGRO ECOLOGICAL ZONES, UGANDA*

ABSTRACT

Despite the economic importance of fruit flies, there is paucity of knowledge on fruit fly host status in Uganda. Previous studies have been conducted in the Kenya and Tanzania under contrasting agro ecological conditions and land use patterns, which potentially could lead to differences in infestation of the different fruit host, and ultimately affect management options. Therefore, this study set out to profile the host range of the main fruit fly pests in the three main mango agro ecological zones; and determine the susceptibility of selected fruits and mango varieties grown. A wide range of fruits was sampled across three agro ecological zones: Western Medium High Altitude Farmlands (WMHF), Lake Victoria Crescent (LVC) and Northern Moist Farmlands (NMF) and reared at the National Agricultural Research Laboratories following standard protocols. Emerging fruit fly species were identified using standard keys and counted. Among the sampled fruits, 633 (35.0%) individual fruits from 15 plant families were positive for fruit fly infestation, *B. invadens* dominated (76.3%) the positive samples. Infestation by native pests, such as *Ceratitis capitata* and *C. cosyra*, was negligible. Annonaceae, Solanaceae, Rutaceae and Anacardiaceae recorded significantly more plant host species, while the number of pest fruit flies per plant species followed a similar trend: Solanaceae > Rutaceae > Anacardiaceae. There was significant (P < 0.0001) variability in mango fruit variety infestation, both within and across zones. For all zones pooled together, Tommy and Kent, and Keitt and Kate were the least and most infested, respectively. Fruit Flies have a diverse range of

commercial and noncommercial hosts in Uganda. IPM strategies should consider management of alternative hosts and integration of mango fruit tolerant varieties.

4.1   Introduction

The fruit industry is one of the most vibrant and dynamic sub-sectors providing income and employment in Africa and the World (Lux *et al.,* 2003; Ekesi and Billah, 2006). Mango (*Mangifera indica* L., Anacardiaceae), the second most internationally traded fruit, constitutes approximately 50% of all tropical fruits traded (Stefan *et al.,* 2003). Unfortunately, fruit flies cause heavy losses to the mango industry in Africa (Ekesi *et al.,* 2006). These losses caused by fruit flies vary according to region, fruit species, fruit varieties and pest species. For instance, Lux *et al.* (2003) reports losses of up to 40% in mango in East Africa, while Vayssie`res *et al.* (2005) recorded loss averages ranging from 12 to 50% for the same host in Benin, depending on the season. In Uganda fruit exports declined from 3,061 tonnes (1,158,000 US$) in 2005, to a mere 2,166 tonnes (722,000 US$) in 2010

(http://www.ugandaexportsonline.com/2009/statistics.php). *B. invadens* accounts for the the greatest losses in fruit production. Rwomushana *et al.* (2008) showed mango and tropical almonds to be the most preferred hosts of *B. invadens*. In terms of varieties, Ambele *et al.,* (2012) reported significant variability in mango varieties susceptibility in Ghana. Consequently, the losses are accorded different economic status in different farming systems in the world (Mwatawala *et al.,* 2009a).

All this notwithstanding, there is paucity of country specific knowledge on fruit fly host status in Uganda. Previous efforts have been mainly at a regional or continent level (De Meyer *et al.,* 2002; Copeland *et al.,* 2002), or country specific (Rwomushana *et al.,* 2008; Mwatawala *et al.,* 2009a). Consequently, these studies mostly fall short of giving any information on host range of the fruit flies particular to Uganda. Previous studies in Uganda, such as those by Nakasinga (2002) were done before the introduction of *B. invadens* in Uganda, while others have concentrated on detection of specific species across the country (Nemeye, 2005; Okullokwany, 2006).

National economic programmes such as National Agricultural Advisory Services (NAADS) and National Agricultural Research Organization (NARO) have introduced improved mango varieties to diversify the country's export base and promote non-traditional export crops in Uganda. However, the relative susceptibility of the different mango varieties to fruit flies has not been studied. Knowledge about the different potential hosts and their relative utilization in a given area and the host partitioning between the different competitive fruit fly pests is crucial in development of a sustainable IPM programme (Mwatawala *et al.,* 2009a). For Uganda, this information is scanty.

Therefore, the specific objectives of this study were:

1. To profile the host range of the main fruit fly pests in the three main mango agro ecological zones; and

2. Determine the susceptibility of selected fruits and mango varieties grown to the various fruit fly pests in the country.

4.2   Materials and Methods

4.2.1   Study Area

The study was conducted in three agro ecological zones: Western Medium High Altitude Farmlands (WMHF), Lake Victoria Crescent (LVC) and Northern Moist Farmlands (NMF) (Wortman and Eledu, 1993). The three zones that represent the major fruit growing regions in Uganda have been described in Chapter 3, Section 3.2.2.

4.2.2   Sampling Procedure

A wide range of fruits, including commercial and non-commercial, but potential hosts, were collected over a period of 24 months (September 2010 – September 2012). As recommended by Mwatawala et al. (2009a), two sampling programmes were adopted: the first focused on intensive random fruit collection from the three zones. The three zones do not harbor similar proportions and types of fruits and hence it was not possible to compare similar fruits across the zones. Therefore, this exercise was intended to obtain a more complete idea of the host range and availability of fruits. Furthermore, the three zones offered different fruit types that matured at different times. Consequently, it was not possible to synchronize sample size and seasonality.

The second sampling strategy, which also lasted 24 months, involved sampling of selected important fruits and mango varieties in each zone. The fruits included sweet orange (*Citrus sinensis* (L.) Osbeck), tropical almonds (*Terminalia catappa* L.), avocado (*Persea americana* Miller, variety 'local'), guava (*Psidium guajava* L., variety 'common guava') and mango (*Mangifera indica* L., variety 'Mixed'). The mango varieties included Apple Mango, Biire, Boribo, Dodo, Glen, Kagogwa, Kate, Keitt, Kent, Tommy Akinson, Palvin and Zillatte. The 12

varieties were classified according to their maturity seasonality into late, mid and early varieties (Ambele *et al.,* 2012). The number of fruits per sample and the number of samples incubated depended on fruit availability and abundance during the season. In each of these zones, fruiting seasons fall on slightly different dates of the year and therefore comparable fruit samples were sampled during the respective fruiting seasons.

All samples comprised either tender skinned mature fruits or tender skinned immature fruits (Mwatawala *et al.,* 2009a). For mangoes, sampling was started early in the phenological cycle to assess the effect of fruit development stage on infestation. Collected fruits were transported to the rearing unit at the National Agricultural Research Laboratories (NARL), where they were labeled, kept in individual rearing buckets and provided with appropriate medium for pupation as recommended by Copeland *et al.* (2002). Conditions at the rearing unit were kept at 28 ± 2^0C and $58\pm5\%$ relative humidity. The tenneral adults that emerged were carefully removed and handled following methods described by White and Elson-Harris (1992) for taxonomic identification.

4.2.3 Fruit Fly Species Identification

To identify the emerging fruit fly species, different types of keys were used: recent systematic revisions for *Ceratitis* and *Trirhithrum* incorporated in a digital multi-entry key, and for dacines and other fruit flies, several other keys were used. For *Dacus taxa*, when in doubt, confirmation of the identification was done at Royal Museum of Central Africa under the guidance of Dr. De Meyer. Voucher specimens are kept in collections at the National Agricultural Research Laboratories, Uganda and Makerere University Zoology Museum and the Royal Museum for Central Africa, Belgium.

4.2.4   Data Analysis

To compare infestation rate in different fruit species and varieties, despite the differences in sizes of individual fruit samples (both in weight as well as in the number of fruit pieces in each sample), two parameters were determined for the selected fruit types and mango varieties: incidence and infestation rate. Incidence is the number of infested or 'positive' samples (= samples from which fruit flies emerged) in comparison to total number of samples per fruit species or variety (Mwatawala *et al.,* 2009a). In addition, the infestation rate, using an infestation index, the number of adult flies per 1kg of fruit was determined (Cowley *et al.,* 1992; Copeland *et al.,* 2002). All variables were tested for normality using Shapiro-Wilk and Anderson and Darling tests and the strongly skewed variables were transformed prior to analyses if necessary to meet the assumption of normality and homogeneity of variances. Percentage incidence (%), were arcsine-square-root (+0.5) transformed; while the number of larvae per kilogramme were log (log (x +1)) transformed. Where log transformation of the raw data was not sufficient to improve data shape, an appropriate nonparametric test such as Kruskal-Wallis was applied.

Differences in mango variety infestation across zones were tested with General Linear Model (GLM) analysis of variance (ANOVA). Where the GLM test indicated Significant differences, post-hoc Tukey (HSD) test was used. Chi-square was used to test for differences in incidence of infestation among fruits, mango varieties and zones. The Bray-Curtis similarity index was used to compare infestation similarities among varieties. The Detrended Correspondence (DCA) was used to assess fruit fly species-plant species associations, as the data format was in frequency form. All these analyses were done using PAST computer programme.

4.3 Results

4.3.1 Fruit fly Host Range

Over the sampling period, 1,812 fruits, belonging to 38 fruit species, from 30 genera in 18 plant families were sampled (Table 4.1). Among these, 633 (35.0%) samples were positive for fruit fly infestation. Among the emerging fruit flies, *B. invadens* was the dominant species: recorded in 29 out of the 38 plant species, while out of the 633 positive samples, 483 (76.3%) were due to *B. invadens*. Plant host infestation incidence for the rest of the fruit fly species ranged between 7.9% in T. *coffeae* to 65.8% in *C. rosa*. However, on the basis of relative positivity, the rest of species ranked very low. The lowest and highest proportion was 7 samples out of 633 (1.11%) for *T. coffeae* and 31 (4.9%) for *C. capitata*, respectively.

All the 29 plant species that *B. invadens* utilized were recorded in 15 plant families, ranking second behind *C. rosa* (16 families) in plant family resource diversity. Other species with relatively more plant family resource diversity included *C. capitata* (14) and *C. anonae* (10), while the least host spectrum species was *T. coffeae* (3). Host range and fruit fly diversity at plant family level were significantly different (Runs Test R value = 3.00, Chi square p value < 0.05). In terms of plant richness, Annonaceae, Solanaceae, Rutaceae and Anacardiaceae recorded more plant species utilized by fruit flies. Pest diversity followed a similar trend; Solanaceae, Rutaceae and Anacardiaceae recorded the highest fly diversity (Figure 4.1).

Table 4. 1. Host plant range (family and species) of fruit flies in Uganda and relative positivity of the various fruit hosts

	Total number of samples	Bactrocera cucurbitae	Bactrocera invadens	Ceratitis anonae	Ceratitis capitata	Ceratitis cosyra	Ceratitis fasciventris	Ceratitis punctata	Ceratitis rosa	Dacus bivittatus	Dacus cilliatus	Trirhithrum coffeae	Fruit fly Richness	Percent +ve Samples
Anacardiaceae													9	
Annacardium occidentale	7	0	1	0	1	0	0	0	1	0	0	0	2	42.9
Mangifera indica L.	320	0	192	5	3	7	2	0	1	4	0	0	7	66.9
Sclerocarya birrea (A. Rich) Hochst	14	0	5	0	0	1	0	1	0	0	1	0	4	57.1
Annonaceae													6	
Annona cherimola Miller	4	0	0	0	1	1	0	0	0	0	0	0	2	50.0
Annona muricata L.	106	1	32	1	1	0	0	0	1	0	0	0	5	34.0
Annona reticulate L.	6	1	0	0	1	0	0	0	1	0	0	0	3	50.0
Annona senegalensis	11	1	4	0	0	1	0	0	1	0	0	0	3	63.6
Annona squamosa	14	1	6	0	1	0	0	0	1	0	0	0	3	64.3
Cananga odorata	5	0	0	0	0	0	0	0	1	0	0	0	1	20.0
Caricaceae													4	
Carica papaya L.	40	1	5	0	1	0	0	0	2	0	1	0	4	25.0
Combretacaea													4	
Terminalia catappa L.	39	0	32	1	1	0	0	0	1	0	0	0	4	89.7
Cucurbitaceae													5	
Momordica charantia	3	0	0	0	0	0	0	0	0	1	0	0	1	33.3
Cucumis melo	22	3	4	0	0	0	0	0	0	1	2	0	3	45.5

Species	1	2	3	4	5	6	7	8	9	10	11	12	n	%
Cucurbita spp.	9	0	2	0	1	0	0	0	1	1	0	0	3	55.6
Euphorbiaceae													**1**	
Drypetes natalensis	9	0	0	0	0	0	0	0	1	0	0	0	1	11.1
Lauraceae													**6**	
Persea americana Miller	100	1	21	2	1	0	2	0	1	0	0	0	6	28.0
Moraceae													**4**	
Antiaris toxicaria	3	0	1	1	0	0	0	0	0	0	0	0	1	66.7
Artocarpus sp.	4	0	0	1	0	0	0	0	0	0	0	0	1	25.0
Ficus sp.	19	0	3	0	2	0	0	2	2	0	0	0	3	47.4
Musaceae													**1**	
Musa sp.	27	0	4	0	0	0	0	0	0	0	0	0	1	14.8
Myrtaceae													**8**	
Acca sellowiana	6	0	2	0	0	0	0	0	1	0	0	0	1	50.0
Eugenia uniflora	10	0	1	1	0	0	0	0	0	0	0	0	1	20.0
Psidium guanjava	100	1	64	4	3	1	2	0	2	0	1	0	8	78.0
Rosaceae													**2**	
Cydonia oblonga	23	0	5	0	1	0	0	0	0	0	0	0	1	26.1
Prunus sp.	27	0	3	0	3	0	0	0	1	0	0	0	2	25.9
Rubiaceae													**5**	
Coffeae arabica L.	81	0	4	2	2	0	2	0	2	0	0	3	5	18.5
Rutaceae													**9**	
Citrus limon	287	0	32	0	0	0	1	0	0	0	0	0	2	11.5
Citrus reticulate	73	0	3	0	0	0	0	0	1	0	0	0	1	5.5
Citrus sinensis	51	0	1	0	1	0	0	0	1	0	0	0	2	5.9
Citrus Spp.	53	1	1	2	1	1	0	0	0	0	1	3	8	18.9
Sapotaceae													**3**	
Chrysophyllum albidum	8	0	0	0	0	0	0	1	1	0	0	0	2	25.0
Manilkara zapota	33	0	1	0	2	0	0	0	1	0	0	0	2	12.1
Solanaceae													**9**	

50

Capsicum annum	123	1	32	0	1	0	0	0	0	0	1	1	5	29.3
Lycopersicon esculentum	85	0	12	0	1	0	0	0	1	1	1	0	5	18.8
Solanum Spp.	24	1	6	1	1	0	1	0	1	0	0	0	5	45.8
Sterculiaceae													**3**	
Theobroma cacao	33	0	4	2	0	0	0	4	1	0	0	0	3	33.3
Verbenaceae													**1**	
Vitex sp.	25	0	0	0	0	0	0	0	0	1	0	0	1	4.0
Vitaceae													**2**	
Vitis vinifera	8	0	0	0	1	0	0	0	1	0	0	0	2	25.0
No. of Plant Species infested		11	29	12	22	6	6	4	25	6	7	3		
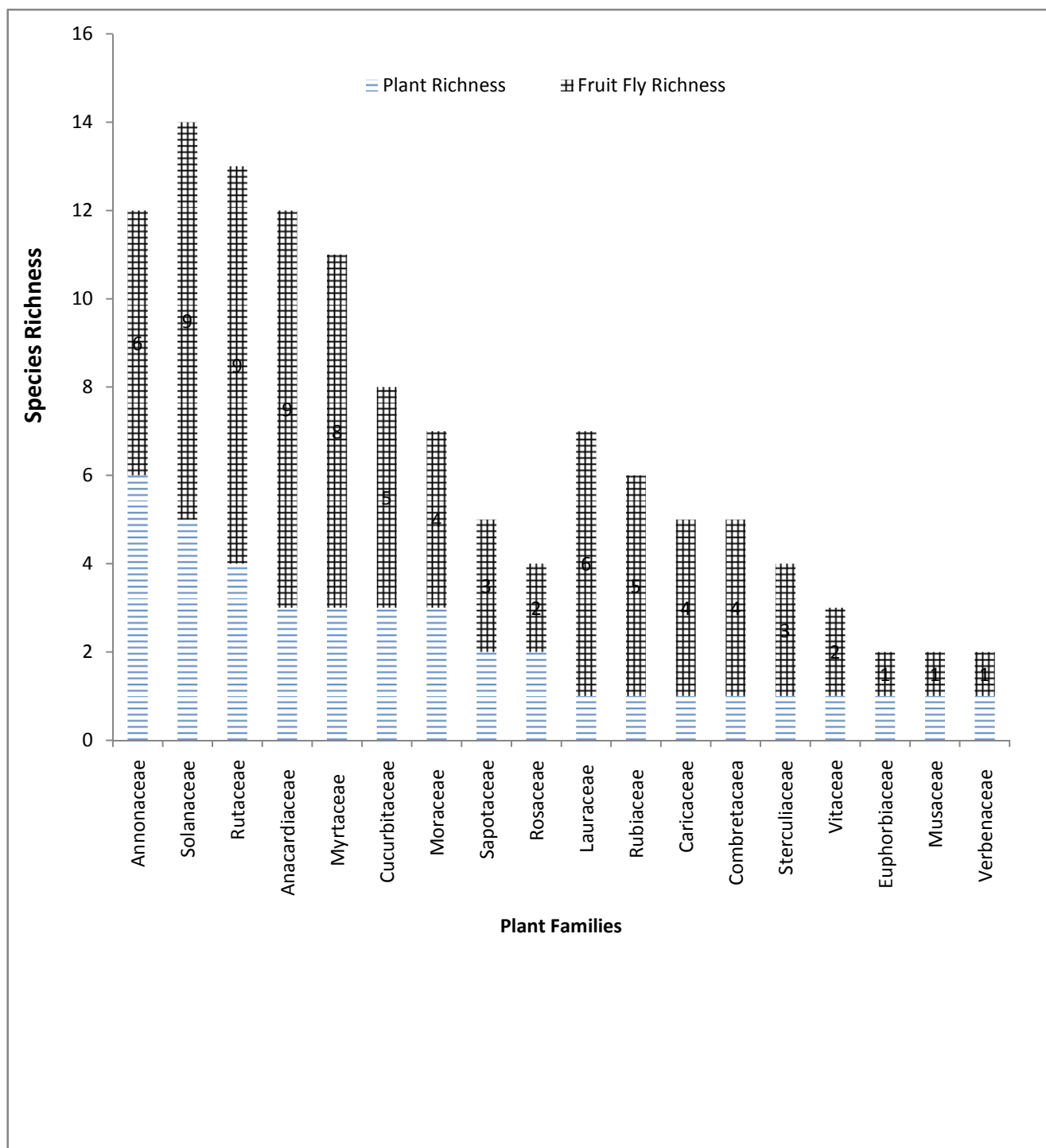
Figure 4. 1: Variability in plant (number of species per family infested by fruit flies) and fruit fly richness (number of fruit fly species utilizing selected plant family) in the study.

4.3.2 Plant Family and Species Host Susceptibility

Plant families showed differences in susceptibility to fruit flies ($F_{17, 36}$ = 9.150, P = 0.0001; Figure 4.2). The explanatory variable (plant families) explained 81.2% and 72.3% in actual and adjusted variability in host susceptibility, respectively. The highest positivity (% infested fruits) were recorded in Combretaceae (89.7±7%), followed by Anacardiaceae (55.6±7%) and Myrtaceae(49.3±0.01%), while least preference was recorded in Verbenceae, Rutaceae and Euphorbiaceae at 4.0, 10.5, and 11.1%, respectively, and each recorded one host species (Figure 4.2).
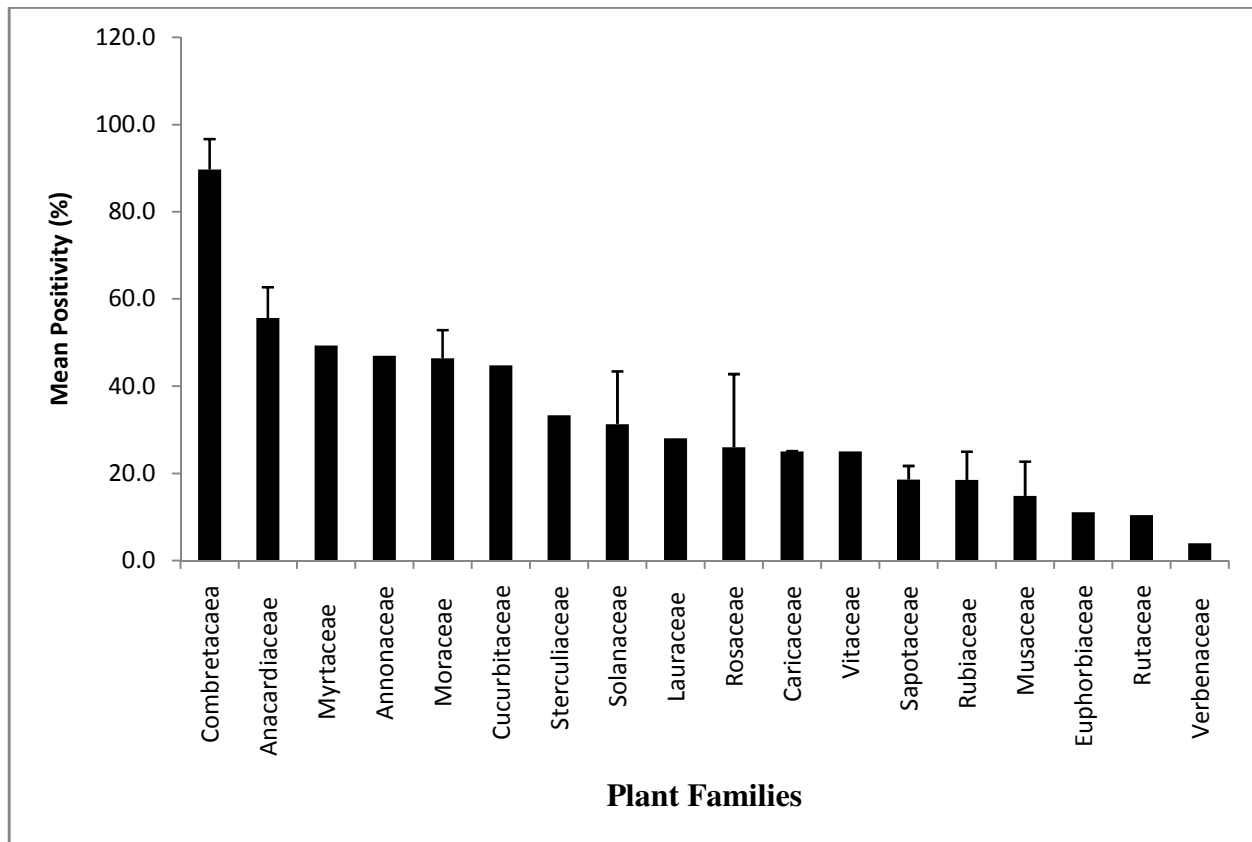
Figure 4. 2: Variability in plant family positivity to infestation by fruit flies

Combretaceae was significantly (P < 0.0001) different from all the other families in infestation positivity (Table 4.2). Anacardiaceae, Myrtaceae, Annonaceae, Caricaceae, Cucurbitaceae, Lauraceae, Moraceae, Rosaceae, Rubiaceae, Sapotaceae, Solanceae and Sterculiaceae were not significantly different but differed significantly with the rest of the families. A high, positive and significant coefficient of the intercept indicated that there were other important causes of variability in infestation other than host family type.

Table 4. 2: Model parameters showing relative plant family preference. Probability values followed by same letters are not significantly different.

Source	Model Value	Standard error	Student t	Probability	Difference
Intercept	25.0	6.9	3.6	**0.00**	
Anacardiaceae	30.6	9.8	3.1	**0.00**	Ab
Annonaceae	22.0	8.5	2.6	**0.01**	B
Caricaceae	0.0	9.8	0.0	1.00	Bcd
Combretacaea	64.7	9.8	6.6	**< 0.0001**	A
Cucurbitaceae	19.8	9.8	2.0	**0.05**	Bc
Euphorbiaceae	-13.9	9.8	-1.4	0.16	Cd
Lauraceae	3.0	9.8	0.3	0.76	Bcd
Moraceae	21.4	9.8	2.2	**0.04**	Bc
Musaceae	-10.2	9.8	-1.0	0.30	Cd
Myrtaceae	24.3	9.8	2.5	**0.02**	B
Rosaceae	1.0	10.9	0.1	0.93	Bcd
Rubiaceae	-6.5	13.8	-0.5	0.64	Bcd
Rutaceae	-14.6	9.1	-1.6	0.12	D
Sapotaceae	-6.5	10.9	-0.6	0.56	Bcd
Solanaceae	6.3	9.8	0.6	0.52	Bcd
Sterculiaceae	8.3	9.8	0.8	0.40	Bcd
Verbenaceae	-21.0	9.8	-2.1	**0.04**	D
Vitaceae	0.0	0.0			

Fruit fly species showed significant (P = 0.0001) plant species association, however, the strength of this fidelity varied across the plant species. The highest plant-pest fidelity was in *T. coffeae* and *D. punctanta,* with 3 and 4 species respectively. Factor 1 (X-component axis) and Factor 2 (Y-component axis) explained discriminate variability of 62 and 14%, respectively, of the total variability due to fruit fly/ plant species association. Factor one, mainly separated plant species on the basis of *B. invadens*; *Terminalia catappa, Mangifera indica, Annona muricata, Persea americana, Psidium guajava*, *Citrus limon, Capsicum annum and Lycopersicon esculentum* were clearly separated from poor hosts such as *A. cherimola, A. reticulate, Cananga odorata, M. charantia, Drypetes natalensis, Artocarpus sp., Chrysophyllum albidum, Vitex sp.* and *Vitis vinifera.* Factor two, segregated mainly cucurbit feeders or dependant species on the basis of orientation from the biplot centre. Close association between Cucurbit spp., *Cucurmis melo*, *Lycopersicon esculentum*, and *Capsicum annum* with *Dacus bivittatus, Dacus cilliatus* and *Bactrocera cucurbitae* is, therefore, not surprising. Likewise, *Ceratitis cosyra* association with *Mangifera indica, and T. coffeae* association with *Coffee Arabica* was expected as these are proven best hosts for the two pests, respectively. Results generally showed that *Drypetes natalensis, Artocarpus sp., Chrysophyllum albidum, Vitex sp.* and *Vitis vinifera* were generally poor fruit fly hosts.

The details of the relative fruit fly infestation for the selected fruits are shown in Figure 4.4. Of the 7 fruits, tropical almond showed the highest incidence (87.9%) and was mainly infested by *B. invadens* (82.1%). Tropical almond was significantly different (P = 0.004) from all the other hosts in the study. Other major hosts that recorded high incidences are guava (*Psidium guajava*) and mango (*Mangifera indica*). The least infested hosts were the Citrus varieties, which did not

55

differ significantly among themselves. *Persea americana* registered intermediate infestation, but was significantly different from the citrus varieties.
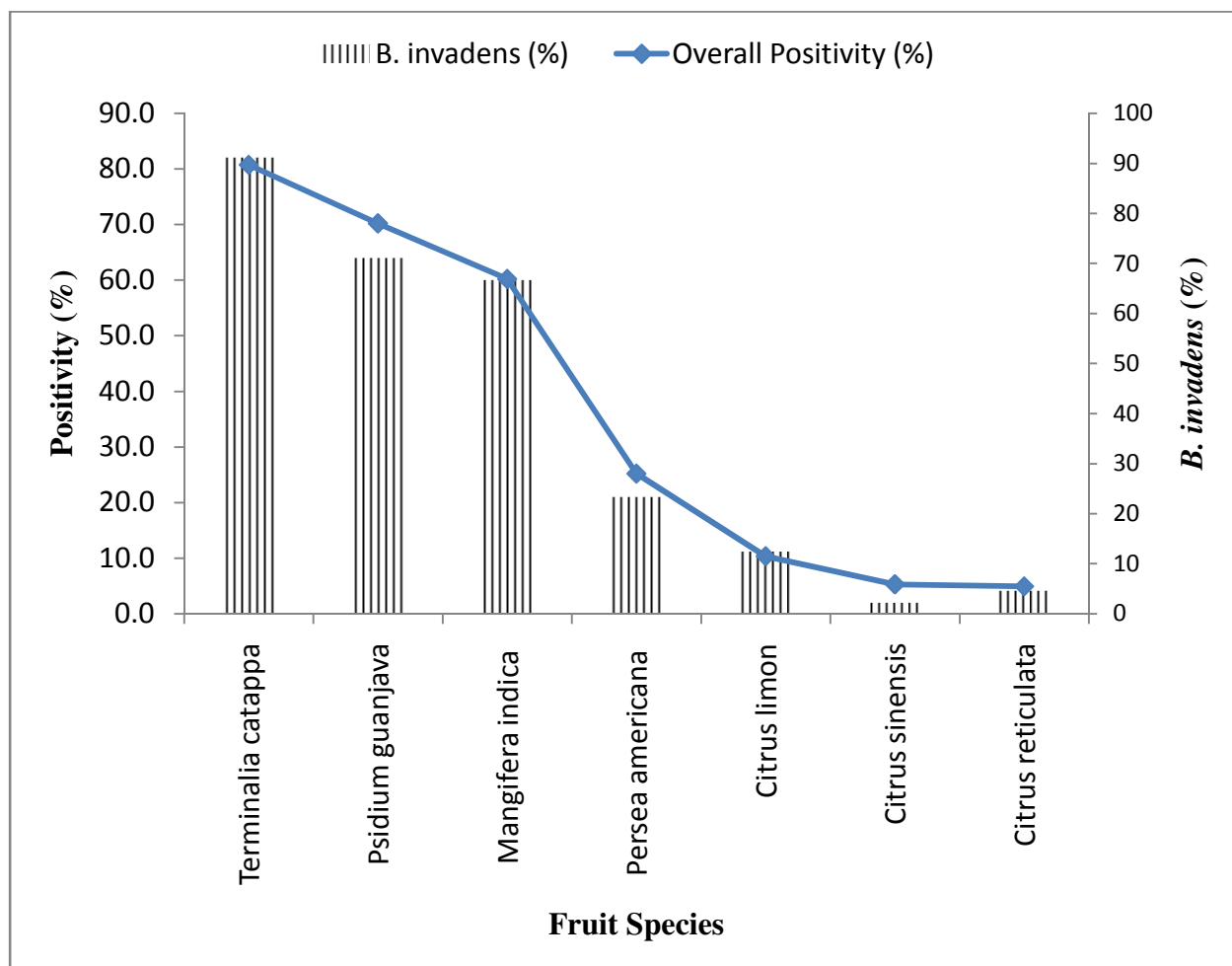
Figure 4. 3: Fruit fly infestation/ incidence by all fruit flies and by *Bactrocera invadens* alone on selected fruit types.

4.3.3   Mango Fruit Host Utilization

Mango fruit fly incidence and infestation intensity across the selected varieties differed significantly across zones. In the LVC, the highest incidence was recorded in Biire (44.7%), Palvin (44.4%), while the least was in Tommy (13.5%), Zillate (22.6%) and Kent (19.5%). In terms of infestation intensity, the difference was significant too ($F_{11,\ 1808} = 7.584$, P = 0.0001; Figure 4.5), with Glen (92.4 larvae/ Kg) and Boribo (86.7 larvae / Kg) being the highest, while

the least infested varieties were Tommy, Keitt, Zillate and Kent. Post-hoc analysis showed that Biire was significantly (P = 0.0001) more infested than all varieties, with the exception of Glen, Boribo and Palvin. Likewise, Glen was significantly different from Tommy (P = 0001), Kent (P = 0.003), Zillate (P = 0.018) and Kagogwa (P = 0.03). Boribo differed with Tommy (P = 0.006) and Kent (0.016), while Dodo was significantly more infested than Tommy (0.0001) and Kent (P = 0.011). Similarly, Kate was significantly different from Tommy (P = 0.0001) and Kent (P = 0.008), while Kagogwa was more infested than Tommy (P = 0.039).

In the NMF, significant difference in variety fruit fly incidence was recorded too. The highest incidence was recorded in Keitt and Kate (75%, each), while the least was in Glen, Boribo and Kent, with 15.4, 16.7 and 22.2%, respectively. There were significant difference in intensity ($F_{11, 243}$ = 2.807, P = 0.0002; Figure 4.5), with Keitt (185.8 larvae/ Kg) and Kate (125 larvae / Kg) being the highest, while the least infested varieties were Tommy, Boribo, Kent, and Palvin. Post-hoc analysis showed that Keitt and Kate were significantly (P = 0.001) more infested than all varieties, with the exception of Apple mango. The latter was significantly (P = 0.03) more infested than Tommy, Glen and Kent, while Dodo was marginally (P = 0.046) more infested than Tommy and Glen. Similar to the LVC, Kagogwa was more infested than Tommy (P = 0.047), but in this case also different from Glen (P = 0.048).

The highest incidence in WMHF was recorded on Zillate and Biire (57 and 44%, respectively), while the least was on Dodo (Figure 4.5). In terms of infestation intensity, the difference was significant too (P = 0.0001), and similarly Zillate and Biire (48and 38 larvae/ Kg, respectively) being the highest, while the least infested varieties were Dodo, Boribo, Apple and Kagogwa.

Post-hoc analysis showed that Zillate and Biire were not significantly different, but differed significantly from the rest of the varieties.

There were significant differences in mango variety susceptibility to infestation for zones data pooled ($F_{11, 720}$ = 11.215, P = 0.0001; Figure 4.6). The highest incidence was recorded in Zillate and Biire (55 and 35%, respectively), while the least was in Dodo, Apple, Boribo, Kate and Kagogwa. A similar trend was followed for infestation intensity: Zillate and Biire (56 and 38 larvae/ Kg, respectively), while the least was in Dodo, Apple, Boribo, Kate and Kagogwa. Post-hoc analysis showed that Zillate was significantly more infested than Biire (P = 0.0001), while the latter two are significantly different in infestation from all the other varieties (P = 0.001). The rest of the varieties did not differ significantly. Across all zones, Keitt, Kate and Biire were the most infested varieties, while Tommy and Kent were the least. Although Palvin recorded significantly low infestation per kilograms, it ranked high in incidence (44%), only comparable to Zillatte, Kate, Biire and Keitt across all zones.
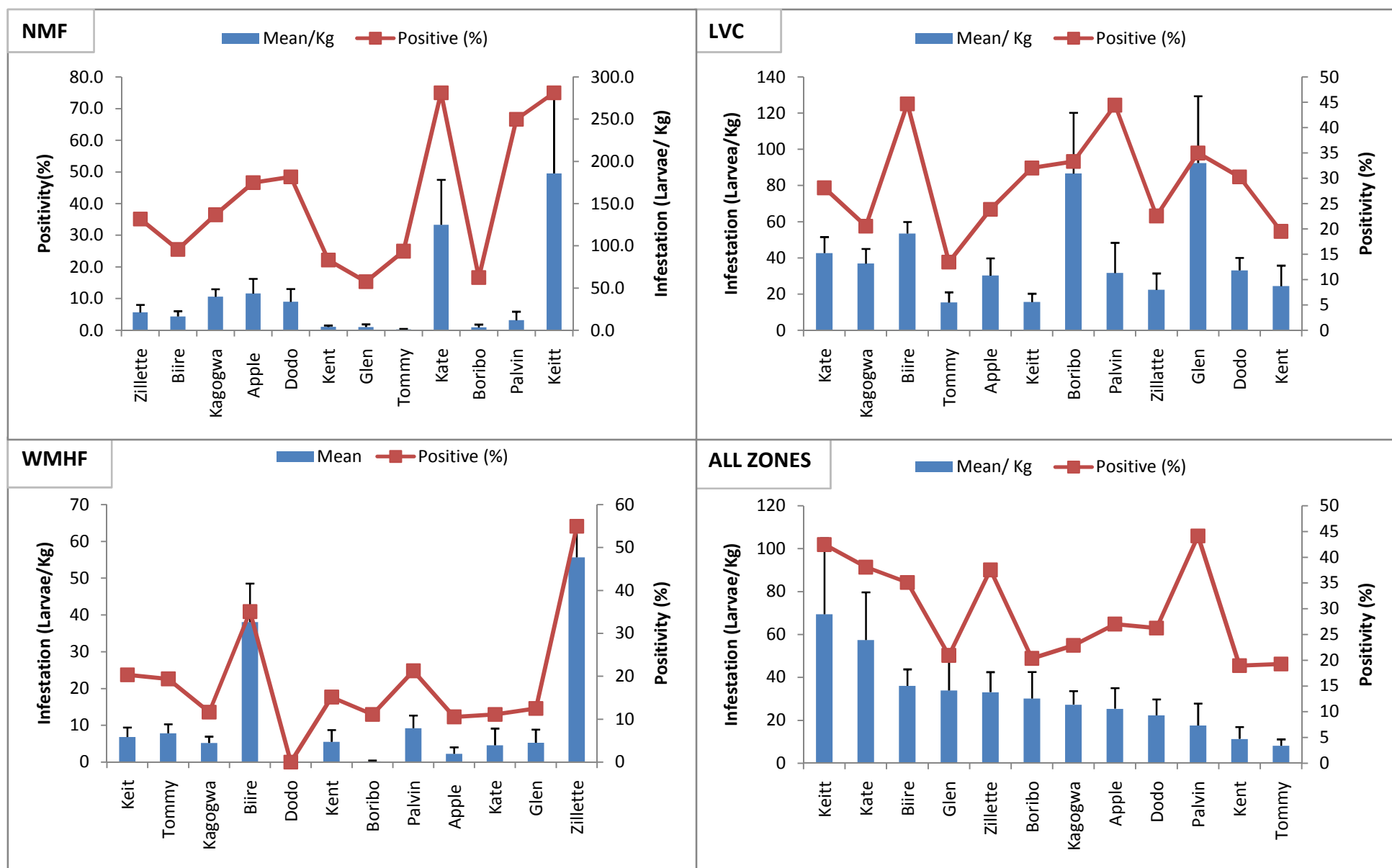
Figure 4. 4: Mean incidence and intensity of infestation across varieties the three zones and average variety infestation across zones

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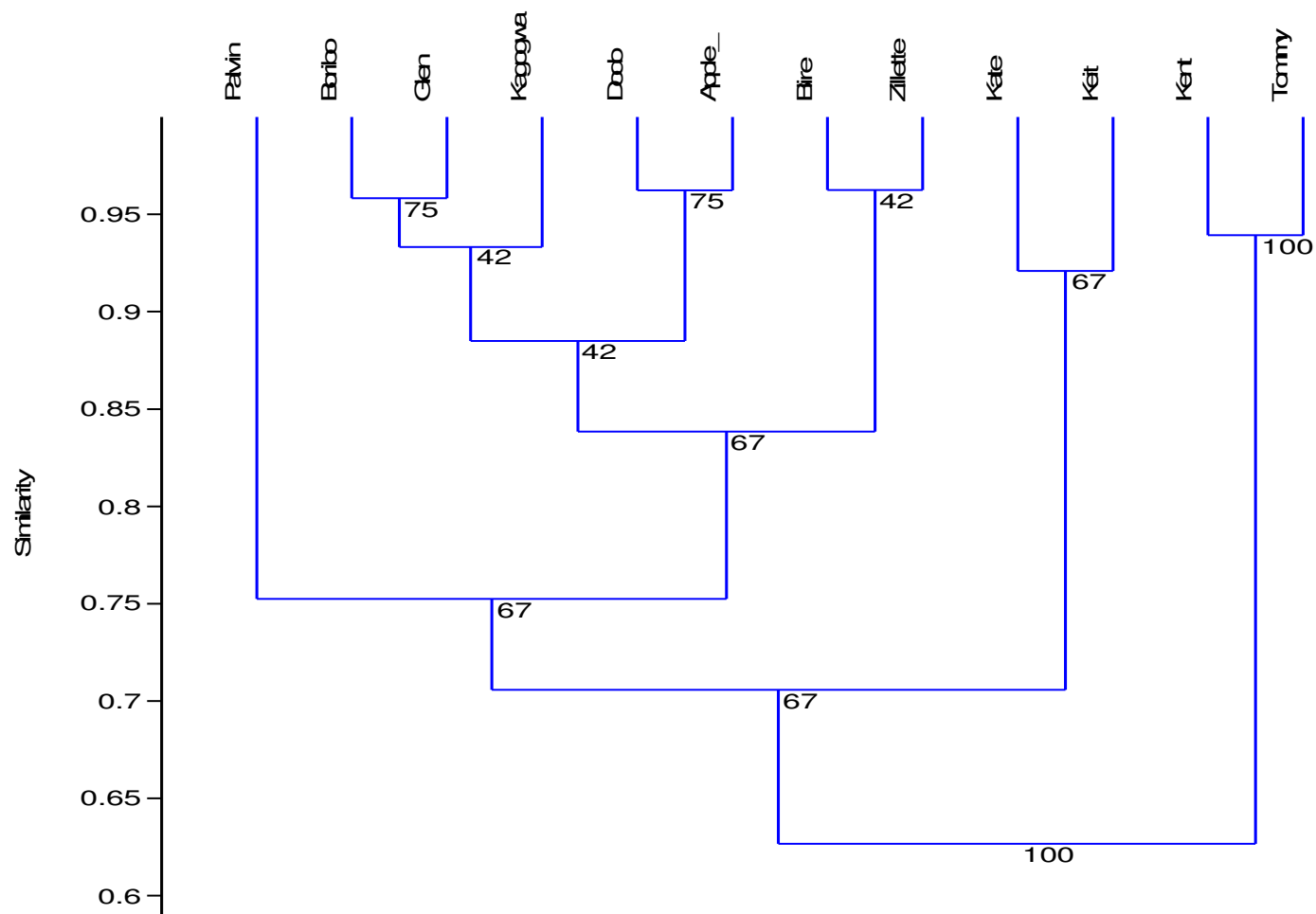
Figure 4. 5: Bray-Curtis similarities in Mango varieties relative infestation. The vertical axis shows percentage similarity between and within clusters, while the number under each cluster indicates cluster homogeneity.

There was significant difference in incidence (%) and total number of puparia recovered (infestation) from mangoes that mature at different stages of the fruiting season (Early, Mid- and Late maturing) (Kruskal-Wallis H' = 15.72, P = 0.0003; Figure 4.8). Early maturing varieties recorded the highest infestation (38.0 Larvae/Kg), while the mid season varieties were the least infested. Late and Early season varieties did not differ significantly (P = 0.5246), but the two were different from mid season varieties (P = 0.0001) in incidence and infestation intensity. Further, as the season progressed, there was orchard level accumulation of incidence and intensity of fruit damage of mango fruits as the population of the fruit flies increased from the onset of the season (Figure 4.7).
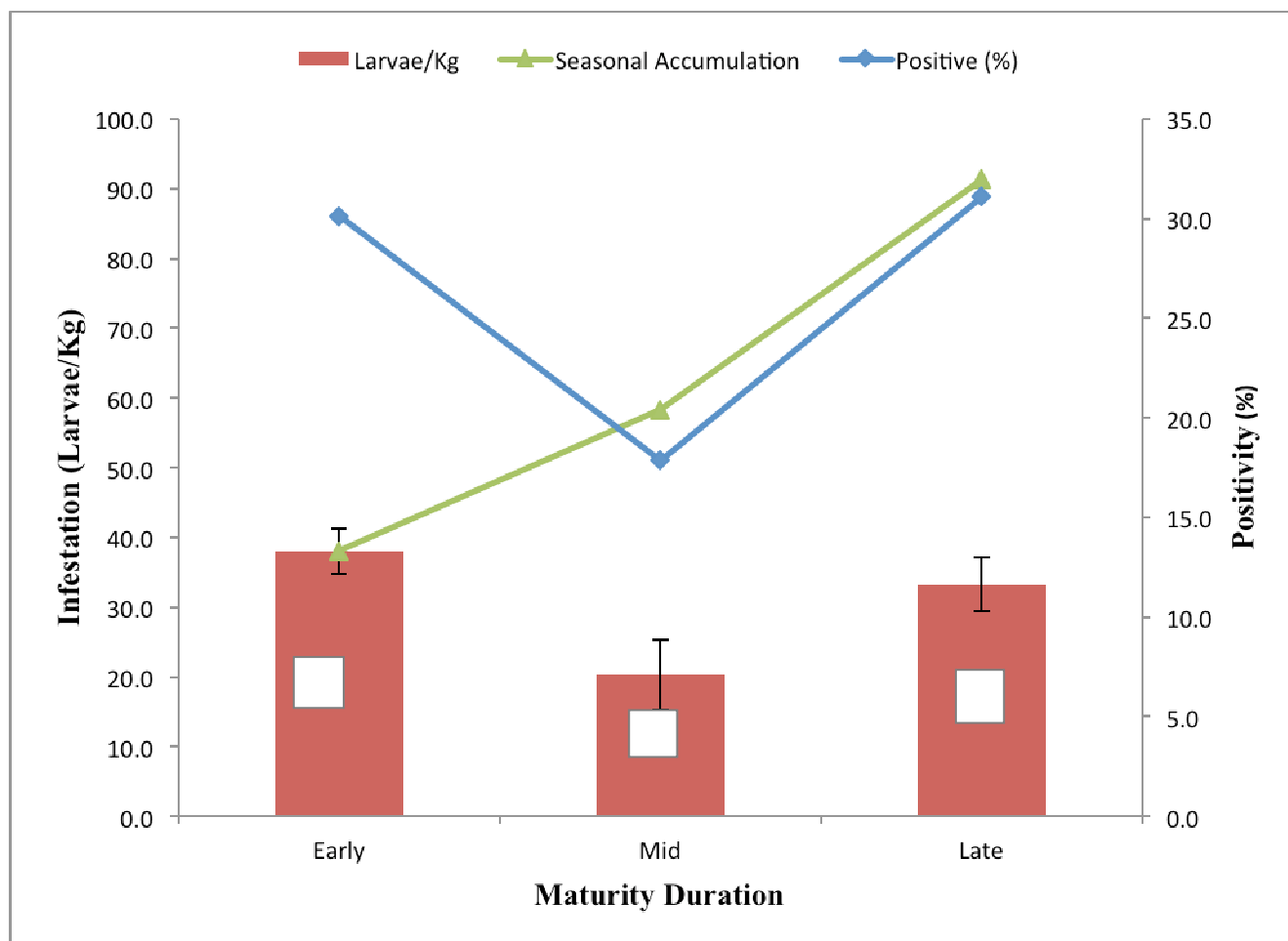
Figure 4. 6: Variations in fruit fly infestation intensity and incidence for mango varieties that mature at different stages of the fruiting season (Early, Mid- and Late maturing).

4.4   Discussion

4.4.1 Fruit Fly Host Spectrum

This study has revealed a high fruit fly plant host range in Uganda, with *B. invadens* as the most dominant. Mwatawala *et al.,* (2009a) reported *B. invadens* in 81.7% of all the positive samples, comparable to the 76.3% in this study. The results also agree entirely with previous studies about the increasingly negligible ecological role of the rest of the species in the ecosystem (Ekesi *et al.,* 2006; Mwatawala *et al.,* 2006a; Rwomushana *et al.,* 2008; Vayssières *et al.,* 2009; Mwatawala *et al.,* 2009a and 2009b).

With a few exceptions, all the major and minor fruit fly hosts encountered in this, have been documented elsewhere (Ekesi *et al.,* 2006; Mwatawala *et al.,* 2006a; Rwomushana *et al.,* 2008; Vayssières *et al.,* 2009; Mwatawala *et al.,* 2009a, 2009b). The study reports for the first time the infestation of B. *invadens* on *T. catappa,* A. *toxicaria*, *E. uniflora*, *A. selllowiana*, *T. cacao* and *C. oblonga* in Uganda. Apart from *T. catappa* whose association with *B. invadens* is widely appreciated, the rest of these new records had low frequency, ranging between one and five fruits. The study therefore recommends more confirmatory assessments, as presence of low numbers of larvae in fruit are not indicative of host acceptability (Aluja and Mangan, 2008).

Notable too in this case, was the positive rearing from *Musa spp.* and *C. Arabica*. These two records are unusual (but not unexpected), and will require further confirmation studies, as they are Uganda's important staple and export crops, respectively. For coffee, similar findings by Mwatawala *et al.,* (2009a) have reported, with up to 370 flies/ Kg of fruit infestation. The bananas (*Musa spp.*) from which the flies emerged in the study were mainly ripe, sometimes ruptured and close to rotting; factors that could have enhanced chances of fruit fly oviposition. It is common for fruit flies to oviposit in hosts that are inadequate for larval development, such as ruptured, tender, but not necessarily appropriate hosts. A high level of acceptance for oviposition for such punctured or intact poor hosts does not necessarily imply suitability of the infested fruit for further larval development or adult emergency (Rengifo *et al.,* 2011). Polyphagous species, such as *B. invadens* circumvent physical resistance to oviposition by utilizing punctures, holes, pecks and crevices (Aluja and Mangan, 2008), which could ultimately explain the *Musa spp.* records here.

The highly polyphagous nature of *C. capitata* (22 hosts) and *B. invadens* (29) recorded in this study could be explained by their ability to oviposit in a wide range of fruit crops (Aluja and Mangan, 2008). Globally, in the genus *Ceratitis*, *C. capitata* is regarded as the most polyphagous species (White and Elson-Harris, 1992; De Meyer *et al*., 2002; Copeland *et al.,* 2002). Earlier on in Uganda, Nakansinga (2002) showed *C. capitata* to be the most polyphagous species among many native *Ceratatis* members, while Copeland *et al.* (2002) demonstrated its wide host spectrum in Kenya (51 indigenous hosts).

Terminalia catappa, the most infested and suitable resource for *B. invadens* in this study, has been reported in many other cases (Rwomushana *et al.,* 2008; Mwatawala *et al.,* 2006a; Mwatawala *et al.,* 2009a; Geurts *et al.,* 2012). This potentially weedy plant is largely planted as ornamental, shade tree, for soil conservation or as a fruit. The higher affinity for *T. catappa* by *B. invadens* has been related to the higher plant concentration of methyl eugenol, a phenylpropanoid compound found in over 200 plant species representing 32 families (Raghu, 2002). After ingesting the compound, males sequester breakdown products of methyl eugenol in the rectal gland and use these metabolites to synthesize a sex pheromone attractive to females (Nishida *et al.,* 1988). Metcalf (1990) adds that plants (like *T. catappa*) that contain methyl eugenol serve as mating rendezvous sites. Hence, the overwhelmingly high incidence (87.9%) and infestation by *B. invadens* (82.1%) in this study should not be surprising. However, the fruit fly management implication is that the plant should be of concern as a reservoir host for *B. invadens*.

Major commercial crops such as mango (Anacardiaceae), avocado and guava (Myrtaceae) showed a high infestation incidence for *B. invadens*, as was the case elsewhere (Ekesi *et al.,* 2006; Mwatawala *et al.,* 2006a; Rwomushana *et al.,* 2008; Vayssières *et al.,* 2009; Mwatawala *et al.,* 2009a, 2009b; Mboyine *et al.,* 2012; Geurts *et al.,* 2012). Among the commercial fruits recorded, oranges do not comprise a favorable fruit fly host in this study (Figure 5.3 and Table 5.2), and the region (Rwomushana *et al.,* 2008; Mwatawala et al., 2009a).

The poor diversity of cucurbits reared in this study could have affected the study's infestation records for this family and hence further efforts are recommended. *C. rosa* was the major infester for high altitude fruits such as coffee and annona fruits (albeit based on few samples) as was the case by Mwatawala *et al.* (2006b). Despite the polyphagous nature of *C. cosyra*, constant displacement by *B. invadens* has mainly restricted its host range to the most favorable and abundant Anacardiaceae hosts, with single samples in five families (Table 4.1). The only two families with two hosts Anacardiaceae and Annonaceae are recognized major host plant families for *C. cosyra* (Mwatawala *et al.,* 2009a).

4.4.2 Mango Varieties

Fruit flies are resilient pests with typical ecological adaptiveness. They may differ in distribution patterns and host utilization preferences but most species can survive, with some degree of flexibility, in a wide range of habitats of varying environmental conditions (Dethier, 1982). Fruit flies can travel over short distances to feed and breed or they can disperse passively over long distances with the aid of wind currents. Since different zones offer these resources in varying proportions, in addition to the obvious ecological and climatic differences and farmer practices

(e.g varietal choices and mixes), it is not surprising that zones ultimately differ in pest infestation (Vayssieres et al., 2009; Ambele *et al.,* 2012),

Across the study zones, the factors that are varying and could explain the observed differences in variety susceptibility include: water or nutrient availability, wind exposure and amount of shade, or the presence of other herbivores (Horner and Abrahamson, 1992). If a mango variety's suitability were phenotypically plastic in response to each of these environmental variables, fruit fly host suitability in a given zone would be increased potentially by orders of magnitude (Cronin and Abrahamson, 1999). Variations in fruit variety infestation among the zones could therefore indicate that varieties have specific adaptations in their ecological requirements. The bulk of mangoes consumed in Uganda are either the local varieties (Kagogwa and Dodo) or the local selections (Kate and Biire). The perception that the exotic varieties introduced in 1990s are generally the most susceptible to fruit flies has been partly disapproved.

Two possible hypotheses may explain the differences in *B. invadens* preference for 'local' selection varieties (Kate and Biire) and in some cases Kagogwa over the "exotics". First, most polyphagous species (like *B. invadens*) adapt quickly to a new host, with larval survivorship increasing linearly over 10 generations in a new host (Hawthorne, 1999). The relatively higher preference of the flies for the local selection and *Kagogwa* varieties may, therefore, be a result of an increase in performance on these hosts. Second, studies by Szentesi and Jermy (1990); Dukas and Bernays (2000); and Egas and Sabelis (2001) have shown that experience or learning may affect host choice of phytophagous insects. *B. invadens* may be probably already adapted for living on these varieties because of their predominant availability in Uganda over the 'exotics'.

Therefore, the country wide distribution of local selections and Kagogwa varieties might have favored adaptation, which may provide an explanation for their high preference.

Several studies have revealed that behavioral and ecological factors (biotic and abiotic) play an important role in host use (Khan *et al.,* 2000). Pre-alighting factors (fruit color, host plant structure, shape and size) and post-alighting factors (e.g pericap toughness), should explain the study's observed variations in infestation among varieties. A conjugation of plant physical and chemical factors influences the choice and balance between positive and negative stimuli, might ultimately determine the final host selection. For instance, peel thickness and firmness (PTF), percent total acids and total soluble solids and developmental times were reported to be the main cause of mango fruit variety variation in fruit fly tolerance in Ghana (Ambele *et al.,* 2012). The latter studies reported peel firmness and thickness, as important determinants for oviposition preference among fruit flies, with female tephritids having oviposition preference for fruits with softer pericarps.

It is therefore possible that *B. invadens* is able to discriminate the mango varieties based on pre-alighting factors such as colors and host colors, which play an important role on oviposition site selection (Katsoyannos, 2001). Saha *et al.* (1996) noted a very low response of adult *B. cucurbitae* for oviposition to yellow, orange or white coloured egging substrates. It may therefore stand to reason that the bright color of Tommy atkin makes the variety to partly escape fruit fly oviposition. Tommy Atkins mangoes, a mid-season variety is green to purplish red and turns a bright yellowish/ orange when ripe.

Mango losses associated with fruit fly infestation are relatively high in relation to the total fruit yields. Thus, understanding the extent of distribution and damage of important species (*B. invadens*) would provide important information required for the design of suitable management strategy. Even though this species is important in terms of losses associated with its infestations; little effort has been made in the past to reduce its population on an area-wide scale. Information on the ecological requirement that is reflected in geographic distribution and population dynamics along the seasons is a prerequisite in priority setting for fruit fly management. Findings from this study have particular relevance to fruit production in Uganda. Results here have revealed that fruit fly infestation builds during fruit growing season, with highest damage to early and late varieties, as demonstrated by Vayssieres *et al.* (2009).

The results also suggest variability in zonal infestation. Orchards in the WMHF recorded low infestation, while the NMF were relatively more infested. It is not clear-cut whether the higher infestation in NMF is due to low investment by farmers in pest control, or harsh environmental conditions capable of reducing plant defense mechanism resulting in higher mango fruit damages and yields losses in this area. It is, therefore, recommended that studies on knowledge, attitudes and practices in the management of fruit flies be conducted to understand the observed differences.

4.5   Conclusion

Fruit Flies have a diverse range of commercial and noncommercial or wild hosts in Uganda. The diverse range is also highly variable across the different zones in the country. This wide and variable spectrum of host range offers management implications, as no single management

package can suffice all the zones hence calling for site-specific approaches. Tropical almonds and *B. invadens* were the suitable host and dominant fruit fly species, respectively. Mango varieties varied in their susceptibility to fruit fly infestation within and across zones, hence making definite conclusions of the most and least susceptible varieties difficult. However, early and late season maturing mango varieties were more susceptible. New fruit fly-host associations in Uganda were also recorded probably due to the adaptative evolution of polyphagous species in highly disturbed ecosystems. Further studies should improve the knowledge of the biology native and other invasive tephritid fruit flies in the different edapho-climatic conditions of Uganda.

CHAPTER FIVE: OVIPOSITION PREFERENCE AND OFFSPRING PERFORMANCE IN PHYTOPHAGOUS FRUIT FLIES (DIPTERA: TEPHRITIDAE): THE AFRICAN INVADER, *BACTROCERA INVADENS**

ABSTRACT

The Preference-Performance Hypothesis (PPH), which states that female insects will evolve to oviposit on hosts on which their offspring fair best, is one of the hypotheses advanced to explain behavioral choices phytophagous insects. The extent to which this is true in the African invader, *Bactrocera invadens (*Diptera: Tephritidae*)* was assessed in this study. Fivehost-plant species: *Terminalia catappa* (Combretaceae), *Persea americana* (Lauraceae), *Psidium guajava* (Myrtaceae), *Mangifera indica* (Anacardiaceae*),* and *Citrus sinensis* (Rutaceae), and 11 mango varieties: Tommy, Zillatte, Keitt, Kent, Kagogwa, Apple, Palvin, Dodo, Kate, Biire and Glen, were tested for their conformity to the PPH. Field collected fruits were incubated, larvae reared and the adults maintained on a honey diet in the laboratory. There was significant variability in host species (P = 0.038) and variety (P = 0.015) preference in the orders: *T. catappa > P. guajava > M. indica > C. sinensis > P. americana*, while Glen and Biire and Zillate and Tommy were the most and least preferred varieties, respectively. Offspring performance (development rate, survival, weights and sex ratio) differed significantly and followed a similar trend only that performance was better on *P. americana* than on *C. sinensis*. The relationship between host preference and the offspring performance measures showed strong support for the PPH with overall coefficient of determination of 75.4% (P = 0.0001) and 65% (P = 0.003) for host species and varieties, respectively. This study has generally increased the knowledge on the role of host species and varieties on the biology of the pest and re-affirmed the growing credence that host-

specific variation in adult performance has an important role in shaping host preference of phytophagous insects as proposed by PPH.

5.1   Introduction

The linkage between preference of ovipositing females for certain plant species and the growth, survival, and reproduction of offspring (performance) on those plants has been a central problem in the theory of insect/plant interactions (Thompson, 1988). Consequently, the preference/performance confront has been subject of several studies and debates on the evolution of host specificity, delimiting of enemy-free space, and host shifts in allopatric and sympatric insect populations (Bonebrake *et al*., 2010; Heard, 2012). Evolutionarily, the relationship between host preference and larval and adult performance has been related to understanding host-plant specificity, diet breadth, host-race formation, sympatric speciation, life-history evolution and the adaptive radiation of herbivore taxa (Mitter *et al.*, 1991). Nonetheless, there is a general paucity of understanding of insect/plant interactions in terms of the preference-performance, especially for the Tephritid fruit flies.

At the wider host preference and larval performance subject level, knowledge gained through the various studies has led to the suggestion of hypotheses on this relationship (Thompson, 1988; Price, 1997), among which is the ideal free distribution theory (IFD) (Fretwell and Lucas, 1970). The IFD theory, which predicts how organisms distribute themselves in a heterogeneous environment, has served as an important foundation for several related theories in ecology (Thompson, 1988). The IFD theory assumes that habitat suitability decreases with increasing

densities of organisms, but that for a given density, habitats differ in their suitability. It then predicts that if organisms have perfect knowledge of their environment and behave ideally, then they should distribute themselves so that the average fitness is approximately equal in all habitats (Ellis, 2008). Among the extensions of this theory, is the oviposition preference–offspring performance (P–P) hypothesis (Thompson, 1988; Nufio and Papaj, 2004; Scheirs *et al*., 2004; Ellis, 2008; Santos and Silveira, 2008; Bonebrake *et al*., 2010; Heard, 2012).This hypothesis predicts how ovipositing females distribute their offspring in a heterogeneous environment (Thompson, 1988; Nufio and Papaj, 2004). Specifically, it predicts that when insects utilize discrete hosts or environments, and have juveniles that have limited ability to disperse, females should evolve oviposition behaviors that maximize offspring growth and survival. The expected consequence is a positive correlation between preference and performance among the available host genotypes (Thompson, 1988).

Several studies have reported strong, positive preference-performance correlations in a number of herbivorous insects (Rossi and Strong, 1991; Hanks *et al.,* 1993). On the contrary, other studies reported results against this pattern: many insects preferentially oviposit on plant genotypes that do not yield highest fitness (Karban and Courtney, 1987; Courtney and Kibota, 1990; Horner and Abrahamson, 1992; Larsson and Ekbom*,* 1995). In response to these inconsistent results, a broad range of hypotheses has emerged to explain the lack of a positive preference-performance correlation. Among these, possible causes for the subvert occurrence of a one-to-one correspondence between preference and performance have included: host confusion and limited discriminatory ability of herbivores (Larsson and Ekbom, 1995), plant appearance (Chew and Courtney, 1991), novel association between host plant and herbivore (Thompson,

1988; Joshi and Thompson, 1995; Larsson and Ekbom, 1995), phenology of herbivore oviposition (Briese, 1996), herbivore abundance (Wiklund, 1982), environmental predictability (Chew and Courtney, 1991; Lalonde and Roitberg, 1992), and parasites and predators (Strong and Larsson, 1994). Earlier on, Jaenike (1986) and recently, Scheirs *et al.,* (2004) also proposed the optima foraging theory as one of the reasons for the subvert occurrence. In this theory, females may select those hosts that are optimal for adult nutrition instead of those that are optimal for their offspring, and hence female insects maximize fitness through the optimization of adult performance. Consequently, there is need for more studies to understand this (PPH) linkage, especially for species where there is lack of information on preference-performance relationship, such as the African invader, (*B. invadens*).

Due to its polyphagous nature*, B. invadens* is a good organism for testing the preference-performance hypothesis. *Bactrocera invadens* infests over 44 known cultivated and wild hosts (Ekesi *et al*., 2006; Mwatawala *et al.,* 2006b; Rwomushana *et al*., 2008; Vayssières *et al*., 2009; Mwatawala *et al.,* 2009a and 2009b). The highly polyphagous nature of *B. invadens* could be facilitated by the higher behavioral adaptation characteristics of polyphagous species. Such adaptations enable these species to oviposit in a wide range of fruit crops (Aluja and Mangan, 2008), offering an opportunity to assess its relative preference and performance in the various hosts. Although studies have suggested that *B. invadens* prefers certain fruit host types (Ekesi *et al*., 2006a; Mwatawala *et al.,* 2006b; Rwomushana *et al*., 2008; Vayssières *et al*., 2009; Mwatawala *et al.,* 2009a; Geurts *et al.,* 2012), or mango fruit varieties (Ambele *et al.,* 2012), most have not adequately demonstrated differences in performance between the fruit species and varieties that may generate pressures necessary for the evolution of these behaviors.

The purpose of this study, therefore, was to examine the effects of fruit species and variety on the performance of *B. invadens*. This study tested the hypothesis that because of its polyphagous nature, *B. invadens* can obscure the expected positive correlation between adult oviposition preference and offspring performance. Specifically, the study aimed at establishing patterns of oviposition in relation to resource type (fruit species and varieties) and comparing offspring performance among the resource types. Results were then used to provide information on the potential roles of resource type (fruit species and varieties) in determining the preference–performance relationship and the evolution of *B. invadens* oviposition preferences. This information may be used as an important means in planning a successful integrated pest management (IPM) program for *B. invadens.*

5.2   Materials and Methods

5.2.1   Study area

The study was conducted in the Lake Victoria Crescent (LVC) agro ecological zone, Uganda (Figure 3.1), as described in Chapter Three.
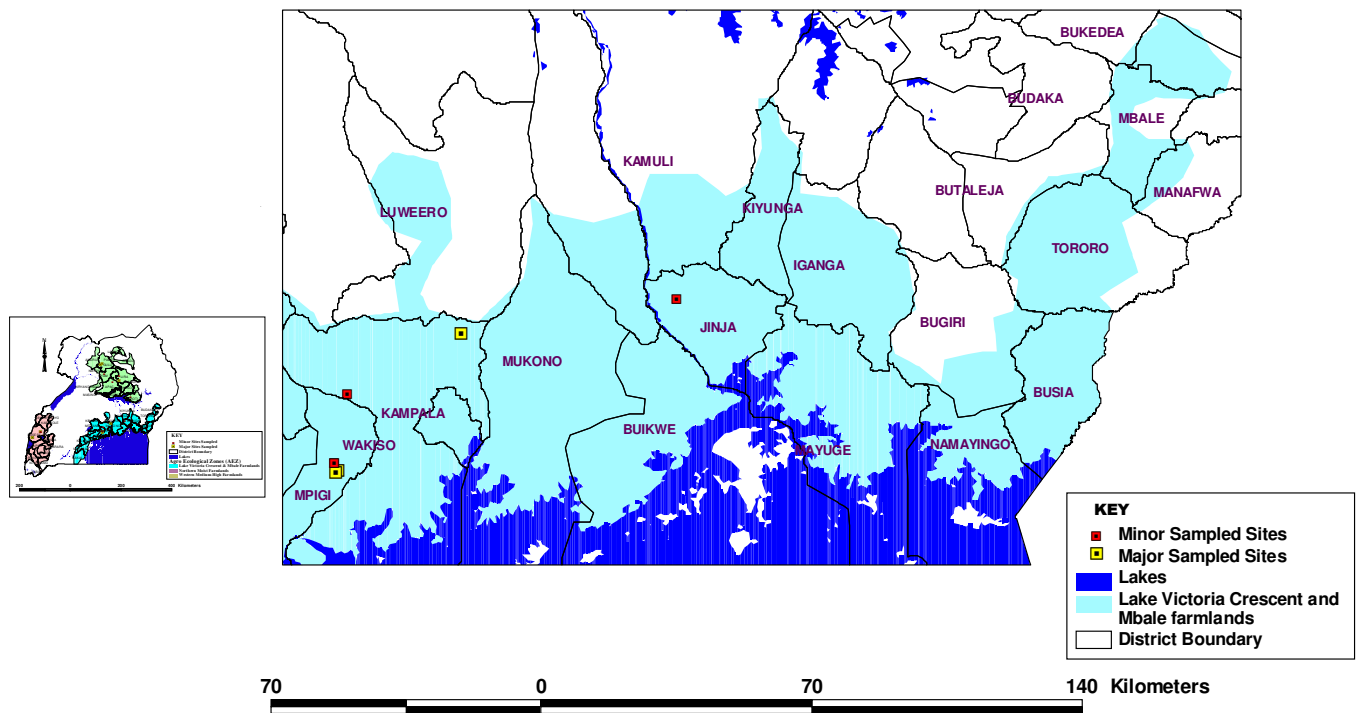
Figure 5. 1:   Map of Uganda showing the location of Lake Victoria Crescent, and the details of the zone with the respective sampling sites.

5.2.2   Host plants

Five host plants were used in this study, including sweet orange (*Citrus sinensis* (L.) Osbeck), tropical almonds (*Terminalia catappa L.*), avocado (*Persea americana* Miller, variety 'Local'), guava (*Psidium guajava* L., variety 'common guava') and mango (*Mangifera indica* L., variety 'Mixed'). These fruits were selected because they are some of the most important economic fruits, and are primary host plants of *B. invadens*. Tropical almonds were included on this list mainly because of its best *B. invadens* host status in the area (Chapter 4). The selected mango varieties included: Tommy, Zillatte, Keitt, Kent, Kagogwa, Apple, Palvin, Dodo, Kate, Biire and Glen. The five species of host plants, including varieties for the mango host, were randomly sampled in the LVC mainly Wakiso, Luwero and Jinja districts (Figure 5.1).

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5.2.3   Host oviposition preference and larval development

Field (both on ground and trees) collected fruits naturally infested by *B. Invadens* were incubated to determined fruit host preference for oviposition (Aluja *et al.,* 2009). For this reason, a few (3) of the fruits were infested by *B. invadens* and some *Ceratitis* species. These samples with double infestations were excluded from the study records for consistence. Collected fruits were transported to the rearing unit at the National Agricultural Research Laboratories (NARL), where they were washed with soap and rinsed, and allowed to air dry before being kept in individual rearing buckets and provided with appropriate medium for pupation as recommended by Copeland *et al.* (2002). Each container was capped with a plastic lid perforated by two-screened 5- by 7-cm ventilation holes, which were covered by a 0.3 mm nylon screen mesh. Containers were placed in an outdoor screen house insectary. The insectary was 15 by 13 m, with half block walls and screen windows and doors. The building was covered with a corrugated iron roof, which provided some degree of shading from ambient sunlight. Temperature conditions in the insectary were very comparable to conditions outside, but experiments were sheltered from wind and precipitation. Temperature was monitored with an onsite thermometer, which gave morning, afternoon and evening readings to average for the day. Conditions at the rearing unit averaged 28 ± 2^0C (range 25.0-30.1^{0}C) and $58\pm5\%$ relative humidity over the experimental period.

Hatched larvae and tenneral adults that emerged were carefully removed and handled following methods described by White and Elson-Harris (1992). The adults were fed with an artificial diet consisting of 10% honey-water solution through a cotton ball in separate plastic transparent buckets (22 X 33cm). These buckets were maintained on countertop space in the laboratory. The larvae and tenneral adults, which varied in number because of differences in emergencies, were

monitored for development and mortality at 12-h intervals. In the meantime, the dishes were cleaned, and cotton ball replaced as needed. The adults were sexed and separately weighed. Developmental time of development stages were measured as time (days) for each pupae to develop into tenneral adult stage. The study was repeated using similar methods for mango varieties (2.2) only that in this case adults were not sexed and weighed, mainly for logistical reasons.

5.2.4   Data Analysis

To compare fruit species and varieties preference, infestation rates were calculated according to Cowley *et al*., (1992). The infestation index values were tested for normality using Anderson and Darling tests and when strongly skewed, they were transformed (log(x +1) prior to analyses if necessary to meet the assumption of normality and homogeneity of variances. Means were separated using the post-hoc Tukey (HSD) Significant differences test at $\alpha < 0.05$. The Kaplan–Meier estimator was used to estimate the survival function from species and varieties fruit fly emergent lifetime data, construct hazard plots and to estimate mean survival times. To test for equality of the survival distribution functions of the Kaplan–Meier survival function, Log-rank tests at $P < 0.05$ were used. For all emergence data, cumulative adult emergence curves over time were constructed. Because cumulative emergence curves were sigmoid, all data were transformed using the inverse cumulative normal distribution function (XLSTAT, 2010). This function transformed the sigmoid proportion data to a linear relationship with time, relating the data to a normal distribution of 3 (for zero) through 3 (for 1), with 0.5 cumulative emergences set equal to zero (the mean of a normal distribution) (Myers *et al.,* 2007). Linear regression analysis was conducted on the transformed cumulative emergence data versus time.

To make pair wise comparisons of regression slopes, t values were calculated using the following formula:

$$T = \frac{(m1-m2)-\mu m1-m2}{\text{Sqrt}([SEm1]^2+[SEm2]^2)}$$

Where T is t value, m1 is slope of regression line 1, m2 is slope of regression line 2, $\mu m1 - m2$ is the hypothesized difference in slopes, SEm1 is standard error of regression slope 1, and SEm2 is standard error of regression slope 2 (Myers *et al.,* 2007). Slopes were considered significantly different if t-value probability derived from T distribution was less than 0.05. To test for the effect of host species on *B. invadens* adult weight, one-way analysis of variance (ANOVA), followed by Tukey HSD's multiple range test at P<0.05 was used, while effect on sex ratio was tested using Kruskal-Wallis test and multiple post-hoc analysis done using Steel-Dwass-Critchlow-Fligner test after application of a Bonferroni correction (XLSTAT, 2010). Linear regression and one-way Multivariate Analysis of variance (MANOVA) were used to test for the ultimate relationship between B. *invadens* host preference and offspring performance indicators (Cronin and Abrahamson, 1999; Scheirs *et al.,* 2004; Santos and Silveira, 2008). All these analyses were done using PAST computer program (Hammer *et al.,* 2002) and XLSTAT2010 (XLSTAT, 2010).

5.3   Results

5.3.1   Fruit Host Preference for Oviposition

In the natural field conditions, numbers of *B. invadens* larvae per fruit on the five fruit types differed significantly (F = 2.759; df = 4, 60; P = 0.038) (Figure 5.2A). *B. invadens* oviposited tropical almond fruit the most (17.38±7.3 larvae, range 0 - 73), followed by guava (13.77±5.8, 0 - 75), and the least on avocado (2.46±1.2, 0 - 13). Tropical almond did not differ significantly

with guava, but was preferred more significantly (P = 0.0001) than mango, avocado and orange (citrus). Mango and guava were not significantly different, but differed significantly with citrus and avocado, which were not different. Similarly, fruit fly oviposition on the 11 mango varieties differed significantly (F = 2.211; df = 10, 2031; P = 0.015) (Figure 5.2B). *B. invadens* oviposited the most on Glen mango fruits (57.8±23.7), followed by Biire (43.1±5.5) and Kate (37.1±8.3), and the least on Zillatte (13.3±5.2) and Tommy (12.0±4.8). Glen, Biire and Kate were significantly different (P = 0.0001) from Zillatte and Tommy, but only Glen and Biire differed significantly with Kagogwa, Kent and Keitt. The latter three were not significantly different from Kate, Dodo, Palvin and Apple varieties. Consequently, the 11 varieties formed three discrete groups: Glen and Biire (most susceptible), Zillate, Tommy and Keitt (least susceptible), while the rest of the varieties were intermediates in susceptibility.
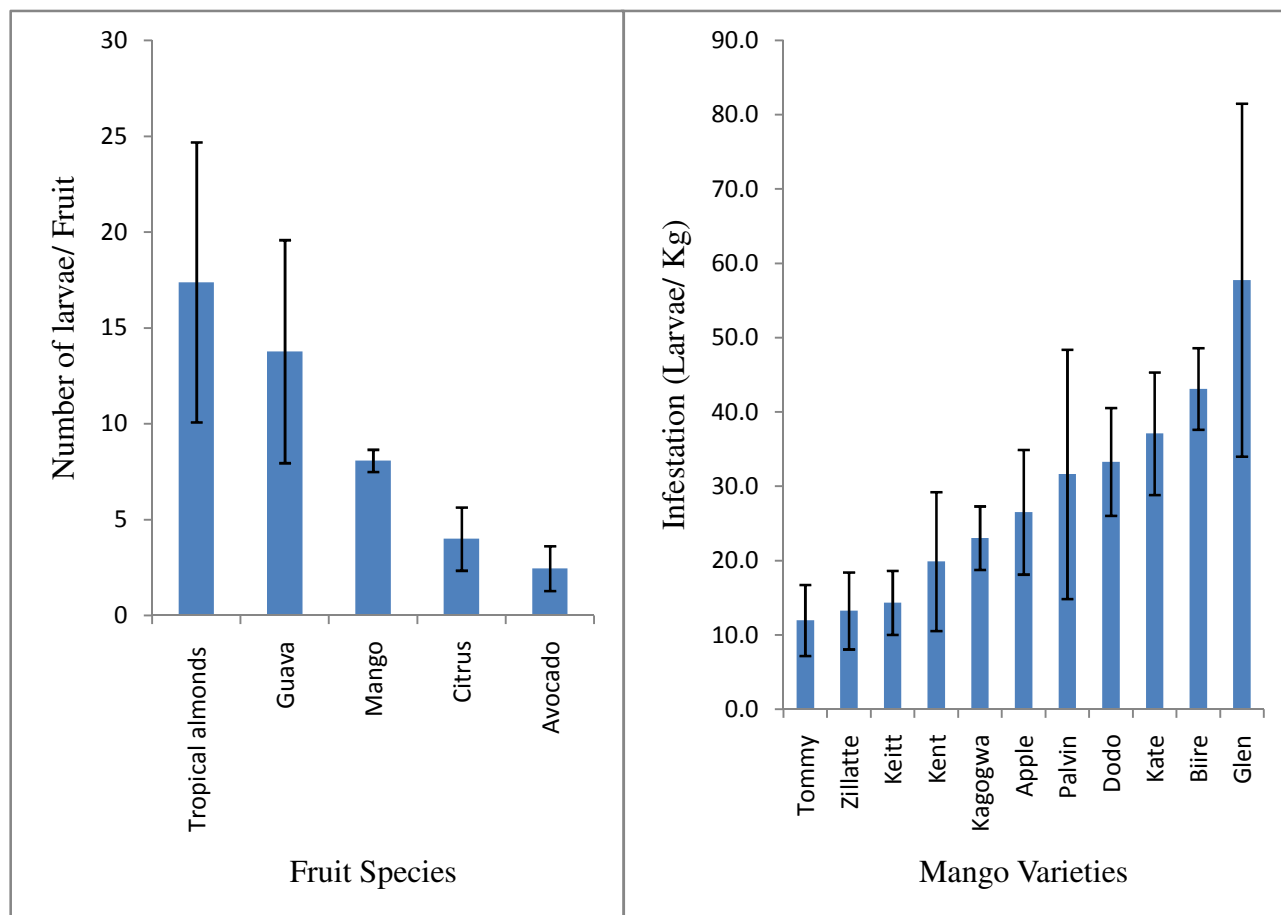
Figure 5. 2: *Bactrocera invadens* larvae relative larval load Mean (±SE) per host plant species (A) and mango host variety (B)

5.3.2 Fruit fly performance on host plants

Effect on pupal development to tenneral adult

Generally, development was significantly affected by host plant type and fruit variety (F = 111.1; df = 4, 60; P = 0.0001) and (F = 123.12; df = 10, 132; P = 0.0001) respectively, with highly susceptible fruits recording faster development rates (Figure 5.3A and B). Transformation of adult emergence curve data from larval studies resulted in highly significant linear relationships between time (accumulated days) and cumulative emergence for all fruit species and mango varieties (Table 5.1). The slope of the transformed emergence curves indicated that development occurred significantly faster on tropical almonds (Slope = 0.832±0.256) and least on citrus (0.404±0.025) (Table 5.1). Post-hoc analysis of the host species effect on emergency time

showed that cumulative development time was not significantly different between tropical almonds and guava, or mango, and guava and mango ($T_{22} = 0.287$, $P > 0.05$, $T_{22} = 0.392$, $P > 0.05$, $T_{22} = 0.212$, $P > 0.05$, respectively). Similarly, guava and mango or avocado were not significantly different ($T_{22} = 0.212$, $P > 0.05$, $T_{22} = 1.568$, $P > 0.05$), while mango vs avocado were also not significantly different ($T_{22} = 0.871$, $P > 0.05$). *B. invadens* on tropical almonds ($T_{22} = 3.776$, $P < 0.05$) and guava ($T_{22} = 3.067$, $P < 0.05$) developed significant faster than on citrus, but paradoxically the latter did not differ in *B. invadens* development rates with mango ($T_{22} = 1.334$, $P > 0.05$) and avocado ($T_{22} = 0.481$, $P > 0.05$).

Table 5. 1: Effect of five host species, mango fruit variety and susceptibility levels on *B. invadens* larval development rates

Fruit Type	Susceptibility*	Fruits	Regression statistics-summary					
			Slope*	SE Slope	R² (%)	df	F value	P value
Species	Very high	Almonds	0.830a	0.256	67.8	11	10.545	0.023
	High	Guava	0.750ab	0.110	92	11	46.171	0.002
	Medium	Mango	0.698abc	0.219	63	11	10.203	0.019
	Low	Avocado	0.472bc	0.139	59	11	11.493	0.009
	Low	Citrus	0.404c	0.025	95.9	11	260.021	0.000
Varieties	Medium	Apple	0.032	0.004	93.5	12	157.238	0.0001
	High	Biire	0.029	0.003	82.3	16	69.597	0.0001
	Medium	Dodo	0.032	0.003	85.4	16	87.436	0.0001
	High	Glen	0.020	0.004	37.7	16	9.069	0.009
	Medium	Kagogwa	0.008	0.002	66.9	16	30.253	0.0001
	Medium	Kate	0.011	0.002	66.5	16	29.76	0.0001
	Low	Keitt	0.025	0.003	85.1	16	85.619	0.0001
	Medium	Kent	0.009	0.002	49.1	16	14.479	0.002
	Medium	Palvin	0.019	0.006	42	16	10.84	0.005
	Low	Tommy	0.004	0.001	67.3	16	30.891	0.0001
	Low	Zillatte	0.006	0.001	68.2	16	32.144	0.0001

*Cumulative development values transformed by inverse cumulative normal distribution function to a linear relationship with time. The 3-point (low, medium and high) susceptibility scale for fruit species was based on relative emergency of *B. invadens* in Figure 5.2.

In terms of mango varieties, emergence was most rapid on Apple, Dodo and Biire (0.032, 0.032 and 0.029, respectively), while the least were in Tommy, Zillatte and Kent (0.004, 0.006 and 0.009, respectively; Table 5.1; Figure 5.3B). Post-hoc analysis of mango variety effect on emergency time showed that cumulative development rates were not significantly different among Apple, Dodo, Biire, Palvin, Keitt and Glen ($P > 0.05$), Kate, Kent and Kagogwa ($P > 0.05$), and between Zillate and Tommy ($P > 0.05$). *B. invadens* development was significantly fastest between Apple or Dodo vs Tommy ($T_{32} = 6.791$, $P < 0.05$), followed by the two (Apple and Dodo) vs Zillate ($T_{22} = 6.306$, $P < 0.05$). Moreover, Kent Kate and Kagogwa did not record significantly ($P > 0.05$) better development rates, while Biire, Keitt, Palvin, and Glen recorded better development rates ($P < 0.05$) than Tommy and Zillatte. Although not significantly different ($K = 4.755$; df = 2; $P = 0.073$), *B. invadens* on highly susceptible varieties developed faster (slope = 0.025±0.005), as compared to medium susceptible varieties (0.019±0.004) and the least susceptible group (0.005±0.001).
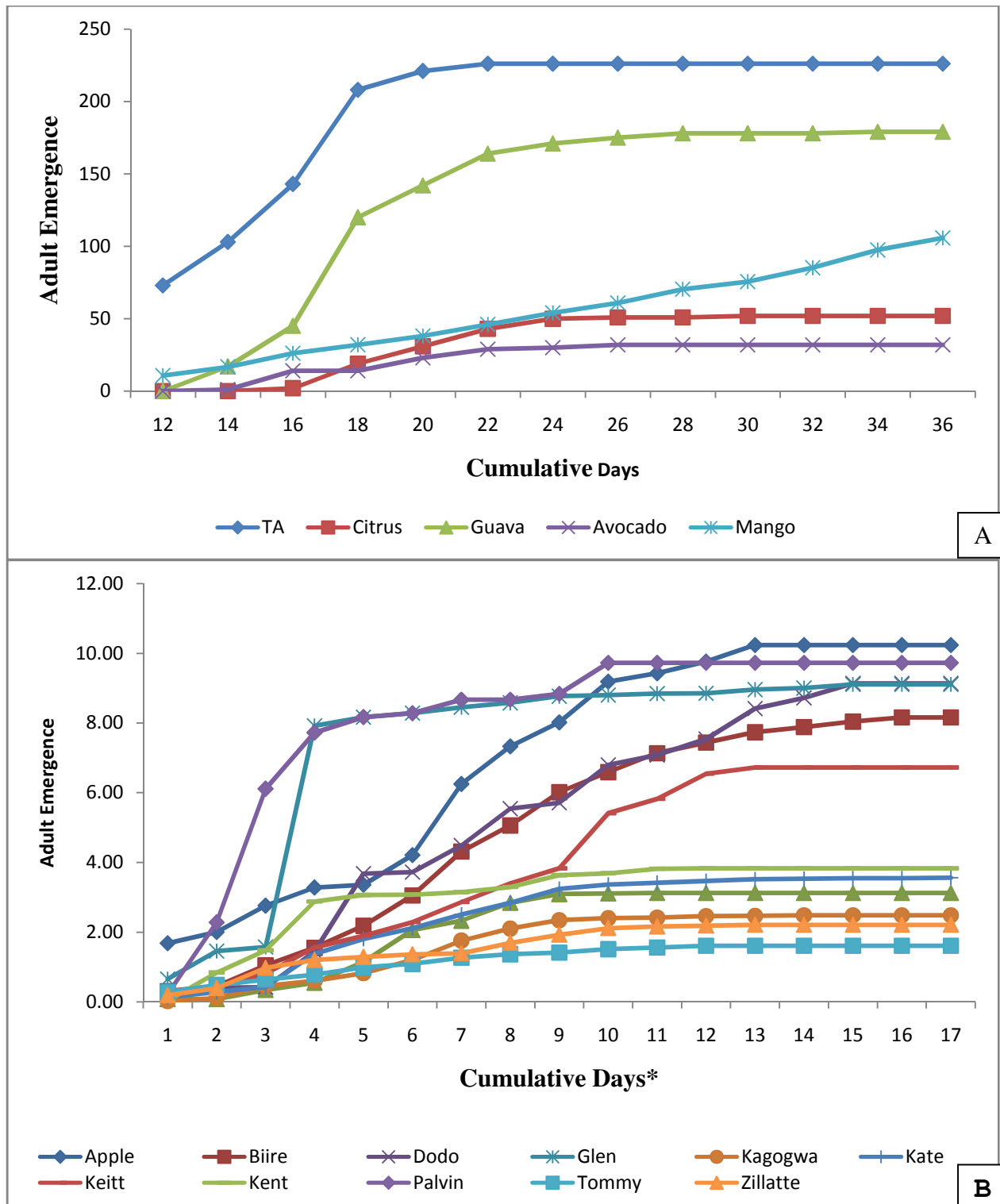
Figure 5. 3: Proportional *B. invadens* larval development rates for the five host types (A) and 11 mango fruit varieties (B). *Cumulative days indicate the days of adult emergency following sample incubation.

Effect on *Bactrocera invadens* adult survival

The survival rates of *B. invadens* adults varied significantly on the five host plants (Log-rank = 54.044, df = 4, P = 0.0001) (Table 5.2; Figure 5.4A). The highest survival rate was on tropical almonds (41.065±1.61 d), followed guava (31.291±1.94 d), and least on citrus (23.133±1.55 d) (Table 5.2). With the exception of guava and mango, the survival rates of *B. invadens* on almonds, avocado and citrus were significantly different (Table 5.2). Similarly, accumulated survival rates of *B. invadens* adults on the eleven mango host varieties differed significantly (Log-rank = 42.268, df = 10, P = 0.0001) (Figure 5.4B), and the highest was on Biire (36.347±2.00 d), followed by Glen (34.095±1.85 d), Kate (33.976±1.70 d), and the least on Tommy (23.580±2.18 d) and Apple (23.609±2.07 d) (Table 5.2). Biire variety conferred significantly more longevity to adult flies than the rest of the varieties, followed by Glen and Kate, the latter two not recording significant differences in longevity (P > 0.005). Kagogwa (31.630±1.72 d) was significantly (P < 0.05) a better host than Tommy, Apple and Keitt, although it did not differ significantly with Palvin, Dodo, Zillatte and Kent (P > 0.05). The latter four varieties recorded varied survival rates, but the difference was not significant (P > 0.05).

Table 5. 2: Longevity (Mean days ± SD) of *B. invadens* reared from five host species and 11 mango varieties

Fruit Type	Fruits	Sample size	Total Censored	Mean survival time (Days±SD)	Boundaries (95%)
Species					
	Citrus	25	22	23.133±1.55d	20.104 - 26.161
	Avocado	451	211	28.098±0.63c	26.870 - 29.327
	Mango	715	404	31.306±0.67b	29.995 - 32.617
	Guava	84	50	31.291±1.94b	27.485 - 35.097
	Tropical almonds	155	121	41.065±1.61a	37.904 - 44.227
Varieties					
	Biire	41	37	36.347±2.00A	32.420 - 40.275
	Glen	41	29	34.095±1.85B	30.461 - 37.728
	Kate	41	28	33.976±1.70B	30.637 - 37.314
	Kagogwa	41	23	31.630±1.72C	28.258 - 35.001
	Palvin	41	18	28.838±1.85CD	25.219 - 32.457
	Dodo	41	17	28.722±1.79CD	25.221 - 32.222
	Zillatte	41	15	26.223±2.09CD	22.122 - 30.324
	Kent	41	14	25.018±2.18CD	20.746 - 29.291
	Keitt	41	7	23.705±1.93D	19.920 - 27.491
	Apple	41	9	23.609±2.07D	19.553 - 27.666
	Tommy	41	14	23.580±2.18D	19.313 - 27.848

*Means within the groups with similar letters are not significantly different (ANOVA followed by Tukey HSD's multiple range test, P<0.05)
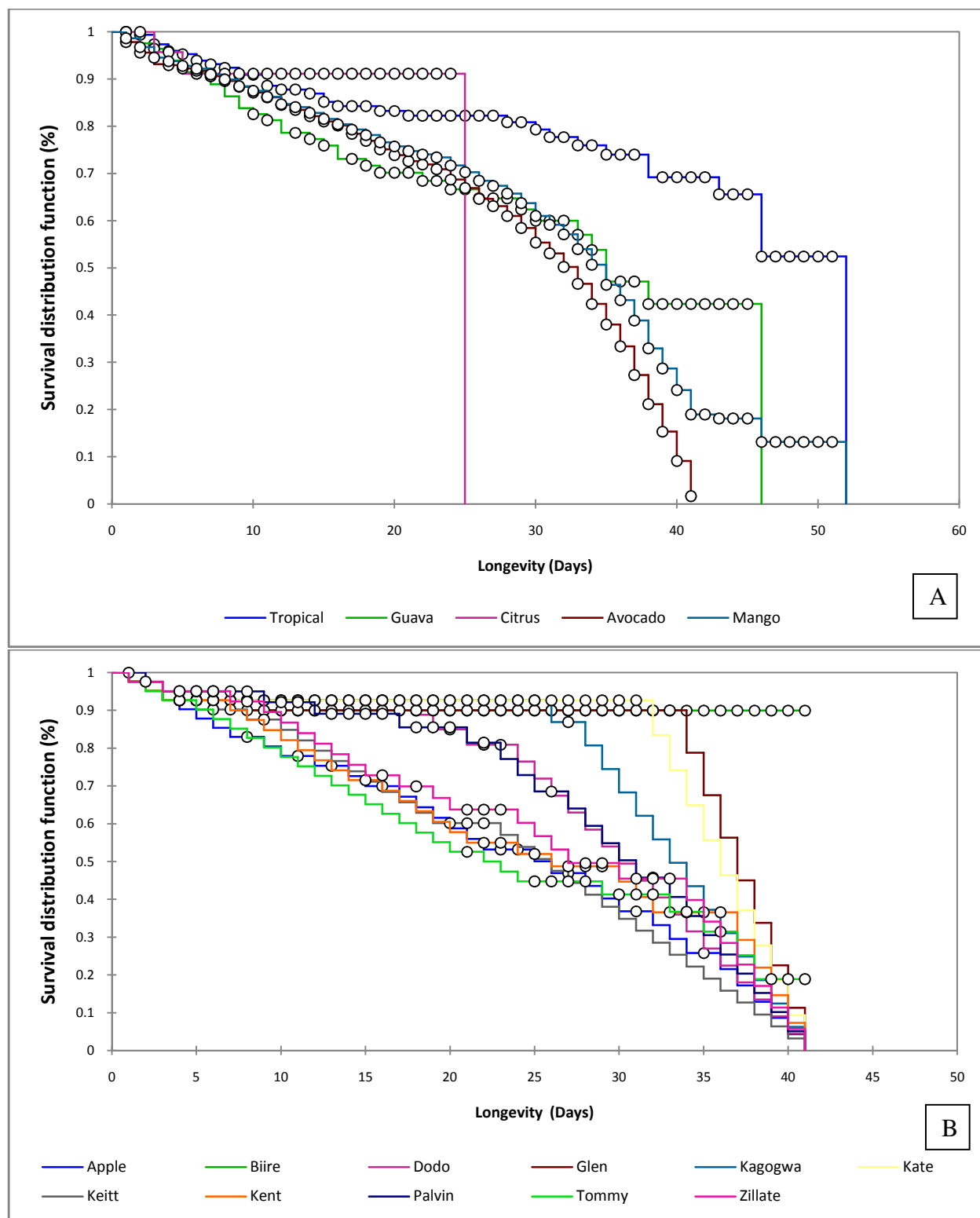
Figure 5. 4: Survival distribution function curves of B. *invadens* adults on five host plants (A) and variety-specific survival on eleven mango varieties (B).

Effect on adult weight and sex ratio

B. invadens adult weights differed significantly depending on the host species on which the larvae were reared ($F_{9,\ 225}$ = 3.052; P = 0.002) (Table 5.3). Female from tropical almonds were heaviest (2.135±0.89mg), but only differed with flies from mango, which weighed the least (1.458±0.39mg). Weight of females from mango was not different from females from guava, avocado or citrus. Males from almonds were heaviest but only different from males reared from citrus; weight of males from citrus was not statistically different from that of males from mango and avocado (Table 5.3). Adults from almonds or guava were heaviest, and the least were flies reared from citrus (Table 5.3).

The level of bias in sex ratios varied significantly across the fruits (P = 0.011). More male adults emerged than female adults when their larvae were reared from all host plants, except tropical almonds that registered relatively more females than males (Table 5.4). The level of sex ratio bias among adults varied significantly, in mango and guavas, approximately one female emerged for every two males (1/2), while in avocado and citrus the ratio was 1 female: 4 males (1/4).

Table 5. 3 Effect of five host species on *B. invadens* adult weight and sex ratio. Means within the groups with similar letters are not significantly different

	Adult weight (mg/ fly ± SD)			Sex ratio
Host plants	**Female**	**Male**	**Overall**	**(Female: Male)**
Tropical almonds	2.135±0.89a	2.118±0.91a	2.127±0.89a	1.0882a
Guava	1.682±0.57ab	1.872±0.74ab	1.812±0.69ab	0.4681b
Mango	1.458±0.39b	1.833±0.48ab	1.703±0.48b	0.5333b
Avocado	1.500±0.71ab	1.636±0.67ab	1.615±0.65b	0.1818c
Citrus	1.500±0.71ab	1.364±0.50b	1.385±0.51b	0.1818c
F. value	$F_{4,82}$=3.951	$F_{4,143}$=2.844	$F_{4,230}$= 5.402	X^2_4 = 13.085
P-value	0.006	0.026	0.000	0.011

5.3.3 Relationship between Fruit fly host preference and offspring performance

Host preference (oviposition) and offspring performance (larval development rates, longevity, adult weights, and gender ratio) were nearly perfectly and significantly correlated (Table 5.4). Host preferences always resulted into significant linear relationships with offspring performance, the former explaining between 73 (female weight) and 87% (development rates) variability in the latter. Adult longevity significantly correlated as well to host preference, but the higher intercept (22.8) recorded might imply that there are other various factors for survival other than host preference (Table 5.4). The relationship between host choice and chances of female offspring (female: male ratio) was also strongly correlated (R^2 = 79.9%, P = 0.000). MANOVA of the relationship between host preference and the offspring performance measures showed support for the preference-performance hypothesis (PPH) with overall coefficient of determination of 75.4% (P = 0.000). In terms of varieties, the results also corroborated the predictions of the PPH. Results of MANOVA of the relationship between host preference and offspring performance measures showed an overall coefficient of determination of 65% (P = 0.003). However, these results indicate that the performance prediction of the PPH was not supported as development

rates of *B. invadens* reared on various mango varieties showed a weaker and insignificant relationship with offspring preference (R^2 = 19%, P = 0.186).

Table 5. 4: Linear regression and Multivariate Analysis of variance (MANOVA) between B. *invadens* host preference and offspring performance indicators of five host species and 11 mango fruit varieties

Performance indicator	Slope	Intercept	R^2	d f	F-value	P (0.05)	Regression equation
Fruit Species							
Development rate	0.03	0.38	0.867	8	84.608	0.0001	Y = 0.026X + 0.384
Survival/ Longevity	0.89	22.84	0.745	8	38.037	0.0001	Y = 0.890X + 22.84
Female weight	0.04	1.31	0.730	8	35.140	0.0001	Y = 0.037X + 1.308
Male weight	0.04	1.41	0.762	8	41.513	0.0001	Y = 0.038X + 1.410
Overall weight	0.04	1.42	0.732	8	35.552	0.0001	Y = 0.036X + 1.422
Sex ratio (female: male)	0.05	0.01	0.799	8	51.664	0.0001	Y = 0.052X + 0.001
MANOVA			0.754	6,8	17680	0.0001	
Mango Varieties							
Development rate	0.00	0.01	0.19	8	02.060	0.186^{ns*}	Y = 0.000X + 0.001
Survival/ Longevity	0.27	21.08	0.65	8	16.700	0.003	Y = 0.268X + 21.08
MANOVA			0.65	2,8	13.110	0.003	

*n.s = not significant

5.4   Discussions

The study tested the hypothesis that because of its polyphagous nature, *B. invadens* can obscure the expected positive correlation between adult oviposition preference and offspring performance for host species types and among selected varieties. In this study, PPH shapes *B. invadens* host preference among fruit species, but not among mango varieties. The perfect correlation between oviposition preference and performance among fruit species indicates that *B. invadens* females maximize offspring fitness by selecting high quality host species for feeding in order to optimize performance of their offspring. This finding is supported by several previous studies (Craig *et al.*, 1999; Rossi and Strong, 1991; Horner and Abrahamson, 1992; Hanks *et al.,* 1993) that showed significant influences of host-plant preference on offspring performance.

The insignificant correlation between oviposition preference and performance among mango varieties could suggest an optimal foraging hypothesis, which ensures that adult females maximize their fitness and not necessarily that of offsprings (Scheirs *et al.,* 2004). This implies that *B. invadens* females may not have a physiological advantage for depending on a given mango variety, but may be well suited for dealing with natural enemies associated with a particular variety (Lill *et al.,* 2002), or that adult adaptation may be slower to evolve than adult preference, a situation which has been found in other taxa (Thompson, 1988). It is also possible that the fruit flies lack the ability to discriminate among varieties.

Mating status influences the response of mature female flies to host fruit (Prokopy and Reynolds, 1998), supposedly due to egg load on females (Rhagu, 2002). *B. invadens* gravid female flies in natural settings may not always be able to find suitable host fruit for oviposition (Aluja *et al.,* 2009), and hence tend to accept unsuitable host fruits under such conditions (Prokopy and Reynolds, 1998). Further, this might be compounded by host confusion and limited discriminatory ability of *B. invadens* for the several mango varieties (Chew and Courtney, 1991; Larsson and Ekbom, 1995), phenological synchronization of the less suitable varieties with *B. invadens* oviposition (Briese, 1996), and the overwhelming *B. invadens* abundance in the region (Wiklund, 1982). Other studies have reported similar findings (Karban and Courtney, 1987; Courtney and Kibota, 1990; Horner and Abrahamson, 1992; Larsson and Ekbom, 1995; Scheirs *et al.,* 2003).

Results of this study also provide some support for the host size models (King, 1990). According to these models, insects provide their offspring with food by ovipositing in or on hosts, and that

the resource available to her offspring may affect which sex ratio a female should produce (Trivers and Willard, 1973). The host size models further suggest that females of solitary species should manipulate the sex of their offspring in response to characteristics of the hosts on which they oviposit. *T*he lower resource availability on relatively poor hosts such as citrus might be expected to increase a female's risks of starvation more than a male's, and hence ovipositing females consider chances of survival to be higher on better hosts like tropical almonds than citrus. Therefore, the observed higher number of male offsprings in the less suitable oviposition hosts (citrus and avocado) in this study could be a result of maternal manipulation. From the results, *B. invadens* manipulate offspring sex ratio in response to host quality in the manner predicted by the host size model, and the findings are consistent with earlier findings that females manipulate offspring sex ratio in response to host size in the manner predicted by the host size model (King, 1990). The research's host ratio results may, therefore, suggest that *B. invadens* manipulation of offspring sex ratio in response to host species may be a response to differences among host plant species. Further studies will be needed to determine more specifically what cues *B. invadens* females are using in their sex ratio response.

5.5   Conclusions

The results of this study showed that *B. invadens* choice of fruit species for female oviposition is guided by the need to maximize offspring performance as proposed by the preference performance hypothesis. However, among varieties of the same species (for instance mangoes), females tend to maximize their fitness and not necessarily that of offsprings as proposed by the optimal foraging hypothesis. The findings should help to understand the biology of *B. invadens* and could help in its management and control, particularly on mangoes.

CHAPTER SIX: GEOMETRIC MORPHOMETRICS OF GEOGRAPHIC AND HOST-ASSOCIATED POPULATION VARIATIONS OF *BACTROCERA* INVADENS IN UGANDA*

ABSTRACT

Fruit flies (Diptera: Tephritidae) are among the most economically important pests of edible fruits worldwide. Because of the cryptic nature of most species in the *Bactrocera* complex, it is not clear whether allopatric and host associated speciation has taken course among *B. invadens* populations in the different zones in Uganda. This would aid the design of efficient management strategies. The morphological variation of 3 allopatric and host-associated populations of *B. invadens* was studied using Euclidean distance matrix analysis and variance structure coordinate system geometric morphometrics techniques. Size and shape were analyzed throughout 15 landmarks for 360 specimens after symmetrization process. Host type significantly affected wing size and shape (P < 0.0001), with CVA showing host population discrimination with very high degree of population classification (95.03%) accuracy. Wings from mango hosts were significantly smaller than tropical almond, but the former did not significantly differ with guava. Similarly, mean wing size and shape among the 3 zones differed significantly (P < 0.0001). *B. invadens* from the WMHF had relatively bigger wings, while the NMF specimens recorded the smallest wings. There was a very high degree of zonal population classification accuracy (95.48%), with the LVC population wing shape being significantly different from NMF (P < 0.0001) and WMHF (P < 0.0001), while the latter two were also different (P < 0.0001). There were significant differences in allometry and developmental instability (fluctuating asymmetry) among fruit hosts in the order: mango > guava > almonds. The results have added to the

evidence that geographic and host-associated adaptation can produce reproductive isolation and produce ecotypes. The ability of *B. invadens* to undergo rapid ecological evolutionary radiations might affect the efficiency of common control methods, and the results of this research should help to recast fruit fly management strategies.

6.1   Introduction

Bactrocera invadens belongs to the *B*actrocera *dorsalis* species complex, which harbors a number of important pest species. The cryptic nature of most species in the *B. dorsalis* complex renders identification of some species particularly difficult even with molecular tools (Clarke *et al.,* 2005). Pest misidentification can have drastic and costly consequences for pest management (Schutze *et al.,* 2012). Identification difficulties chiefly arise due to broad intraspecific morphological variation between members of the *B. dorsalis* complex, which can seriously confound their identification (Drew *et al.,* 2008).

Tephritids are believed to have speciated prolifically throughout the tropics and subtropics (Clarke *et al*., 2005), with groups of sibling species identified (Drew and Hancock, 1994). Host fidelity and consequent sympatric speciation have also been reported in the genus *Rhagoletis* (Diptera: Tephritidae*)* (Feder *et al.,* 1993). Nevertheless, many of these sibling complexes remain difficult to identify (Schutze *et al.,* 2012). Tephritids are highly diverse in ecological and biological traits (Clarke *et al.,* 2001; Schutze *et al.,* 2012), which posses profound pest management implications (Dyck *et al.,* 2005). Where such variation occur with-in species (intraspecific variation), biotypes, host or pheromone races may emerge with variable responses

to management options (Menken *et al.,* 1996). It is not clear whether site and host specific phenotypic variations has taken course among *B. invadens* populations in Uganda, yet this would be important in the design of efficient management strategies.

Where recent radiations or variation occur, they are fine-scale, requiring high resolution and discriminating techniques to elucidate (Shaffer and Thomson, 2007). Among such techniques is Geometric Morphormetrics (GM) (Rohlf, 1999). GM involves measuring the relative positions of homologous structures or shape (e.g. landmarks or outlines) and then comparing variation among individuals or groups (Gilchrist and Crisafulli, 2006; Bouyer *et al.,* 2007; Marsteller *et al.,* 2009). As shape is regarded as a sensitive and more heritable character, GM has greater ability in detection of differences among populations or species following recent evolutionary radiations compared to traditional techniques (Dujardin *et al.,* 2003; Bouyer *et al.,* 2007).

It is believed that *B. invadens* invaded Africa recently (Drew, Tsuruta and White, 2005). Given its diverse host range and distribution, it is possible that it has undergone phenotypic variations, synonymous with *B. dorsalis* species (Clarke *et al*., 2005). This study aimed at assessing the variations among three geographic and hosts (*Terminalia catappa, Psidium guajava* and *Mangifera indica) B. invadens* populations in Uganda. The study also examined fluctuating asymmetry (FA): small random departures from perfect symmetry among individuals. This was to determine whether individuals reared from different hosts and geographical regions would show significant variability in FA.

6.2   Materials and Methods

6.2.1   Study area

The study was conducted in three agro ecological zones, Western Medium High Altitude Farmlands (WMHF), Lake Victoria Crescent (LVC) and Northern Moist Farmlands (NMF) as described in Chapter Three.

6.2.2   Sampling Procedure

In each AEZ, three orchards or areas with substantial mango growing and other potential hosts were selected to represent the major sampling sites. An insecticide strip of dichlorovos was placed in each trap to kill the adult flies. The traps were emptied on a fortnightly basis and the lures renewed bi-monthly. Fruit flies were collected and placed into vials containing 70% alcohol for preservation, labelled and transported to the National Agricultural Research Laboratories (NARL) for identification using multi-entry keys. During the same period, fruits of three types: *Terminalia catappa* (Combretaceae), P*sidium guajava* (Myrtaceae) and *Mangifera indica* (Anacardiaceae*)* were collected for assessment of *B. invadens* emergence*.* Flies emerging from these fruits constituted the host population. Confirmation of the identification of the *B. inva*dens that were trapped or emerged from fruits was done at the Royal Museum of Central Africa under the guidance of Dr. De Meyer. Voucher specimens are kept in collections at the NARL, Makerere University Zoology Museum and the Royal Museum for Central Africa, Belgium.

6.2.3   Specimen choice, slide preparation and measurement

Thirty specimens from each of the three geographical and host populations were sampled. With a few exceptions due to damage two wings were taken from each specimen for analysis. wings are

the structures recommended for studies on morphological variation using GM techniques (Klingenberg, 2003; Guan et al., 2015) because wing shape exhibits heritability, wing venation provides stable morphological landmarks that can be used to describe wing shape (Moraes et al., 2004). All specimens were assigned a unique code number, which was affixed to both the slide-mounted wing and the alcohol-preserved voucher specimen. Each wing was mounted in Canada balsam and dissected using a Leica MS5 Microscope (Schutze *et al.,* 2012).

Each specimen was imaged at $10 \times$ magnification using a Sony camera (Model No. DSC-H10, Sony Corp., Japan) mounted onto the microscope and saved in JPEG format. Fifteen type 1 landmarks on the wing were selected for each wing (Bookstein *et al.,* 1991). A total of 105 distances between the landmarks following vein junctions, vein terminations or vein suture were also computed to characterize the wing as estimations of the size and shape differentiation in the specimens (Figure 6.1). The selected landmarks included: 1 basal junction of veins of cell bm; 2 anterior-most point of the suture located towards the base of vein sc; 3 inner antero-distal corner of cell bc; 4 junction of veins A_1 and CuA_2; 5 junction of CuA_1 and CuA_2; 6 junction of vein CuA1 and dm-bm cross vein; 7 junction of vein M and dm-bm cross-vein; 8 junction of vein CuA_1 and dm-cu; 9 junction of vein M and dm-cu; 10 junction of vein M and r-m cross-vein; 11 junction of vein R_{4+5} and r-m cross-vein; 12 junction of vein R1 and costal vein; 13 termination of vein M; 14 termination of vein R_{4+5}; and 15 termination of vein R_{2+3} (Schutze *et al.,* 2012). Measurements and land mark digitization were done using tpsDig, Version 2.16 software programme (Rohlf, 1999), for which landmark x, y coordinates were generated and saved as a text file.
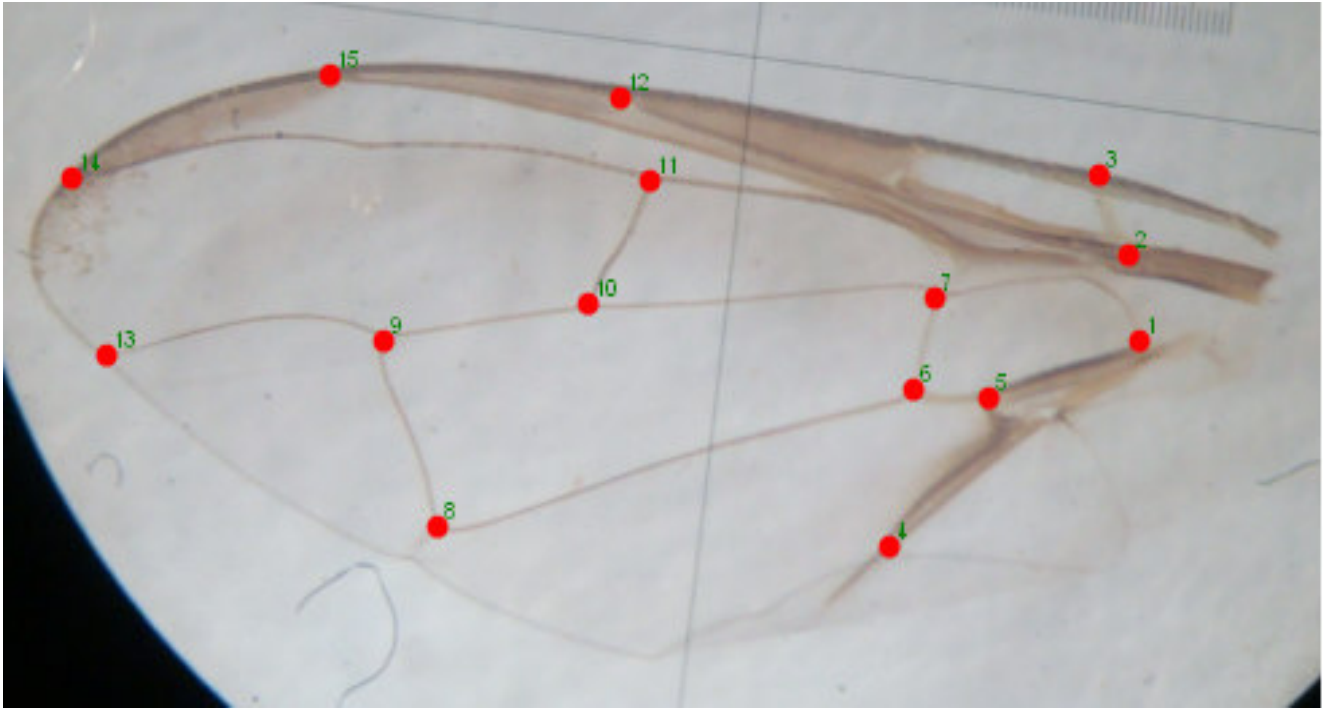
Figure 6.1 Left-hand wing of a *Bactrocera invadens* individual showing each of the 15 landmarks used in the geometric morphometric analysis. 1, basal junction of veins of cell bm; 2, anterior-most point of the suture located towards the base of vein sc; 3, inner antero-distal corner of cell bc; 4, junction of veins A1 and CuA_2; 5, junction of CuA_1 and CuA_2; 6, junction of vein CuA_1 and dm-bm cross vein; 7, junction of vein M and dm-bm cross-vein; 8, junction of vein CuA_1 and dm-cu; 9, junction of vein M and dm-cu; 10, junction of vein M and r-m cross-vein; 11, junction of vein R_{4+5} and r-m cross-vein; 12, junction of vein R_1 and costal vein; 13, termination of vein M; 14, termination of vein R_{4+5}; 15, termination of vein $R_{2+3.}$

6.2.4   Data analysis

Raw landmark co-ordinate data were imported into PAST and MORPHOJ (v 1.02E) programmes and aligned using a generalized Procrustes analysis procedure, which is a process for removing non-shape variation i.e. rotation, translation and scale from the data (Rohlf, 1999).

Centroid size was used as the measure of size for each fly wing (Hammer *et al*., 2002). Tests for significant differences among samples for each geographic or host population were undertaken using a one-way ANOVA with a Tukey post-hoc test. As wing size can also significantly influence the shape of the wing allometry, multivariate regression of the dependant variable (wing shape) on centroid size independent variable using the software package MORPHOJ was done (Klingenberg, 2011; Schutze *et al*., 2012). The statistical significance of the regression was tested by permutation tests (10,000 replicates) against the null hypothesis of independence. Procrustes transformed coordinate data was then used for subsequent Multivariate Analysis of Variance (MANOVA) and canonical variates analysis (CVA) and also for group assignment tests based on Mahalanobis distances. Individuals were a priori defined as members of one of the 3 geographic or host populations based on collection locality or fruits from which it was reared.

Following CVA, intraspecific groups were combined according to populations to form homogenous groups, for which the assignment test was undertaken to determine the number of individuals from any one sample being reassigned to their a priori population. Within populations of animals with an essentially bilaterally symmetrical body plan, minute deviations to the left or right, or the difference in value between paired bilateral traits, are known as fluctuating asymmetry FA (Van Valen, 1962; Markow, 1995). Three Fluctuating asymmetry (FA) indices FA3, FA7 and FA9 recommended by Palmer and Strobeck (1986) were computed. FA was calculated as the absolute value of the difference between the left (L) and right (R) sides for the wing size trait. FA3 is the mean of the absolute value of the unsigned difference between left and right (FA =|L − R|), divided by the population trait size mean, FA7 is the variance of the

signed difference, divided by the population trait size mean, while FA9 contains the square of correlation coefficient $1-r^2$.

6.3   Results

6.3.1   Effect of host type on intraspecific wing size and shape variation

There were significant differences among the three hosts ($F = 21.31$, df = 2, 159, $P = 0.0001$) for the effect of host type on wing size. Wings of flies from mango were significantly smaller than for those of flies from tropical almonds, but did not differ from flies from reared from guava (Figure 6.2). Inter-landmark or Euclidean distance (EDMA) between the 15 landmarks indicated significant differences among the three hosts (Wilks' $\lambda=0.07186$, df = 202, 118, $P = 0.003$), with subsequent CVA showing appreciable host population discrimination (Figure 6.3). The first two canonical variates contributed a total variability of 97.8% (CV 1 = 70.5% and CV 2 = 27.3%) to the total variance, with a very high (95.03%) degree of host population classification accuracy. The overall population dissimilarity was 1.678, and subsequent pair wise differences following the order: Guava vs. Mango > Mango Vs Almonds > Guava Vs Almonds. Mean Landmark distances of the ten distances contributing most to the differences among the three hosts are shown in Table 6.1 below. Distances between landmarks 2-4, 1-4 and 3-4 contribute highest to the differences.

Table 6. 1: Mean Landmark distances of the ten distances contributing most to the differences among the three hosts

Distance	Contribution	Cumulative %	Guava	Mango	Almonds
2_4	0.03443	10.2	0.275	0.274	0.267
1_4	0.03406	10.0	0.248	0.249	0.242
3_4	0.03253	9.6	0.300	0.298	0.292
4_8	0.03114	9.2	0.291	0.292	0.297
4_5	0.02929	8.6	0.138	0.137	0.131
4_7	0.02857	8.4	0.189	0.187	0.180
4_13	0.02813	8.3	0.540	0.539	0.545
12_15	0.02513	7.4	0.196	0.204	0.194
4_9	0.02446	7.2	0.361	0.360	0.361
4_6	0.02442	7.2	0.121	0.120	0.115
11_15	0.02366	7.0	0.235	0.245	0.246
14-15	0.02322	6.8	0.189	0.182	0.183

Similarly, there were significant differences among the three hosts (F = 21.31, df = 52, 4134, P = 0.0001) for the effect of host type on wing shape. The first two principal axes showed partial or fuzzy separation of the populations (Figure 6.4). Subsequent projection onto the first two canonical axes distinctly separated the populations into the three hosts clusters (Figure 6.5). The first two canonical variates contributed a total of 100.0% (CV 1 = 72.5% and CV 2 = 27.5%) to the total variance (Figure 6.5). Post-hoc analysis showed that wing shapes of flies from almonds were significantly different from guava (Mahalonobis distance = 2.0680, P = 0.0001) and mango (Mahalonobis distance = 1.4315, P = 0.0001), as was the case between guava and mango (Mahalonobis distance = 1.6063, P = 0.0001).

Figure 6. 2 Effect of host type on fruit fly wing size (centroid). Box plots show median, mean, 25th and 75th percentiles, and 10th and 90th percentiles.
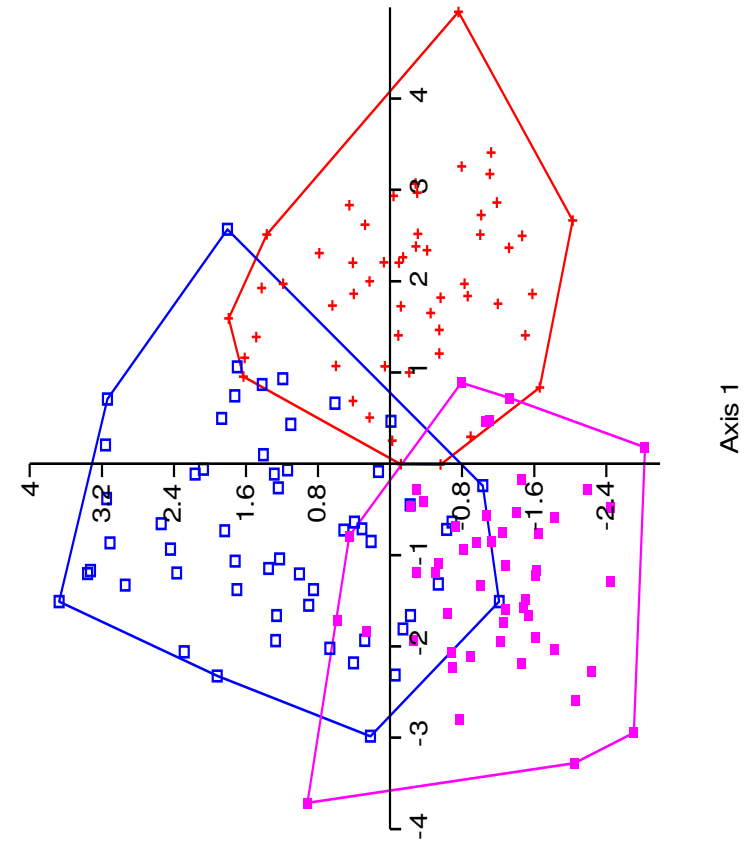
Figure 6. 3 Canonical Variates Analyisis of the effect of three hosts on wing size (centroid) for the three populations showing the three clusters.. (Blue squares: Mango; Pink squares: Tropical almonds; Red crosses: Guava). The ellipses show convex hulls for each population.
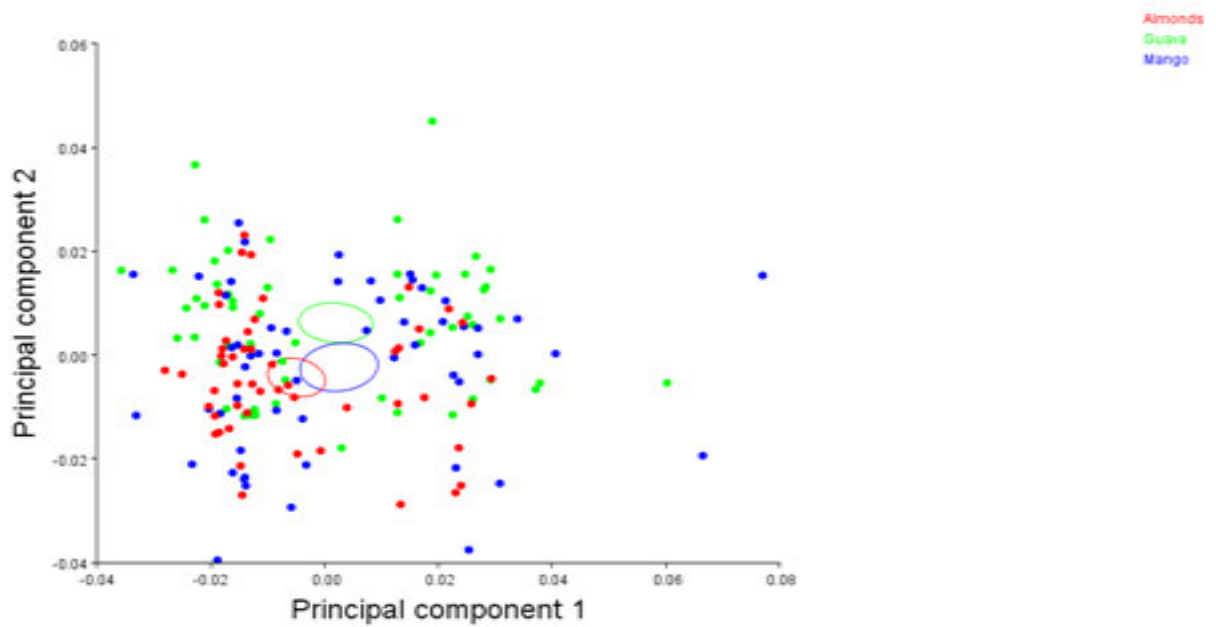
Figure 6. 1. Projection of wing data of the three *B. invadens* populations on the first two principal components showing a fuzzy separation of the populations. Blue points: Mango; Pink points: Tropical almonds; Red points: Guava. The ellipses show 90% means confidence intervals for each population.
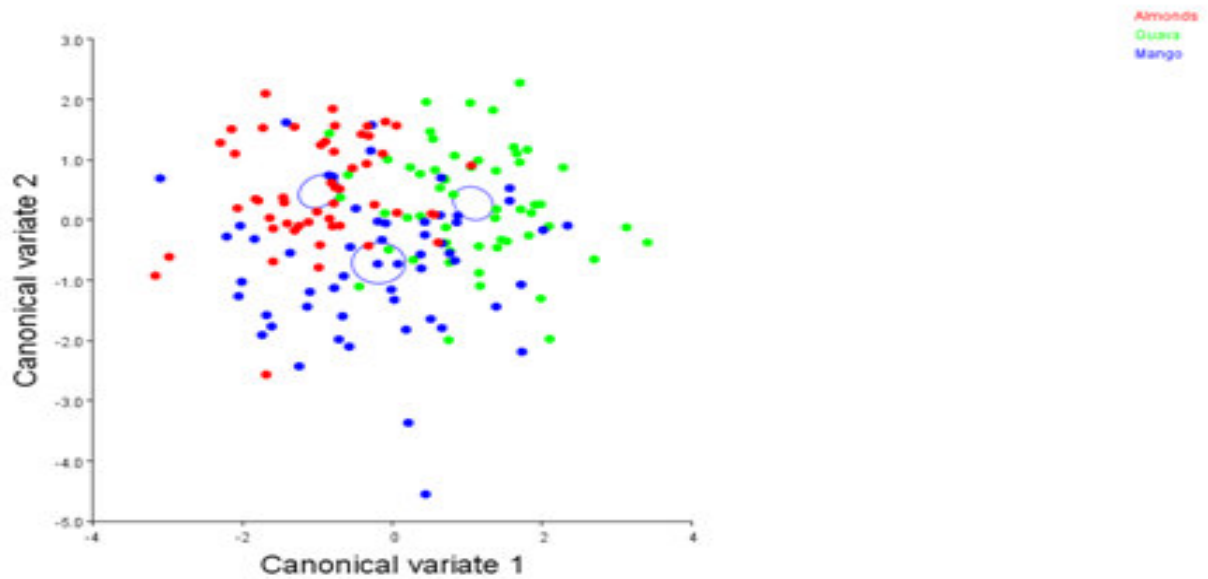
Figure 6. 2. Canonical Variates Analyisis of the effect of the three hosts on wing centroid size showing the three clusters. (Blue points: Mango; Pink points: Tropical almonds; Red points: Guava). The ellipses show 90% means confidence intervals for each population.

Discriminant function analysis with mahalanobis and procrustes distances confirmed the significant differences between the three host populations (P = 0.0001). Success rate for the reassignment of individuals from any one sample ranged from 74.55% (Mango) to 86.61% (Guava), with Almonds being intermediate to the latter two (79.41%). Multiple regression revealed appreciable allometry as the relationship between wing shape and centroid size was significant (P = 0.0052). Post-regression analysis of the means of the three host's shape-centroid size relationships showed that Almonds and Guava did not differ significantly in shape, but the two were significantly different from Mango shapes (Figure 6.6). Indeed of the three host's wing shapes, wing size significantly affected most the Mango reared *B. invadens* than the other two hosts, as indicated by the highest means confidence ellipse (Figure 6.6).
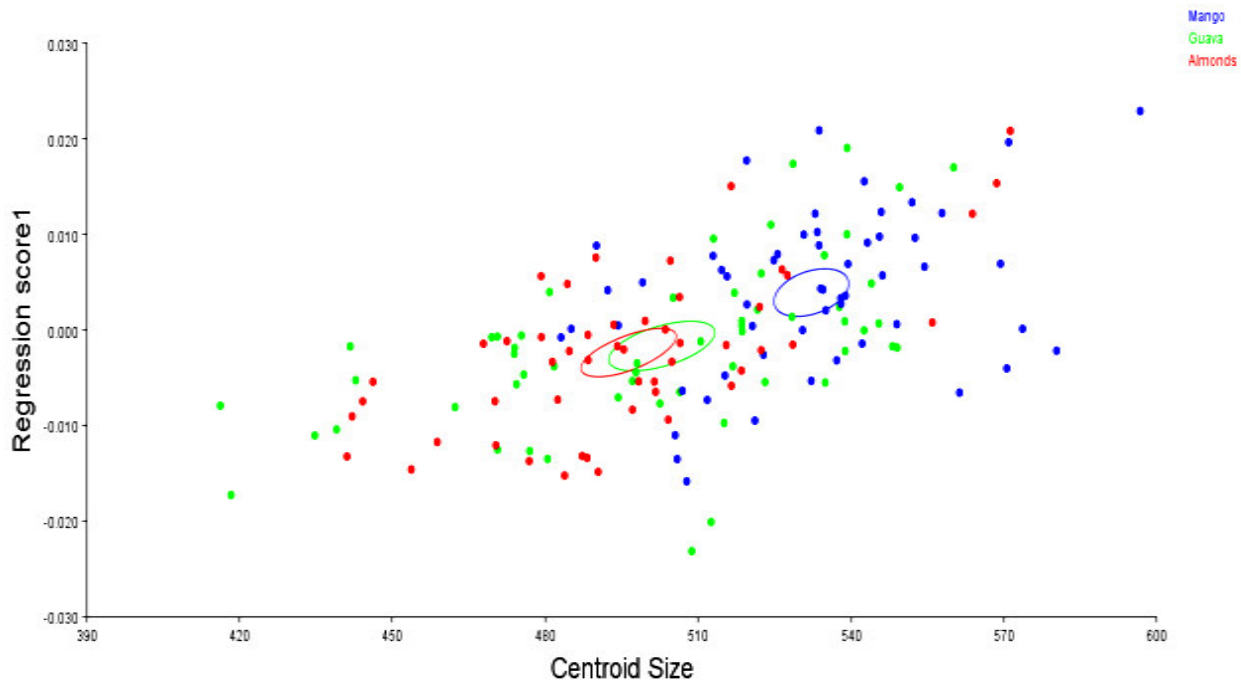
Figure 6. 3: Multivariate regression of wing shape Regression scores 1 on centroid size wing size. Blue points: Mango; Pink points: Tropical almonds; Red points: Guava. The ellipses show 90% means confidence intervals for each population.

6.3.2 Intraspecific Geographical Variations in Wing size and Shape

Mean wing size among the three zones differed significantly (F = 8152.27, df = 2, 152, P = 0.0001). *B. invadens* from the WMHF had relatively bigger wings, while the NMF specimens recorded the smallest wing sizes (Figure 6.7). Similarly EDMA distances between landmarks indicated significant wing differences among flies from the three zones (Wilks' λ=0.0517, df = 202, 96, P = 0.008), depicting significant ecotype clusters (Figure 6.8). CVA discriminant functions showed a very high degree of host population classification (95.48%) accuracy. The first two canonical variates contributed a total of 100% (CV 1 = 58.91% and CV 2 = 41.09%) to the total variance. The Bray-Curtis dissimilarity measure showed overall population dissimilarity of 1.582, with subsequent pair wise differences following the order: NMF vs. LVC > WMHF Vs LVC > WMHF Vs NMF. Mean Landmark distances between landmarks 12-13, 1-12 and 7-12 contributed highest to the differences in zonal populations (Table 6.2).

Table 6. 2: Mean Landmark distances of the 20 wing distances contributing most to the differences among the three populations.

Taxon	Contribution	Percent (%)	WMHF	NMF	LVC
12_13	0.02924	6.6	0.181	0.181	0.193
1_12	0.02631	5.9	0.188	0.193	0.186
7_12	0.02611	5.9	0.238	0.238	0.240
10_13	0.02495	5.6	0.539	0.541	0.546
3_4	0.02372	5.3	0.562	0.562	0.566
5_12	0.02299	5.2	0.597	0.598	0.603
2_4	0.02262	5.1	0.361	0.365	0.371
1_4	0.02251	5.1	0.244	0.243	0.239
11_13	0.02242	5.0	0.360	0.362	0.354
4_13	0.02159	4.9	0.449	0.448	0.452
6_12	0.02147	4.8	0.237	0.239	0.239
11_12	0.02134	4.8	0.384	0.384	0.376
4_12	0.02090	4.7	0.497	0.496	0.500
11_14	0.02081	4.7	0.455	0.455	0.458
3_11	0.02037	4.6	0.478	0.478	0.482
4_5	0.02022	4.6	0.383	0.381	0.386
4_7	0.01995	4.5	0.425	0.426	0.418
9_13	0.01905	4.3	0.28	0.278	0.271
3_10	0.01876	4.2	0.344	0.347	0.341
7_10	0.01873	4.2	0.315	0.311	0.312

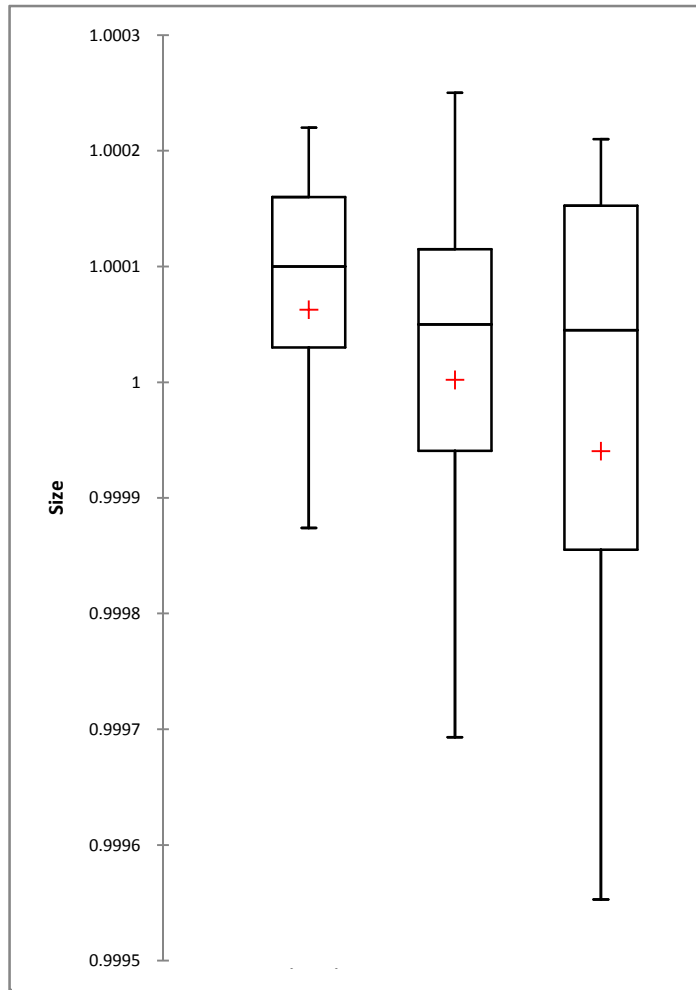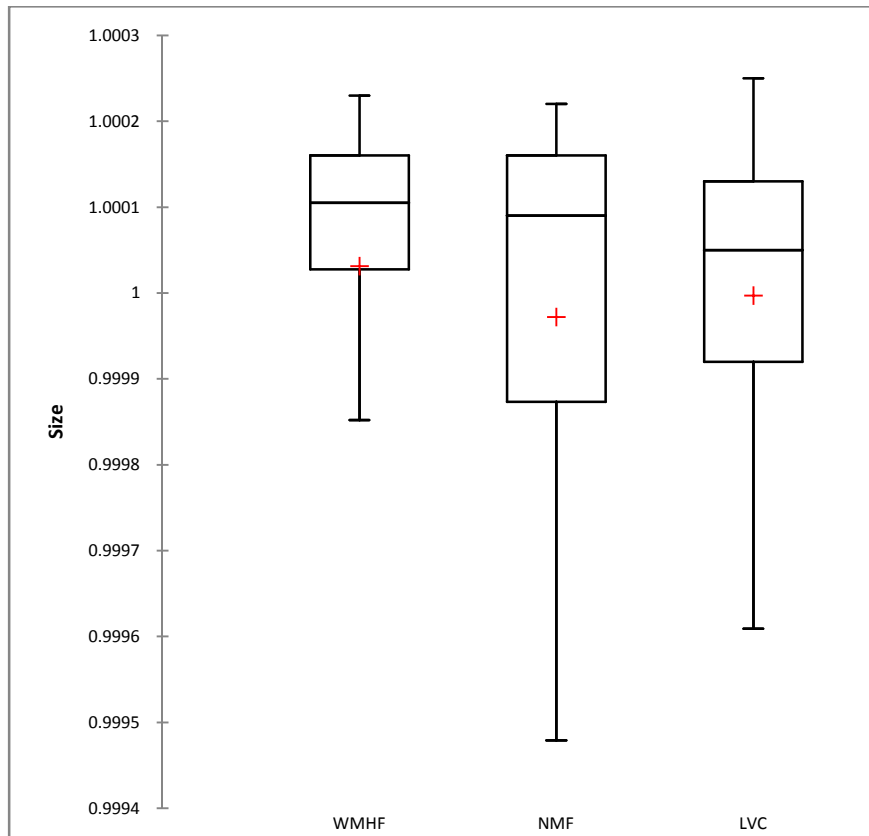**Figure 6. 4** Effect of zones on fruit fly wing size (centroid). Box plots show median, mean, 25th and 75th percentiles, and 10th and 90th percentiles.
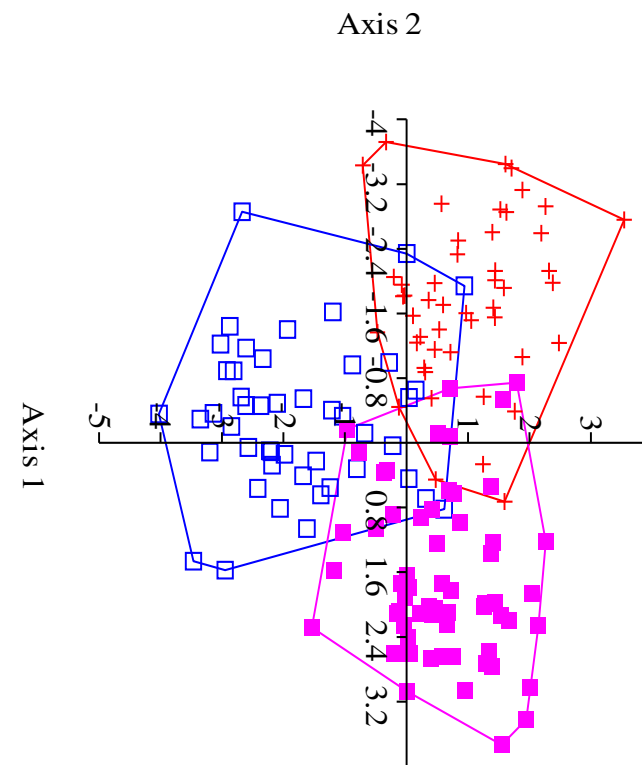
Figure 6. 5 Canonical Variates Analyisis of the effect of three zones on wing centroid size showing the three clusters. The ellipses show convex hulls for each population. (Blue squares: LVC; Pink squares: WMHF; Red crosses: NMF).

Geographical location significantly affected wing shape across the three zones ($F = 2.77$, df = 52, 3952, $P = 0.0001$). The first two principal component axes with fuzzy separation of the populations are shown in Figure 6.9. The first two canonical axes distinctly separated the populations into their respective ecotypes, with the first two canonical variates contributing a total of 100.0% (CV 1 = 53.608% and CV 2 = 46.392%) to the total variance (Figure 6.10). Post-hoc analysis showed that LVC population wing shapes were significantly different from NMF (Mahalonobis distance = 1.6673, $P = 0.0001$) and WMHF (Mahalonobis distance = 1.5955, $P = 0.000$), while the latter two were also different (Mahalonobis distance = 1.6985, $P = 0.0001$). Wing shapes discriminant function reclassification success rate for the three populations ranged from 81.67% (LVC) to 84.78% (WMHF), with NMF being intermediate to the latter two (83.68%). Multiple regression revealed significant allometry across the three populations ($P = 0.046$). Post-regression analysis showed that LVC and NMF did not differ significantly in allometry, but the two were significantly different from WMHF (Figure 6.11).
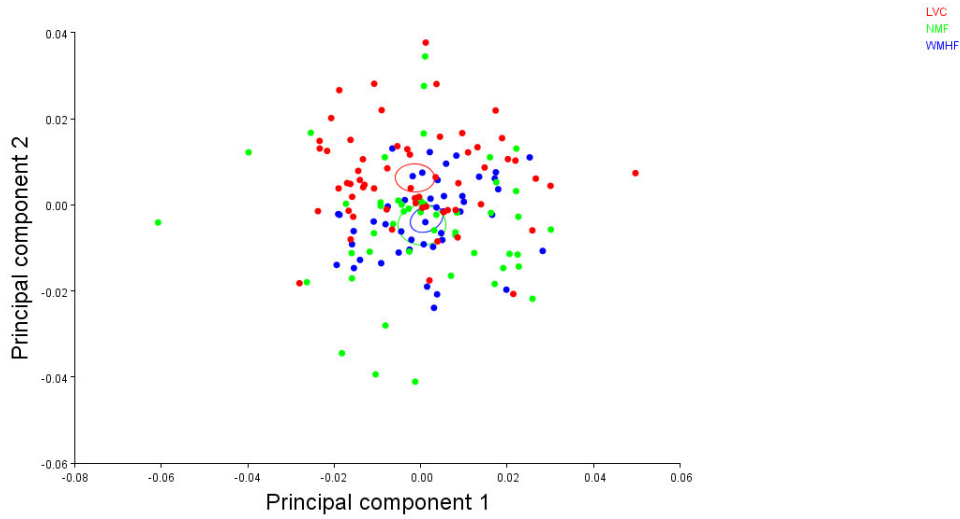
Figure 6. 6: Projection of wing data of the three *B. invadens* zonal populations on the first two principal components showing a fuzzy separation of the populations. Blue points: WMHF; Red points: LVC; Green points: NMF. The ellipses show 90% means confidence intervals for each population.
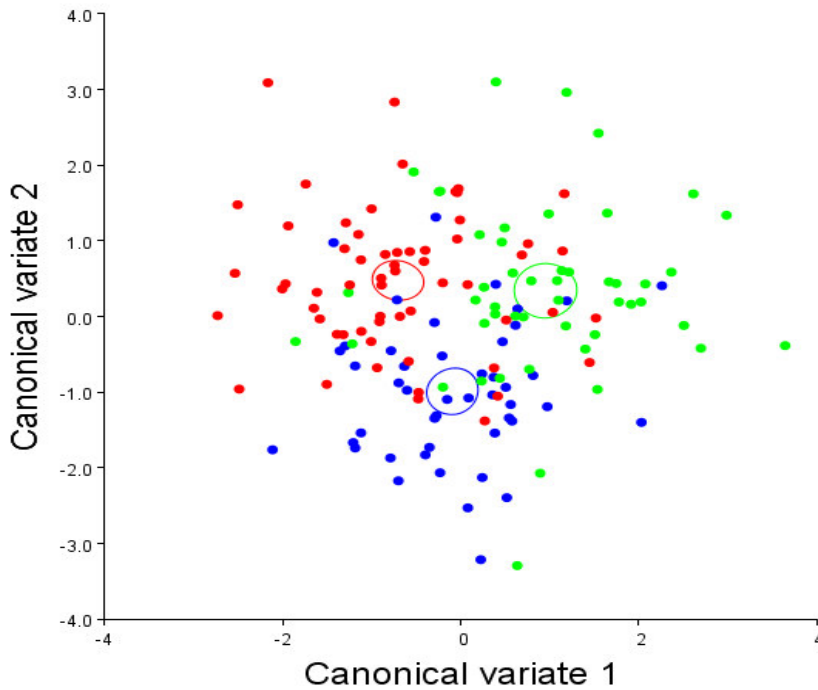
Figure 6. 7. Canonical Variates Analyisis of wing shapes for the three populations. (Blue points: WMHF; Red points: LVC; Green points: NMF). The ellipses show 90% means confidence intervals for each population.
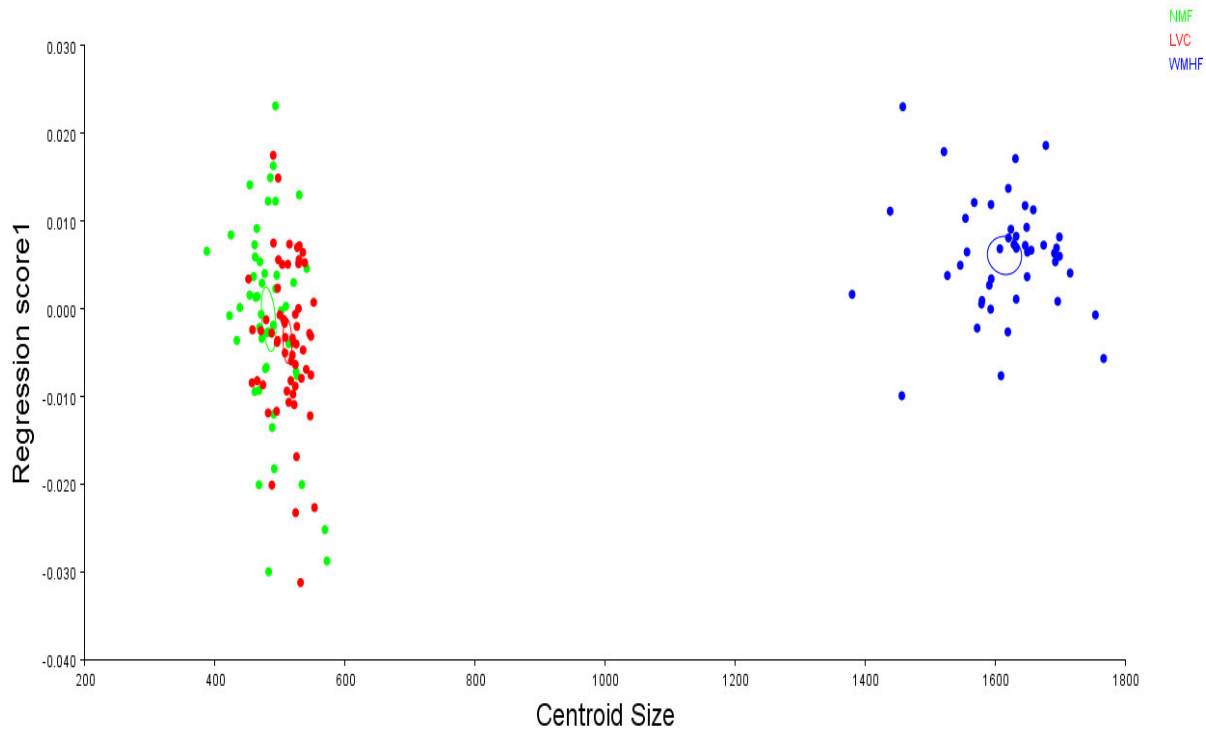
Figure 6. 8. Multivariate regression of wing shape Regression score 1 on centroid size wing size of three populations of *B. invadens*. Blue points: WMHF; Red points: LVC; Green points: NMF. The ellipses show 90% means confidence intervals for each population.

6.3.3. Fluctuating Asymmetry (FA) in Zonal and Host *B. invadens* Populations:

There were significant differences in FA among the 3 hosts (Table 6.3). Fruit fly wings from mango exhibited significantly higher FA than those from almonds or guava. Generally, there was a significant inverse relationship between absolute FA and wing size (right wing: r = -0.609, P = 0.0001, R^2 = 37.1%), and left wing: r = -0.551, P = 0.0001, R^2 = 30.4%). The relationship was strongest in the NMF (r = -0.825) and least in WMHF (r = -0.741). All fruit host populations depicted a bilateral FA type (Table 6.3; Figure 6.12).

Wings from specimens drawn from different zones did not show significant differences in FA, although there were variations in levels of bilateral FA in the order: NMF > LVC > WMHF

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(Table 6.3). There were differences in forms of bilateral asymmetries: specimen from LVC followed bilateral fluctuating asymmetry type; NMFs depicted a skewed antisymmetry form, while the WMHF followed a directional asymmetry form (Figure 6.12).

Table 6. 3: Comparison of fluctuating asymmetry FA among fruit hosts and zones.

	N	FA3 $\mu|R - L| / \mu TS$	FA7 $\sigma^2 R\text{-}L/\mu TS$	FA9 $1\text{-}r^2/\mu TS$	Asymmetry FA type
Hosts					
Guava	28	1.845E-04 ab	9.447E-08 ab	0.98814a	Fluctuating
Mango	25	2.695E-04 b	1.767E-07 b	0.97451a	Fluctuating
Almonds	23	1.168E-04 a	2.939E-08 a	0.98913a	Fluctuating
Test/ Prob.		F=2.662, P = 0.042	X^2 = 15.838, P = 0.000		
All Fruits	76	1.903E-04	1.031E-07	0.98748	Fluctuating
AEZs					
WMHF	15	1.895E-04 a	1.078E-07 a	0.99998a	Directional
NMF	18	2.056E-04 a	7.863E-08 a	0.80638a	Antisymmetry
LVC	29	1.928E-04 a	8.934E-08 a	0.99188a	Fluctuating
Test/ Prob.		F = 0.021, P = 0.979	X^2 = 9.991, P = 0.808		
All Zones	62	1.957E-04	9.10749E-08	0.97422	Fluctuating

Subscript R ± L denotes difference in value measured on left and right side, TS denotes trait size = average of values of left and right side (Palmer and Strobeck, 1986).
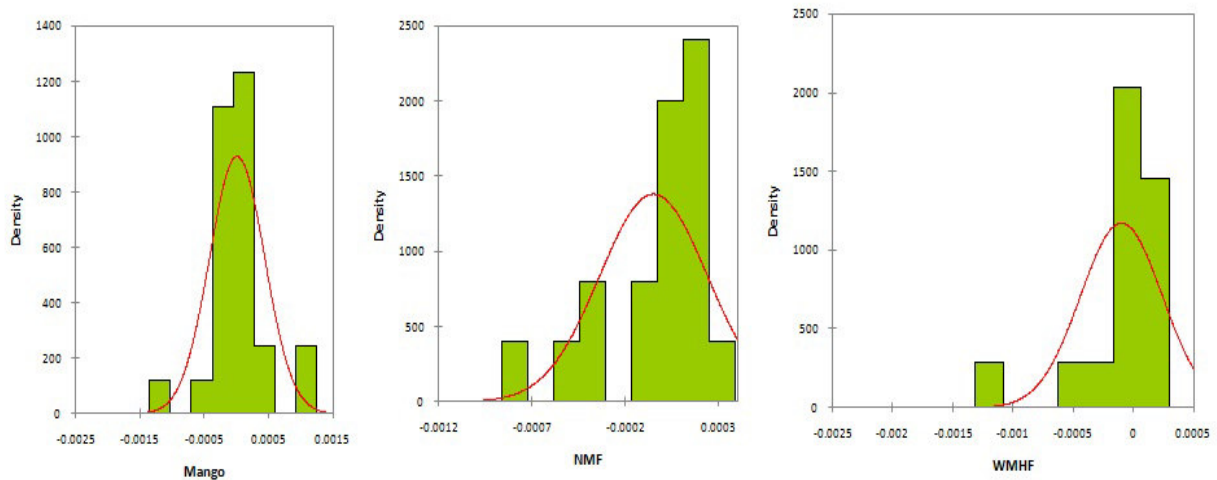
Figure 6. 9:Histograms depicting the three forms of bilateral asymmetry: Fluctuating asymmetry (Mango), Antisymmetry (NMF) and Directional asymmetry (WMHF).

6.4   Discussion

6.4.1   Host and Zonal Wing Size and Shape Variation

This study assessed phenotypic variations among three geographic and host populations. Generally, the results showed significant wing size and shape variations among the various populations. This may suggest that there is enough genetic variation in natural populations of *B. invadens* to show alternative phenotypes in response to host and geographical variations. The *B. invadens* population host overlaps and the resultant variations observed may lead populations to adapt and survive in difficult conditions, which maybe why it is largely invasive.

Similar results on intraspecific morphological variations or polymorphism have been reported among *B. dorsalis* complex members, of which *B. invadens* is part (Drew *et al.,* 2008; Adsavakulchai, 1998; Schutze *et al.,* 2012). Dacinae fruit flies are projected to have originated in the Papua New Guinea area and speciated prolifically across major world regions (Drew, 1989). This rapid speciation has led to emergence of cryptic and sibling species, with high levels of similarity in external morphology but with geographic/host variation in morphology within each species (Drew, 1989; Adsavakulchai, 1998).

Although the three zones share similar fruit types, the fruit proportions and environmental conditions differ, hence the intraspecific variations (Hooper, 1978; Krainacker *et al.,* 1987). Where post-hoc differences were not significant (eg mango Vs. guava), it is likely that such hosts expose the larvae to similar environmental conditions, possibly resulting in adults of similar size (Schutze *et al.,* 2012). Indeed specimens from mango and guava have similar EDMA distances between the major landmarks 2-4, 1-4, 3-4, 4-8 compared to almonds (Table 6.1).

The higher degree of phenotypic discrimination based on wing shape and size is evidence of environmental and host induced fine scale intraspecific variations, which should not be construed to imply species evolutionary radiation at this stage (Schutze *et al.,* 2012). Such early stage phenotypic differences due to shape sensitivity to environmental and host differences may be hard to resolve with other techniques such as molecular analysis. Therefore, rather than provide fine answers, the study has set the debate that will require more studies with other taxonomic techniques to elucidate and resolve the fine identities of the various phenotypes.

6.4.2 Host and Zonal-associated Fluctuating Asymmetry

The results indicated high level of kurtosis and skewness among zonal and host populations, evidence of FA and developmental instability (DI) among *B. invadens*. FA is commonly used as a measure of developmental instability (DI), which refers to an individual's inability to produce a specific phenotype under a given set of environmental or other forms of stress conditions (Demontis *et al.,* 2010). FA is known to be elevated due to environmental stresses and genetic factors and is often seen as an indicator of 'overall quality' or 'general health' (Zakharov, 1992; Lens *et al.*, 2000; Andersen *et al.,* 2002; Kristensen *et al.*, 2004; Røgilds *et al.,* 2005; Pertoldi *et al.,* 2006; Petavy *et al.,* 2006; Soderman *et al.,* 2007; Krag *et al.,* 2009). The idea behind this concept is that individuals of low quality cannot control their development precisely and consequently more often develop different phenotypes on both sides (Windig and Nylin, 2000).

Although *B. invadens* was able to propagate and survive in each of the three hosts, DI was significantly higher in mangoes than in guava or almonds (Table 6.3). The reason for the low DI

among the almonds population could be attributed to the host inferred buffering mechanism against random noise in the cellular processes among species reared from almonds. Cellular buffering mechanisms are involved in the development of morphological structures (Lynch and Force, 2000). DI has been attributed to the inability of an organism to buffer its developmental processes against environmental and genetic disturbances to ensure bilateral symmetry, which would logically imply that mango hosts are relatively poor hosts as they seem not to infer such abilities (Demontis *et al.,* 2010).

The inverse relationship between wing size and FA widely recorded in this study would have implied that *B. invadens* populations reared from mangoes would exhibit less FA, but it was the converse (Figure 6.2). The mango paradox potentially means that increase in FA in mango might not be due to environmental factors in isolation, but maybe attributed to genetic incompatibilities in mango reared flies (Demontis *et al.,* 2010). Therefore, the incomplete knowledge of the genetic mechanisms behind FA and host utilization makes clarification of the paradox a challenge, and calls for more efforts to understand how the two processes responsible for intraspecific variation are controlled and interact.

The three indices used in this study support the suggestion that for one to determine the relationship between asymmetry and environmental stress, a large set of indices should be used, as FA might vary significantly under different variables and indices sets (Palmer and Strobeck, 1986; Windig and Nylin, 2000; Hogg *et al.,* 2001). The failure to detect the relationship between FA and host types by the FA9 index could be masked by the low sensitivity of the index (Palmer and Strobeck, 1986; Windig and Nylin, 2000) or due to effects of other non-measured factors

(Hogg *et al.,* 2001). Consequently, when populations are compared, as was the case in this study, one of the difficulties is to select the characters to be used and to know if results will vary depending on the character (Lajus, 2001). The choice of centroid size in this study could have masked other development variations, with implications on finding differences in zonal populations (Table 6.3). Therefore, the study recommends the use of multiple traits, but see Hogg *et al.* (2001). The research findings that indicated relationship between host or site quality support the use of FA as an easy and efficient method to assess the population status under varying stress conditions and, therefore, FA could be a useful indicator tool to integrate in the management of fruit flies.

6.5 Conclusions

Bactoreca invadens displays wide range of phenotypic variations under different environmental and host conditions. The results have added to the evidence that geographic and host-associated adaptation can lead to polymorphs and phenotypes. Such phenotypic variations by *B. invadens* are likely to affect the efficiency of common control methods, and this research's results should help to recast fruit fly management strategies. The prevalence of fluctuating asymmetry, particularly among hosts, calls for more work to quantify the environmental and host based stress thresholds where population developmental patterns are highly unstable. This could aid in the development of management strategies. Further behavioral and ecological research to confirm or refute whether the observed host and geographic differences have genetic and behavioral confirmatory basis is recommended.

CHAPTER SEVEN: MODELING THE POTENTIAL GEOGRAPHICAL DISTRIBUTION AND ECOLOGICAL NICHE OF SELECTED FRUIT FLY (DIPTERA: TEPHRITIDAE) SPECIES IN UGANDA*

ABSTRACT

Despite their overwhelming economic importance, efforts to model the distributions of fruit fly (Diptera: Tephritidae) species in Uganda have been minimal. Potential geographical distributions and envelope of 10 selected fruit fly species were modeled using predictive models. Two presence-only methods, Maxent and Bioclim, were run using 19 bioclimatic parameters at a resolution of 30 arc seconds. New detections and existing records of fruit flies were used in the model. The climatic profiles of the selected fruit flies are described and the relative importance of the bioclimatic variables is explored. Precipitation (PC-1 = 61.4190%) and temperature (PC-2 = 29.214%) significantly shaped fruit fly niches across Uganda. There was a close agreement by the two models about the distribution and suitability patterns matching the main fruit agro ecological zones. Central and mid north zones provided the most suitable niches, while the western, northeastern and areas around Albert Nile were characterized as marginal areas. The models were mostly robust in performance (AUC: 0.815 – 0.974). Model test performance ranged from random (C. *capitata*: 0.486) to excellent (*C. cosyra:* 0.965). Areas believed to be marginal, such as higher altitude zones matched negative areas of the models, which reflected higher model prediction abilities. These results provide an initial insight into the bioclimatic tolerance ranges of fruit flies in Uganda, and should assist in identification of sites for future sampling efforts and management planning.

7.1   Introduction

Several fruit fly species constitute a potential threat to the fruit industry in Uganda. However, exhaustive studies on their distribution in the country are lacking. This knowledge on geographical suitability of the pest in the country is needed in the design of spatially explicit management strategies for the pest, to guard against losses in the fruit industry. Uganda is ecologically diverse with each agro ecological zone varying in endowment in natural resources and with a multitude of land use patterns across the country. This ecological diversity could lead to differences in spatial suitability of fruit flies in the country, with consequent implications on management.

The distribution of a given species in a region is determined among other things by the species' ecological requirements that constitute a species' fundamental niche or the intersection of necessary multiple environmental conditions (Hutchinson, 1957). A region may have suitable environmental conditions, but if other biological and historical realities are not in place, the species may not occupy its full potential geographical distribution (Brown *et al.,* 1996). For instance, a fruit fly's utilization of its ecological potential may be modified by interactions with other species (e.g. competitors, parasitoids, or parasites), reducing its fundamental niche into a fraction or subset that can be exploited — the realized niche (Hutchinson, 1957). Additionally, historical factors have often acted to restrict a species' distribution (Patterson, 1999; Peterson *et al.,* 1999).

Innovative new methods, such as niche-based modelling provide an option for potential species distribution. These techniques allow obtaining on the range of environmental conditions/

tolerance (climatic envelope-CE) and areas in which a given species would be able to survive (Pearson, 2007; Rubio and Acosta, 2010). The methods assume that the resulting predictive distribution model is a function of the way in which species respond to the environmental variables, thus reflecting a subset of their fundamental niche.

This study therefore set out to determine the geographical regions that are ecologically suitable for their establishment through modeling the potential distribution of selected fruit fly species in Uganda. Specifically, the study explored the climatic profiles underpinning the selected species distribution, to understand their niche requirements – a fact almost completely ignored for the selected species in Uganda.

7.2   Materials and Methods

7.2.1   Selected Species and Occurrence data

The specimens belong to 10 species in four genera: *B. invandens*, *C. anonae*, *C. cosyra*, *C. capitata*, *C. fasciventris*, *C. rosa*, *T. coffeae*, *B. cucurbitae*, *D. bivittatus* and *D. cilliatus.* The species were identified as the most economically important fruit fly species in the three most important mango growing agro-ecological zones in Uganda (Chapter 3). Additional countrywide records were obtained through deliberate detections and from existing scientific literature. The main sources documented data were the Royal Museum for Central Africa (http://projects.bebif.be/enbi/fruitly) and the Global Biodiversity Information Facility (http://www.gbif.org/). Records were georeferenced either in situ, using a Map-60 Garmin-GPS, or with the use of different digital gazetteers available in the Internet (mainly Google Earth ©). They were plotted on maps and inspected visually to detect obvious errors. Multiple records with the same coordinates remained as one record in the analysis.

7.2.2   Environmental Variables

Nineteen (19) environmental variables were used to characterize the climatic conditions across Uganda (Table 7.1). These had a 30 arc-seconds (~1 km²) partial resolution and were derived from the WorldClim project (http://biogeo.berkeley.edu), following guidelines by Hijmans *et al.* (2005). These variables were chosen because such a combination thoroughly and effectively represents environmental dimensions with maximum relevance to the ecological distributions of the fruit flies. These parameters (bioclimatic variables) fall into two broad categories – temperature or precipitation variables (Table 7.1). Elevation data were available for reference purposes but not used as a predictor by themselves (Rubio and Acosta, 2010).

7.2.4   Model Building

The geographical range of the 10 species was modeled with two widely used, presence-only methods, Maxent and Bioclim. The Maxent algorithm (Phillips *et al.,* 2006) yields results ranging from 0 to 1, indicating relative suitability of a given grid cell (high values indicate a higher presence probability) (Graham and Hijmans, 2006; Phillips *et al.,* 2006). To estimate the relative contribution of each variable in the final model a jackknife analysis was applied (Phillips *et al.,* 2006).

The Bioclim model was built using its implementation in DIVA-GIS 5.4 (Hijmans and Graham, 2006; Ward, 2007; Rubio and Acosta, 2010; Echarri *et al.*, 2009). Bioclim is a frequency distribution based algorithm, which extracts values of each bioclimatic variable that define the bioclimatic profile of each species, delimiting the so called "envelope", i.e. the climatic conditions that bound all occurrence localities (Guisan and Zimmermann, 2000; Ward, 2007). In

the potential distribution maps, grid cells are scored as suitable (if within the envelope; i.e. the presence of the species can be expected) or unsuitable (if outside the envelope) (Rubio and Acosta, 2010).

7.2.4   Model Evaluation and Bioclim Envelope Assessment

The original data set was split and a subset of 25% presence points was set apart as a test sample. The model was then run using the remaining 75% of the original presence data (training sample), randomly resampled in 20 repetitions (Pearson, 2007; Rubio and Acosta, 2010; Echarri *et al.,* 2009). Models obtained in these repetitions were superimposed to get a first visual evaluation of their mutual consistence and with the model built using the full dataset. Subsequently, the accuracy of the model was evaluated by calculating the AUC (area under curve) in a receiver operating characteristic plot. AUC values vary from 0.5 (model not better than random) to 1.0 (perfect accuracy as indicative that the model can discriminate perfectly between presences and absences of records (Graham and Hijmans, 2006). To assess the influence of bioclim variables on fruit fly species niches, Principal Component Analysis (PCA) of the bioclim envelopes was done. PCA in this case was used for reduction of the data set to only two variables (the two first components), using Pearson (n-1) correlation type and varimax rotation (Kaiser normalization) rotation.

7.3   Results

7.3.1   Records of Fruit Flies and Potential Distribution in Uganda

Existing and new records of fruit fly distribution in Uganda are given in Figure 7.1. Positive detections were made across the country in most of the major agro-ecological zones, with the

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exception of the North eastern short grass plains, Northern grass-farm bush transition, North central farm bush, in northern Uganda, and in the western zones of Bushenyi, South western-medium-high farmlands, Kabale-Rukungiri highlands and the Kisoro-Kibale highlands zones. In all the zones, detection for *Bactrocera invadens* was very predominant.
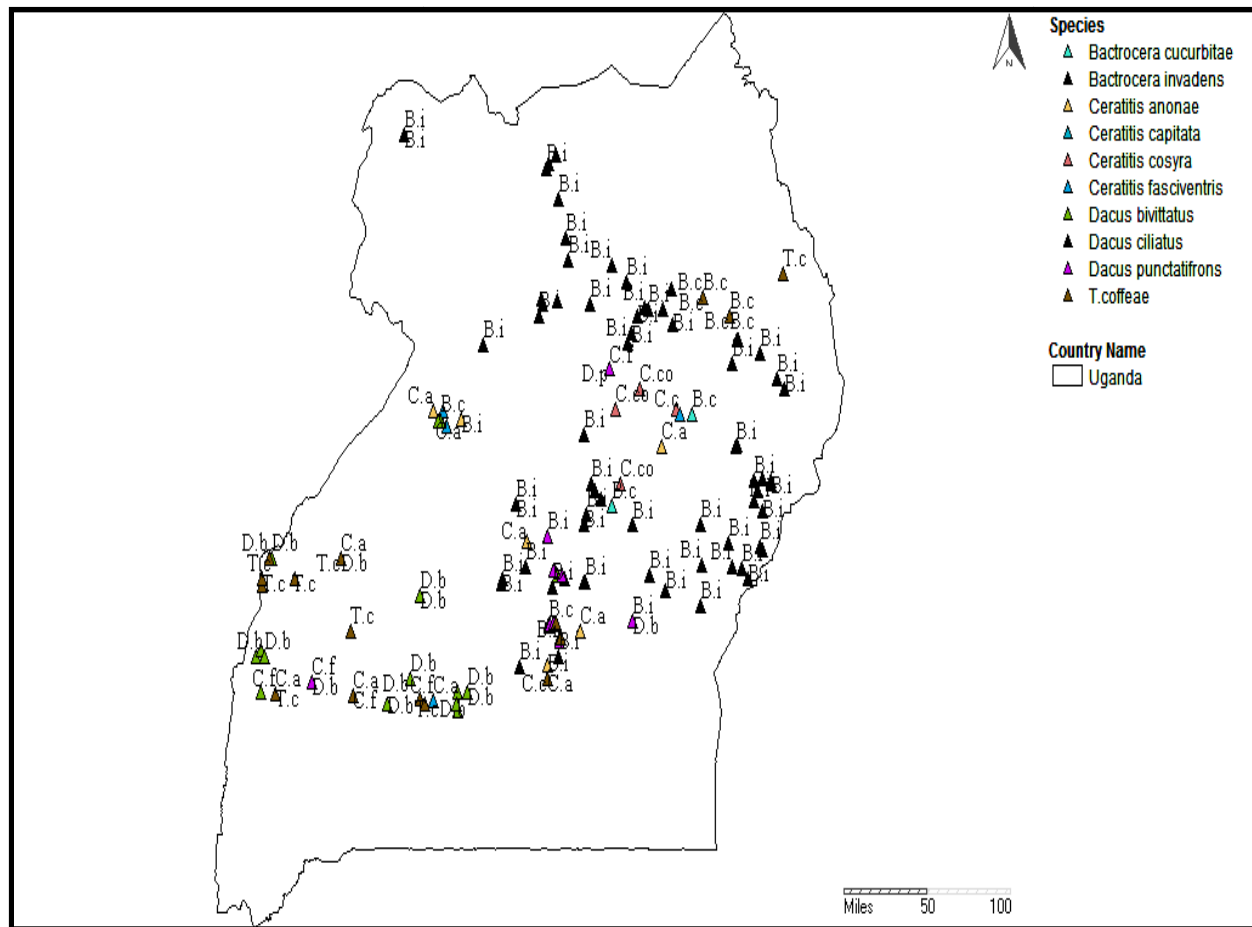
Figure 7. 1: Map of Uganda showing the distribution of sampling points and the respective fruit fly species. The key to the species abbreviations is given in the map legend.

7.3.2 Bioclimatic profiles of fruit fly species

The bioclimatic profiles of the niches of the ten species are summarized in Tables 7.2 to 7.4. All the 19-bioclimatic variables were classified into two principal components: precipitation (PC-1 = 61.4190%) and temperature (PC-2 = 29.214%) and overall cumulative variability of the first

three components accounting for 96.814% (Table 7.1). PC-3 was related to elevation and temperature seasonality; hence temperature variability was increasingly significant along the altitude gradient. Some temperature variables were correlated with precipitation components (Bio2, Bio4, and Bio7), while precipitation seasonality (Bio15) was correlated significantly on the temperature component.

Generally, all species were responsive to both precipitation and temperature variables, though at varying degrees and orders. However, *D. cilliatus* was significantly negatively correlated with the precipitation component (PC1) and the temperature component (PC2).

Table 7. 1: Environmental variables used in BIOCLIM and Maxent ecological niche models.

		Factor Loadings		
Variable	**Variable type**	PC-1	PC-2	PC-3
Bio1	Annual mean temperature	0.087	**0.900**	0.005
Bio2	Mean diurnal range: mean of monthly)	**0.633**	0.093	0.216
Bio3	Isothermality: (P2/P7) × 100	0.021	**0.928**	0.043
Bio4	Temperature seasonality (SD × 100)	**0.309**	0.157	**0.521**
Bio5	Maximum temperature of warmest month	0.086	**0.902**	0.005
Bio6	Minimum temperature of coldest month	0.014	**0.982**	0.003
Bio7	Temperature annual range (P5 – P6)	**0.572**	0.211	0.167
Bio8	Mean temperature of wettest quarter	0.013	**0.982**	0.004
Bio9	Mean temperature of driest quarter	0.067	**0.931**	0.001
Bio10	Mean temperature of warmest quarter	0.062	**0.935**	0.001
Bio11	Mean temperature of coldest quarter	0.013	**0.983**	0.003
Bio12	Annual precipitation	**0.980**	0.014	0.005
Bio13	Precipitation of wettest month	**0.980**	0.014	0.005
Bio14	Precipitation of driest month	**0.980**	0.014	0.005
Bio15	Precipitation seasonality	0.118	**0.263**	0.171
Bio16	Precipitation of wettest quarter	**0.980**	0.014	0.005
Bio17	Precipitation of driest quarter	**0.980**	0.014	0.005
Bio18	Precipitation of warmest quarter	**0.980**	0.014	0.005
Bio19	Precipitation of coldest quarter	**0.980**	0.014	0.005
C. anonae		0.781	0.364	0.201
D. bivittatus		1.184	0.400	0.002
C. capitata		0.519	0.404	0.016
D. cilliatus		0.218	2.831	0.000

T. coffeae	0.680	0.282	0.023
C. cosyra	1.570	0.113	0.166
B. cucurbitae	1.184	0.400	0.002
C. fasciventris	0.463	0.329	0.003
B. invadens	0.545	0.384	0.183
D. punctatifrons	1.353	0.156	0.195
Eigen-value	**11.670**	**5.551**	**1.174**
Variability (%)	**61.419**	**29.214**	**6.180**
Cumulative %	**61.419**	**90.633**	**96.814**

Table 7. 2: Mean (average) temperature (0^c) and rainfall (mm) bioclimatic variables/ conditions of the niches of 10 fruit fly species

Bioclimatic variable	*C. anonae*	*D. bivittatus*	*C. capitata*	*D. cilliatus*	*T. coffeae*	*C. cosyra*	*B. cucurbitae*	*C. fasciventris*	*B. invadens*	*D. punctatifrons*
Annual mean temperature	28.1	30.5	29.7	21.5	29.3	27.9	30.5	29.4	29.1	27.6
Mean diurnal range: mean of monthly)	13.5	15.3	14.9	14.0	14.8	14.3	15.3	14.7	14.6	13.4
Isothermality: (P2/P7) × 100	94.3	94.9	94.8	97.5	94.9	95.1	94.9	94.8	94.9	94.5
Temperature seasonality (SD × 100)	106.1	72.0	133.9	303.6	146.0	484.0	72.0	132.9	238.2	105.3
Maximum temperature of warmest month	29.0	31.4	30.6	22.1	30.2	28.7	31.4	30.2	30.0	28.4
Minimum temperature of coldest month	14.7	15.3	14.9	7.7	14.7	13.7	15.3	14.8	14.5	14.2
Temperature annual range (P5 – P6)	14.3	16.1	15.7	14.4	15.6	15.0	16.1	15.5	15.4	14.2
Mean temperature of wettest quarter	15.2	15.7	15.5	8.3	15.2	14.1	15.7	15.3	15.0	14.8
Mean temperature of driest quarter	27.1	28.7	28.1	20.6	28.0	26.4	28.7	27.9	27.6	26.5
Mean temperature of warmest quarter	26.7	28.3	27.7	20.3	27.6	26.1	28.3	27.5	27.2	26.2
Mean temperature of coldest quarter	14.1	14.6	14.4	7.7	14.1	13.1	14.6	14.2	14.0	13.7
Annual precipitation	1191.9	1015.1	1081.4	1145.0	1063.6	1296.1	1015.1	1084.0	1192.4	1253.9
Precipitation of wettest month	889.1	757.3	806.7	854.2	793.4	966.9	757.3	808.7	889.5	935.4
Precipitation of driest month	997.6	849.6	905.1	958.4	890.2	1084.9	849.6	907.3	998.1	1049.5
Precipitation seasonality	305.7	258.5	322.7	183.8	362.4	267.8	258.5	333.7	285.5	150.3
Precipitation of wettest quarter	793.8	676.1	720.2	762.6	708.4	863.2	676.1	721.9	794.1	835.1
Precipitation of driest quarter	932.1	793.8	845.6	895.4	831.7	1013.6	793.8	847.7	932.5	980.6
Precipitation of warmest quarter	817.6	696.4	741.8	785.5	729.6	889.1	696.4	743.6	818.0	860.2
Precipitation of coldest quarter	612.6	521.8	555.8	588.5	546.7	666.2	521.8	557.2	612.9	644.5

Table 7. 3: Maximum temperature (0^c) and rainfall (mm) bioclimatic variables/ conditions of the niches of 10 fruit fly species

Bioclimatic variable	C. anonae	D. bivittatus	C. capitata	D. cilliatus	T. coffeae	C. cosyra	B. cucurbitae	C. fasciventris	B. invadens	D. punctatifrons
Annual mean temperature	31.1	31.6	31.6	27.5	31.0	31.7	31.6	31.6	34.1	30.8
Mean diurnal range: mean of monthly	14.2	15.3	16.3	13.3	15.3	15.9	15.3	15.6	17.5	15.0
Isothermality: (P2/P7) × 100	93.8	94.5	95.1	94.4	94.7	94.8	94.5	94.7	95.1	94.5
Temperature seasonality (SD × 100)	106.1	72.0	133.9	303.6	146.0	484.0	72.0	132.9	238.2	105.3
Maximum temperature of warmest month	32.0	32.5	32.6	28.3	31.9	32.7	32.5	32.6	35.1	31.7
Minimum temperature of coldest month	16.9	16.3	15.5	14.3	15.8	15.9	16.3	16.1	16.7	15.8
Temperature annual range (P5 – P6)	15.1	16.2	17.1	14.1	16.2	16.8	16.2	16.5	18.4	15.9
Mean temperature of wettest quarter	17.2	16.6	15.9	14.9	16.3	16.3	16.6	16.5	17.4	16.2
Mean temperature of driest quarter	29.1	29.4	29.5	26.9	29.4	29.6	29.4	29.5	31.5	28.9
Mean temperature of warmest quarter	28.7	29.0	29.1	26.6	28.9	29.2	29.0	29.1	31.1	28.5
Mean temperature of coldest quarter	16.0	15.5	14.8	13.8	15.1	15.2	15.5	15.3	16.2	15.1
Annual precipitation	1383.0	1240.0	1383.0	1361.0	1391.0	1622.0	1240.0	1383.0	1622.0	1383.0
Precipitation of wettest month	1031.7	925.0	1031.7	1015.3	1037.7	1210.0	925.0	1031.7	1210.0	1031.7
Precipitation of driest month	1157.6	1037.9	1157.6	1139.2	1164.3	1357.6	1037.9	1157.6	1357.6	1157.6
Precipitation seasonality	327.1	276.5	345.3	196.7	387.7	286.6	276.5	357.1	305.5	160.8
Precipitation of wettest quarter	921.1	825.8	921.1	906.4	926.4	1080.3	825.8	921.1	1080.3	921.1
Precipitation of driest quarter	1081.5	969.7	1081.5	1064.3	1087.8	1268.4	969.7	1081.5	1268.4	1081.5
Precipitation of warmest quarter	948.7	850.6	948.7	933.6	954.2	1112.7	850.6	948.7	1112.7	948.7
Precipitation of coldest quarter	710.9	637.4	710.9	699.6	715.0	833.7	637.4	710.9	833.7	710.9

Table 7. 4: Minimum temperature (0^c) and rainfall (mm) bioclimatic variables/ conditions of the niches of 10 fruit fly species

C: Bioclimatic variable	C. anonae	D. bivittatus	C. capitata	D. cilliatus	T. coffeae	C. cosyra	B. cucurbitae	C. fasciventris	B. invadens	D. punctatifrons
Annual mean temperature	26.2	27.3	26.2	21.5	24.6	16.4	27.3	25.8	16.4	25.8
Mean diurnal range: mean of monthly	13.2	13.1	12.0	14.0	14.4	13.0	13.1	14.9	13.0	14.9
Isothermality: (P2/P7) × 100	94.9	94.3	93.8	97.5	96.4	99.1	94.3	96.3	99.1	96.3
Temperature seasonality (SD × 100)	106.1	72.0	133.9	303.6	146.0	484.0	72.0	132.9	238.2	105.3
Maximum temperature of warmest month	27.0	28.1	27.0	22.1	25.4	16.8	28.1	26.6	16.8	26.6
Minimum temperature of coldest month	13.0	14.3	14.2	7.7	10.4	3.7	14.3	11.1	3.7	11.1
Temperature annual range (P5 – P6)	13.9	13.9	12.8	14.4	14.9	13.1	13.9	15.5	13.1	15.5
Mean temperature of wettest quarter	13.9	14.9	14.9	8.3	10.9	4.1	14.9	11.9	4.1	11.9
Mean temperature of driest quarter	25.4	26.5	25.4	20.6	23.8	15.3	26.5	25.2	15.3	25.2
Mean temperature of warmest quarter	25.0	26.1	25.0	20.3	23.5	15.1	26.1	24.9	15.1	24.9
Mean temperature of coldest quarter	12.9	13.8	13.8	7.7	10.2	3.9	13.8	11.0	3.9	11.0
Annual precipitation	842.0	904.0	904.0	1145.0	707.0	1226.0	904.0	842.0	768.0	1142.0
Precipitation of wettest month	628.1	674.4	674.4	854.2	527.4	914.6	674.4	628.1	572.9	851.9
Precipitation of driest month	704.8	756.6	756.6	958.4	591.8	1026.2	756.6	704.8	642.8	955.9
Precipitation seasonality	248.6	210.2	262.4	149.5	294.7	217.8	210.2	271.4	232.2	122.2
Precipitation of wettest quarter	560.8	602.1	602.1	762.6	470.9	816.5	602.1	560.8	511.5	760.6
Precipitation of driest quarter	658.4	706.9	706.9	895.4	552.9	958.7	706.9	658.4	600.6	893.0
Precipitation of warmest quarter	577.6	620.1	620.1	785.5	485.0	841.0	620.1	577.6	526.8	783.4
Precipitation of coldest quarter	432.8	464.7	464.7	588.5	363.4	630.2	464.7	432.8	394.8	587.0
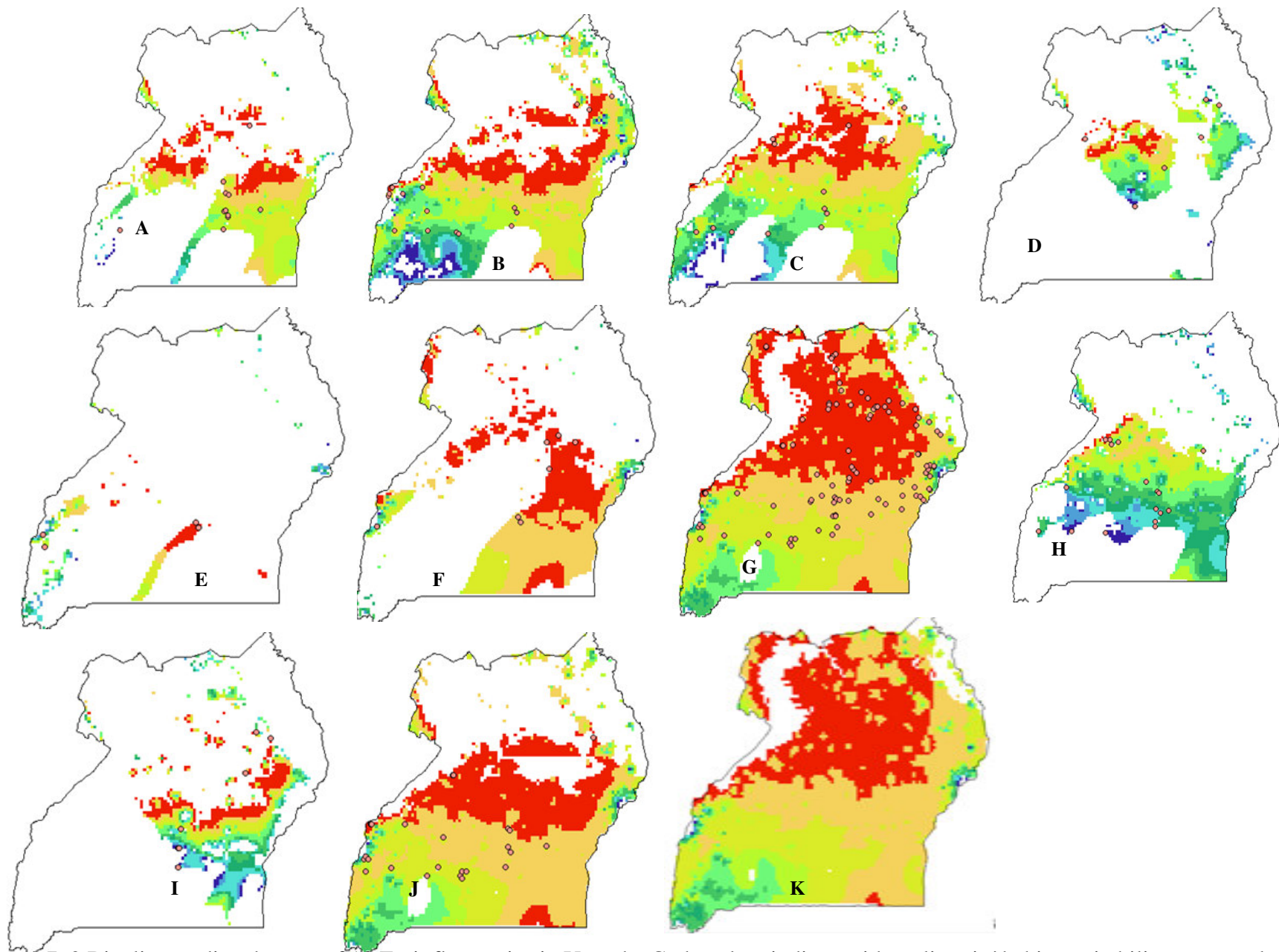
Figure 7. 2 Bioclim predicted range of 10 Fruit fly species in Uganda. Code colors indicate either climatic/ habitat suitability, as cumulative distribution percentile): red (20–34), orange/ yellow (10–20), green (5–10), light blue (2.5–5), and white (low potential areas). A (*D. punctatifrons*), B (*T. coffeae*), C (*C. fasciventris*), D (*B. cucurbitae*), E (*D. cilliatus*), F (*C. cosyra*), G (*C. capitata*), H (*D. bivittatus*), I (*C. anonae*), J (*B. invadens*) and K (ALL).

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7.3.3 Potential Range

Generally, there was close agreement by the two models (Maxent and Bioclim) about the distribution probability and suitability patterns (Figures 7.3, A-K and 7.4, All). Both model outputs (Figures 7.3, A-K and 7.4, All) generally match the main fruit agro ecological zones (Lake Victoria Crescent, Northern Moist Farmlands and Western Mid Altitude High Farmlands (Wortman and Eledu, 1999). The central and mid northern areas consistently recorded the highest probability and suitability, as was the case for most incidence records (Figure 7.2). Northern moist farmlands, Central farm and bush lands and Usuk sandy farm-grasslands (here after all referred to as Northern moist farmlands, NMF), Central Buruli farmlands, Western clay loam farmlands and Central wooded savanna (here after referred to as Central wooded savanna, CWS), Southern and Eastern lake Kyoga basin (SELKB), and Jinja and Mbale farmlands and Lake Victoria Crescent (Lake Victoria Crescent and Mbale farmlands, LVCMF) recorded the highest suitability ranges for all the species.

There was a fair amount of interaction between species and model type prediction of the area with highest climatic suitability: *B. invadens, T. coffeae, C. fasciventris, C. cosyra*, and *D. bivittatus*, suitable areas were higher under Bioclim, while *C. anonae, C. capitata, C. cosyra B. cucurbitae* and *D. punctatifrons* showed higher suitability under Maxent (Figures 7.2, A-K). Range varied, with highest range recorded for *B. invadens>D. bivittatus>T. coffeae>C. fasciventris*, medium range (*C. anonae, C. cosyra, C. capitata* and *D. punctatifrons*), while *B. cucurbitae* and *D. cilliatus* had restricted or low range. Among all the zones, SELKB recorded highest suitability for all species, with the exception of *D. cilliatus, C. anonae* and *B. cucurbitae* (Figures 7.2, A-K). Similar findings were made for the NMF, except that in this case *B.*

cucurbitae bioclimatic requirements were fairly suitable. Bioclim also indicated potential distribution of fruit flies in the poorly sampled Karamoja region, initially thought to be too warm for establishment (Figures 7.2, K). Likewise, both models predict species ranges north- and north western, albeit with differing intensities (Figures 7.2, A-K). The predictions into the north and south western are more continuous though with low probability in, but with fairly good representation in Bioclim (Figure 7.2, B, C, D, G, and H).

In western Uganda, south western-medium-high farmlands, Kabale-Rukungiri highlands and the Kisoro-Kibale highlands zones are generally unfavorable sites for fruit fly distribution, but they are potential habitats for *T. coffeae*, *D, bivittatus* and *B. invadens* (Figure 7.2, B, H and J). Generally, drier and high altitude zones were unsuitability for all the species in the study. Indeed, with the exception of C. *capitata*, *C. faciventris*, *C. cosyra* and *T. coffeae*, Maxent model gives low probability for high altitudes, and the extreme northern and southern zones, while Bioclim remarkably ranks the same sites as a marginal sites (Figures 7.2, A-K). Both models over predict suitability for some species, including over water bodies. Based on temperature and precipitation (variable used in building the models), Lake Victoria would offer suitable fruit fly habitats; implying that the islands in the Lake Victoria may harbor fruit fly species modeled in this study.
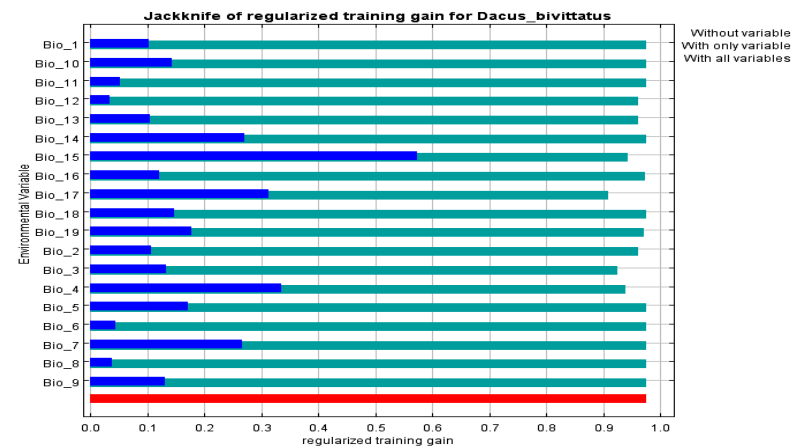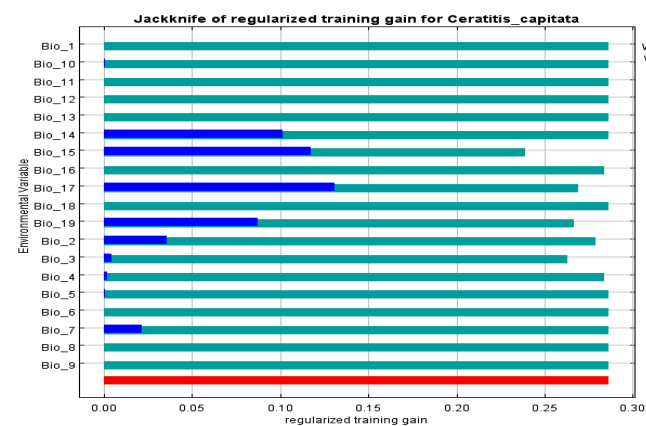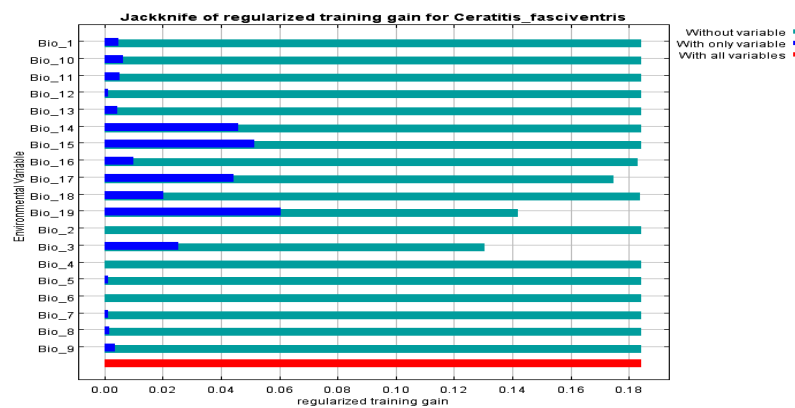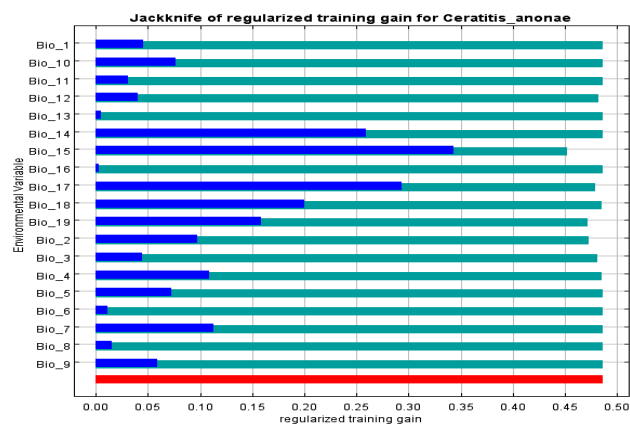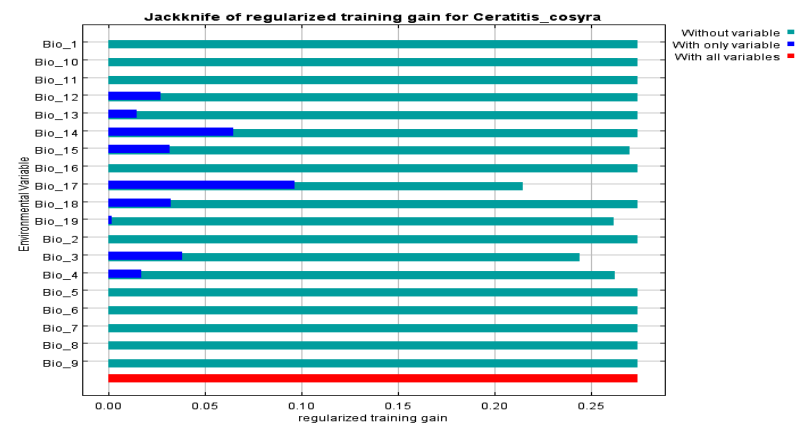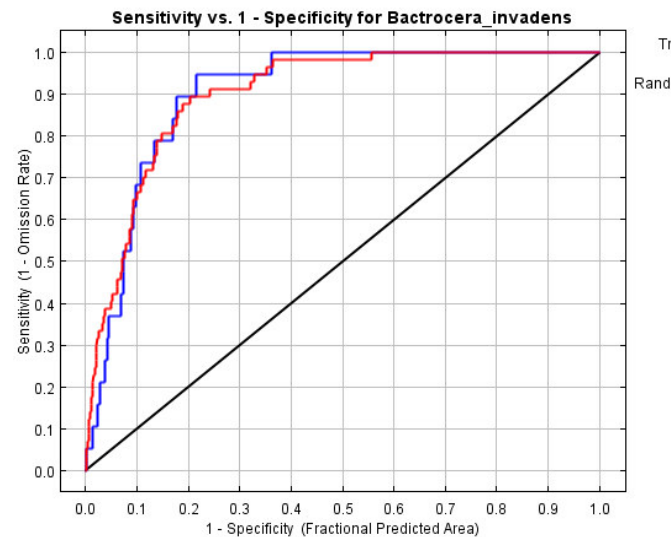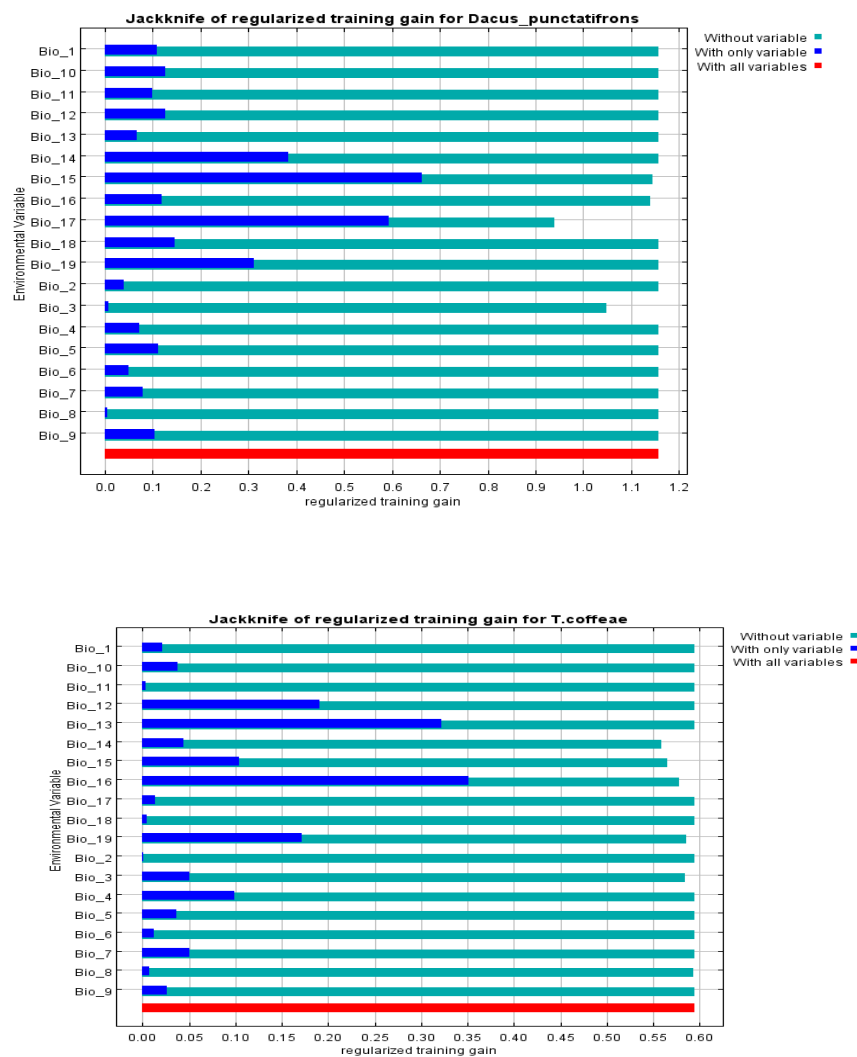
Figure 7. 3 Maxent based Jackknife of the regularized training gain: without variable (light blue), with only variable (blue), with all variables (red) and the 10 Receivership Operating Curves (ROC). Code colors in the close-up maps indicate suitability shown as probability: red (0.90–1), orange (0.80–0.90), green (0.65–0.80), and light blue (0.50–0.65). ROC curves show the area under curves both for training and test data. The Receivership Operation Curve (ROC) for *B. invadens* is indicated in the lower bottom corner.

7.3.4 Limiting Factors and Relative Importance of Variables

Precipitation variables played the highest role in clearly restricting distribution of *B invadens*: the five most limiting factors (Bio12 > Bio13 > Bio 18 > Bio16 > Bio 19) were all precipitation variables, while some temperature variables also affected site suitability (Bio4 > Bio3 > Bio9 > Bio 6). Extreme lows and highs of the limiting variables associated with the far north eastern (Northern short grass plains), areas surrounding the Albert Nile and the South western grasslands are unfavorable, while SELKB, NMF, CWS and some parts of LVC attain the optimum precipitation, temperature seasonality and Isothermality conducive for *B. invadens* (Figures 7.2 and 7.3).

Precipitation variables were critically more important in the distribution of *D. punctatifrons*, but the effect was more to annual precipitation distribution than annual means (Figure 7.3). Quarter (Bio19 and Bio17), monthly means (Bio14) and precipitation variability/ seasonality (Bio15) were the most limiting variables. Low monthly (Bio14) and quarterly (Bio17) precipitation of the driest period significantly negatively affected *D. punctatifrons*, while increasing precipitation seasonality beyond 33 (coefficient of variation) leads to low suitability, just like extremes of precipitation of coldest quarter. Two more variables linked to temperature (Bio3: Isothermality) and precipitation (Bio16: Precipitation of wettest quarter) are relevant when the range is considered in totality, i.e. they show the higher decrease in the gain when omitted (Figure 7.3). Consequently, the importance of temperature and precipitation variables in constraining the climatic niche of *D. punctatifrons* into central and marginally western Uganda is emphasized (Figure 7.2A).

Precipitation variables played a significant role in restricting the distribution of *C. anonae* too: the six most limiting factors (Bio15 > Bio17 > Bio 14 > Bio18 > Bio 19 > 12) were all precipitation variables, with a few temperature variables also affecting site suitability variability (Bio7 > Bio4 > Bio2). The optimum conditions for *C. anonae* are in sites with low precipitation seasonality (Bio15), annual temperature consistence (low range: Bio7), small temperature seasonality (Bio4) and diurnal temperature (Bio2). Low monthly (Bio14), precipitation of warmest quarter (Bio18) and quarterly (Bio17) precipitation of the driest period significantly negatively affected *C. anonae*. Unit increase up to optimum (sigmoid) increases the probability of incidence, while extremes annual precipitation (Bio12) and precipitation of coldest quarter (Bio19) lead to low site suitability (Figure 7.3).

Higher temperature means of the driest quarter (Bio9), temperature annual range (Bio7) and temperature seasonality (Bio4), coupled with low Isothermality (Bio3) and precipitation of warmest quarter and moderate precipitation of coldest quarter increase suitability for *B. cucurbitae*. This combination of bioclimatic variables restricts *B. cucurbitae* to central and sporadic distribution to the east and northeastern Uganda (Figure 7.3). The three most important precipitation variables for *C. capitata* were Bio14, Bio15 and Bio17, while the temperature variables included Bio2 and Bio3. Low values of Bio15 and the temperature variables increased *C. capitata* suitability, while the reverse was the case for Bio14 and Bio15. The observed wider range distribution predicted for *C. capitata* under the Maxent model (Figure 7.3) is commensurate with this requirement, while eastern Uganda distribution is also predicted under the Bioclim model (Figure 7.2G).

Areas with higher volumes of precipitation of driest month and quarter were associated with higher probabilities of *D. bivittatus*, *C. cosyra* and *C. fasciventris* incidence, while increased precipitation seasonality negatively affected suitability for the three species. Precipitation of warmest quarter increased, while higher levels of Isothermality decreased *C. cosyra* suitability. Likewise, precipitation of coldest quarter (Bio19) decreased, while increased Isothermality increased *C. fasciventris* suitability. The moderate precipitation of coldest quarter and temperature seasonality increased, while increased temperature annual range and maximum temperature of warmest month decreased suitability of *D. bivittatus*. Areas suitable for *T. coffeae* were characterized with comparatively low Bio12, Bio13, Bio15, Bio16, Bio19, and Bio4 (Figure 7.3).

7.3.5 Model Performance

The Maxent generated models proved mostly excellent in performance both for the training and test data (Figure 7.4). The Area under Curve values (AUC) were of high accuracy ranging between 0.815 – 0.974 in *C. capitata* and *C. cosyra*, respectively for training data. However, for some species test data AUC were random: *B. cucurbitae* (0.500) and C. *capitata* (0.486), while excellent AUC for test data were recorded with *C. cosyra* (0.965), *D. punctatifrons* (0.904) and *B. invadens* (0.902). The modeling proved to be in agreement with the expected fruit fly habitats. Higher altitude zones in Kasese, Kisoro, Mbale and Karamoja farmlands (Figure 7.2 and 7.3) matched negative areas of the models, which reflected a correct prediction in this sector.

7.4   Discussions

Potential Distributions of the 10 species in Uganda

This study presents the first local attempt at understanding the spatial suitability of fruit fly distribution in Uganda. The results suggest higher suitability for most species considered (Figure 7.2). The 10 species modelled are predicted to be ubiquitous in the central (LVC and MF) and mid-northern regions, areas associated with favorable precipitation (1215 mm - 1328 mm) distribution and optimum temperatures for fruit flies. These suitable areas receive two rainy seasons with the main season from March to May with a peak in April and secondary season from August to November with a peak in October/November. The areas experience a main dry season December to February, and a secondary dry season between June and July. Temperature ranges from 15 – 32.5 °C, while altitude ranges from 914 – 1,800 m ASL (Wortman and Eledu, 1999), encompassing the majority of the fruit fly species envelopes.

Most species have the potential to extend north and southwestwards and inhabit considerable areas there. The suitability of the extreme south, northeastern, Rwenzori, Elgon and around Albert Nile is low for most species; hence these areas might remain restrictive to the spread of fruit flies. These distributions correspond very well to an intolerance of high altitudes, aridity potentials (north-eastern) and precipitation extremes (Stephens *et al.,* 2007; De Meyer *et al.,* 2010; Geurts *et al*., 2012).

Bactrocera invadens is the most serious fruit fly pest in Uganda (Nemeye, 2005; Okullokwany, 2006) and the region (Mwatawala *et al.,* 2006a; Rwomushana *et al*., 2008; Geurts *et al.,* 2012), and has the widest host/ distribution range because of its ability to tolerate diverse environmental

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conditions (De Meyer *et al.,* 2010). The predicted distribution of *B. invadens* in this study is consistent with other regional studies (Ekesi and Billah, 2006; De Meyer *et al.,* 2010). The observed ardent response of *B. invadens* to precipitation and some temperature variables confirmed the earlier findings that *B. invadens* prefer hot and humid environments (De Meyer *et al.,* 2010). Annual precipitation must be high, although it does not have to be continuous, as was the case of *B. invadens* quarterly and monthly means relevance in the findings. Suitable habitats for *B. invadens* identified in central and mid north zones are characterized by moisture rich short dry season, a distinct dry period with driest-month precipitation (Wortman and Eledu, 1999), favorable for *B. invadens* (Ekesi and Billah, 2006; De Meyer *et al.,* 2010. Mwatawala *et al.* (2006a).

With slight exceptions mainly with *C capitata* and *D. cilliatus*, habitat suitability breadth of the other 8 species in this study agree with the earlier potential distribution predictions by Ekesi and Billah (2006)(Figure 7.2 and 7.3). This is probably because the latter in most cases used few incidence points and modeled at a larger African scale. For instance, their predictions for *D. cilliatus* and *D. punctatifrons* were all based on one incidence point, while most of the other species were based on less than five points in the Ekesi and Billah (2006) study. The latter also predicted a more northern extent for *C. capitata* (based on two points), while this study's results showed a more central and wider extent. The use of fewer sample points may be the reason, although according to Pearson (2007), Maxent can still retain high success rates and predictive qualities when the sample sizes are small. Central and mid-northern zones were found to be highly suitable for *C. capitata*, but differences between the two models were evident, as De Meyer *et al.* (2008) too reported.

Principal Component Analysis (PCA) and Jackknife tests indicated that precipitation and temperature variables have strong influence on potential distributions and suitability of all fruit fly species in this study. Temperature has a significant influence on fruit fly physiology, such as population density, gross fecundity, female and male longevities and generation time (Duyck and Quilici, 2002). The relationship between temperature variables and suitability observed for *C. capitata* has already been reported elsewhere (White and Elson-Harris, 1992; Duyck and Quilici, 2002). A humid atmosphere and soil, instead of precipitation directly has been reported to influence the survival of fruit fly pupae, which favors desiccation resistant species such *C. capitata* to survive in drier zones, hence increasing their breadth (Duyck *et al.*, 2006).

Significant overlap appears to exist in the potential distributions of most species. For example, *B. invadens, T. coffeae, D. bivittatus* and *C. faciventris* seem to have broadly similar potential ranges (Figure 7.2, B, C, H, J). An obvious difference in the predictions among these species is the extreme wetter southern zones slightly suitable for *B. invadens* and *D. bivittatus*, but poorly tolerant to the other two. Low temperatures and higher humidity in those sites, favour *B. invadens* over *T. coffeae* and *C. faciventris* that is more sensitive to low humidity (Rwomushana *et al.,* 2008); which would support the study's predictions. Rwomushana *et al.* (2008) also reported significant differences in performance of *C. cosyra* over *B. invadens* under fluctuating temperature. *B. invadens* was more competitive under low temperatures (characteristic on the southern parts of Uganda), which might explain the absence of *C. cosyra* suitable niches in this region. This might provide some insight into the unknown extent to which these species co-exist in the wild.

Precipitation distribution is an important determinant fruit fly species ranges, assuming optimum temperature. Much of Uganda has meaningful altitudinal differences in short distances, so that both temperature and precipitation gradients are common. This probably limits distribution on northeastern and mostly western zones. The close relationship of *Bactrocera* and *Ceratitis* species ranges depicted in the models in this study most likely influences partitions and suitability between the two genera (Rubio and Acosta, 2010; Geurts *et al*., 2012).

Bioclim and Maxent Predictive Capabilities

Superiority of the Maxent algorithm over Bioclim (Figures 7.2 and 7.3) is consistent with related studies (Elith *et al,* 2006; Phillips *et al.;* 2006; Rubio and Acosta, 2010). This is partly because the prediction maps made by Maxent are generally suitable for processing at a high threshold, which gives a qualitative suitability judgment for each grid (Rubio and Acosta, 2010). However, the bioclimatic analysis proved to be a valuable means to get insight of the fundamental niche features of the ten fruit flies in Uganda, which will improve the ecological knowledge of Tephritids in Uganda.

One of the fundamental criticisms of Ecological Niche Modelling (ENM), particularly Bioclim method is that models are not built with vegetation variables, as was the case in this study. Vegetation is generally assumed to be a critical determinant in the presence or absence of most species (Rubio and Acosta, 2010). In that sense, the correct prediction of absence, as matched in most northeastern, Elgon and Rwenzori peaks, offers further support of the soundness of the models. This predictive value is appreciated as a remarkable strength of ENM (Muñoz *et al.*, 2009; Rubio and Acosta, 2010).

It has been suggested that no single modeling method has the complete truth (Elith *et al.*, 2006), so that comparative analyses like this may help to gain a better understanding. Habitat ranges obtained with Bioclim, despite its lower computational performances, probably look biogeographically more realistic for fruit fly species that have been previously predicted to be ubiquitous across Uganda. However, Bioclim appears to be more sensitive to the record set bias and to concentrate more in areas where point density is higher, such as central Uganda, which has been reported elsewhere (Rubio and Acosta, 2010). Both Bioclim and Maxent agreed in detecting presumable high suitability zones, where detection has not been done yet.

7.5   Conclusions

Fruit flies pose a significant threat to Uganda; countrywide potential distribution of native and exotic species was demonstrated. Precipitation and temperature significantly determined fruit fly distribution. There was close agreement by the two models about the distribution and suitability patterns matching the main fruit agro ecological zones. Central and mid north zones provided the most suitable habitats, while the western, north eastern and areas around Albert Nile were characterized as marginal. Further studies of the species are warranted, particularly their ecological impacts. These results provide an initial insight into the bioclimatic tolerance of fruit flies and they also help in identifying sites for future sampling and management efforts. Several avenues exist to improve the accuracy and value of habitat suitability maps for these species, including, inclusion of edaphic and host plant data. There is also an urgent need for widespread monitoring surveys to ground-truth the suitability across Uganda. Such information will help evaluate modeling performance and also reduce apparent commission errors.

CHAPTER EIGHT: PROJECTIONS OF CLIMATE-INDUCED FUTURE RANGE SHIFTS AMONG FRUIT FLY (DIPTERA: TEPHRITIDAE) SPECIES IN UGANDA*

ABSTRACT

The potential impact of climate change (CC) on future distribution of selected fruit fly species in Uganda was assessed. Predictions were made using two general circulation models from two prediction centres: HADCM and CCMA from United Kingdom and Canada, respectively. Two future predicted CO_2 emission scenarios: A2 and B2, for increased and decreased future carbon emissions, respectively, were used to assess pest variations under both full (populations are able to migrate entirely into their future climate habitat) and no dispersal (unable to migrate quickly enough) modes. Fruit fly ranges were projected to decline at an average of 25.4% by 2050 future period. In the full-dispersal mode, only 3 species' (*D. cilliatus > C. cosyra > B. invadens)* ranges are predicted to increase, while the rest are likely to decrease at an average of 17.2%. In the no-dispersal scenario, a significant average decrease in size of niches by 67.9% is predicted. *Dacus bivittatus >Bactrocera cucurbitae >Ceratitis anonae* were the least likely climate change resilient species: their future niches were projected to decrease by more than 75%. *D. cilliatus* was predicted to have the highest potential (249.3%) climate change resilience. Habitat losses were higher under B2 (30.56±22.6%) but not significantly different with A2 (16.38±21.8%). Species richness was predicted to decrease significantly differently across zones (P = 0.003), with the WMHF recording the highest decrease (55%), while the least was in the NMF (12%). Future niches are predicted to shift northwards, mainly to the Northern Moist Farmlands. The results should assist in the development of climate change adaptative pest management strategies.

8.1   Introduction

Global climate continues to change (IPCC, 2007). For agriculture, climate change will be significant, as such changes are associated with shifts in pest and disease ranges, posing new risks to food and farming (Cooper *et al.,* 2013). To better understand the potential impacts of the current warming trend, considerable effort has gone into predicting the effect of future climate scenarios (Walther *et al.*, 2002; Chambers *et al.*, 2005; McKenney *et al.,* 2007). McKenney *et al.* (2007) examined the potential redistribution of North American plants as a result of climate change and dispersal, and found major redistribution pressures under the different scenarios. Mean temperature increases of about 5 ºC in Peru during the 1997 El Niño, resulted into decreased infestation by the leaf miner fly (*Liriomyza huidobrensis)*, and an increased infestations by the bud midge (*Prodiplosis longifila)* (Cisneros and Mujica, 1999).

A study examining nearly 100 species of drosophilid fruit flies showed that these fruit flies are constrained within an evolutionary strait jacket and cannot readily adapt to climate change-related temperature increases (http://www.ecology.com/2012/09/18/fruit-flies-cant-adapt-climate-change/). Habitat shifts among tephritid fruit flies also have been predicted to alter considerably with climate change. For instance, *Bactrocera cucurbitae*, *B. dorsalis* and *B. latifrons* have been projected to shift ranges throughout the Japanese Archipelago due to climate change (FAO, 2008). Climate change has also been demonstrated to affect tephritid fruit flies at the regional scale (Stephens *et al.,* 2007; De Meyer *et al.,* 2010) or in other countries (Khalil *et al.,* 2010; Liu *et al.,* 2011).

In East Africa too, climate change has been reported: temperatures are rising, rainfall is

increasing in some areas and declining in others, seasonal patterns and pest and disease distribution are changing, and extreme weather events are becoming more frequent and severe (Cooper *et al.,* 2013). The tropical climate in Uganda reported to be changing, characterized by increasing temperatures and infrequent rainfall peaks, especially in the drier periods (Climate Change Unit, 2014). If species range shifts are the likely dominant species response to future climate change, then explicit planning will be fundamental to estimating the rate and direction of pest species movements to ensure real time response.

Future range shifts in response to climate change have been hypothesized to fall either under full or no dispersal scenarios (McKenney *et al.,* 2007). A full dispersal scenario is one in which populations are able to migrate entirely into their future climate habitat, while a no dispersal situation is where they are unable to migrate quickly enough and thus survive only in areas that overlap with their current climatic range (Thuiller *et al.,* 2006; McKenney *et al.,* 2007). Similarly, due to uncertainties in the future CO_2 emission hypothesis, several gas emission scenarios have been suggested (Nakicenovic and Swart, 2000). Among these scenarios, A2 assumes a higher human population, less-forested land, greater pollution, and higher CO_2 emissions; while scenario B2 assumes an acceleration of energy and resource conservation efforts during the early decades of this century, such that CO_2 emissions will decline by mid-century.

Current and future climate variables are generated by two general circulation models (GCMs), which model the complex relationship between atmospheric flow and radiative energy (Hayhoe et al. 2006). Among GCMs, the Canadian GCM/ CCCMA (Boer et al. 2000) and the UK-based

Hadley GCM/ HADCM (Gordon *et al.,* 2000) are some of the most robust and commonly used models. In choosing a set of climate variables interpolated from these GCM one looks for the smallest set that defines important climatic constraints on insect survival and growth, to avoid superfluous climatic requirements and reduce multicolinearity variance inflation (Box, 1981; Beaumont *et al.,* 2005). On this basis, 2 variables that summarize two important climatic gradients for insect pests—heat and moisture are normally used to construct climate envelopes (CE) that bound the climate tolerance range of each species (Woodward, 1987; Shao and Halpin, 1995; Stephenson, 1998; McKenney *et al.,* 2007).

This study therefore set out to predict the potential impacts of climate change on the climatic ranges of 10 fruit fly species in Uganda under two dispersal and CO_2 scenarios interpolated through two GCMs. Specifically, this study aimed at understanding how local-level distribution patterns may be expected to change under future climate change and the comparative potential range shifts among the 10 species. It is hoped that the study will gauge the proportion of species under severe threat of exacerbation under the projected climate change scenarios and dispersal options, and the implications of these patterns for pest management in Uganda.

8.2   Materials and Methods

8.2.1   Selected Species and Occurrence data

The species assessed were: *Bactocera invandens*, *Ceratitis anonae*, *Ceratitis cosyra*, *Ceratitis capitata*, *Ceratitis fasciventris*, *Dacus punctatifrons*, *Trirhithrum coffeae*, *Bactrocera cucurbitae*, *Dacus bivittatus*, and *Dacus cilliatus.* Species data and model building are described in Chapter Seven, Sections 7.1-7.3.

8.2.2 Climate Envelope Approach

Climatic controls on current fruit fly distributions were summarized using the climatic niche of the 10 species using the climate envelope (CE) approach (Nix, 1986). The study analyzed CEs for full and no dispersal extreme scenarios. A CE was generated for each fruit fly species by first generating an estimate of minimum and maximum values of each climate variable of interest at each location where a species was observed. The climate variables used represented the mean values of humidity/moisture and temperature at a given location. For heat, annual mean temperature and mean temperature of wettest quarter were chosen, while moisture gradients were represented by mean annual precipitation and precipitation of coldest quarter.

From the extent of the current CE for each fruit fly species, areas of suitable climate habitat were delineated on maps for the 2000 – 2050 periods. Future climate variables were generated by two GCMs: HADCM and CCCMA under emission scenarios A2 and B2. To generate the future climate grids, average change surfaces were generated for the 2050 time period by interpolating the changes predicted by each GCM and emission scenario (McKenney *et al.,* 2006; McKenney *et al.,* 2007). Thus, the climate variables represent Ugandan climatology as provided by the existing network of climate stations in combination with the broad scale average changes predicted by the climate-change scenarios.

In the full-dispersal scenario, changes in CE area were calculated by expressing the future CE area as a percentage of the current CE area. For the no-dispersal scenario, future maps were overlaid on current maps and only the area of overlap was taken as the future distribution. Once

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the future CE was defined in this way, change metrics were calculated in the same way as for the full-dispersal scenario.

Two measures were used to assess the potential impact of climate change on fruit fly species composition in the three main mango-growing regions: Northern Moist Farmlands (NMF), Western Mid-altitude High Farmlands (WMHF), and the Lake Victoria Crescent. First, the predicted current and future local species richness each of the regions were calculated by summing the current and predicted future species for each dispersal and emission scenarios. Current and future species richness was then compared. Second, the number of species predicted to arrive newly (species gain [SG]) and the number of species predicted to no longer be present in the future (species loss [SL]) in each site were calculated, and thus estimated the percentage of species turnover as:

$$\textbf{Species turnover} = \textbf{100} \: \textbf{\textit{x}} \: \textbf{1} * \frac{\textbf{\textit{SG}} + \textbf{\textit{SL}}}{\textbf{\textit{SR}} + \textbf{\textit{SL}}}$$

where SR is the current species richness (Peterson *et al.*, 2002; Buisson *et al.,* 2010). A turnover value of zero indicated that the predicted assemblage in the future would be the same as the current assemblage, whereas a turnover value of 100 indicated that the assemblage would be completely modified under climate change (Buisson *et al.,* 2010). Both the change in species richness and the turnover rate were related to the latitudinal gradient using regression models in order to highlight potential spatial patterns in the response of species assemblages to future climate change. To assess the potential reorganization of fruit fly species, CE richness maps were generated for the current time period and the 2050 time period under the A2 and B2 scenarios. The CEs for all species were overlaid, counted the number of CEs that fell in any given grid cell, and then averaged the results cross the two GCMs (McKenney *et al.,* 2007).

8.2.4 Data Analysis

All variables were tested for normality using Shapiro-Wilk test and the strongly skewed variables were transformed prior to analyses if necessary to meet the assumption of normality and homogeneity of variances. Variables expressed as percentages (such as species turnover) were arcsine-square-root (+0.5) transformed, while the number of species or counts of individuals were log (log(x +1)) transformed. Wilcoxon (V) test was used to compare medians of dispersal and emission scenarios, while Kruskal-Wallis compared zones and models. Where transformation was not sufficient to improve data shape, an appropriate non-parametric test was applied. Differences among the three zones were tested with General Linear Model analysis of variance in R. Where the GLM test indicated Significant differences, post-hoc Tukey (HSD) test was used.

8.3 Results

Effect of dispersal on future climate envelope size and location

In the full-dispersal scenario, three species CEs increased in size, while the rest decreased, with an overall average of 17.2% increase (Table 8.1). There were significant differences ($X^2 =$ 30.830, df = 9, P = 0.0001) among the 10 species in their potential response to climate change. Particularly, *B. invadens* is projected to increase its current range in the face of climate change (3.1%), while *C. cosyra* showed better resilience to climate change, with projected increase rate of 26.4% by 2050 (Figure 8.2). The most concerning species with highest rate of dispersal potential under changing Uganda climate is *D. cilliatus* (Table 8.1). The species is predicted to increase its current range from 1.61 to 10.4 million hectares (545.5%) across the country by 2050

(Figure 8.2). Among those with projected declining CEs, *Dacus bivittatus* >*Bactrocera cucurbitae* >*Ceratitis anonae* were the most climate change vulnerable; their future CEs are projected to decrease by more than 75% in size (Table 8.1).

The average centres of future CEs are predicted to generally shift northwards for most species, with some species registering drastic shifts (Figure 8.3). The highest future species richness and suitability was projected in the Northwestern Wooded Savanna (NWS), Northern Moist Farmlands (NMF), Arua Farmlands (AF), and Western Mid-Altitude Farmlands (WMHF), and in the some parts of Central Buruli Farmlands (CBF) (Figure 8.1). These zones are predicted to offer the future ecological niches of *C. anonae, D. bivittatus, B. cucurbitae*, and *D. punctatifrons*. The Karamoja region will generally be unsuitable for the future distribution of most species, as it is currently (Figure 8.2 and 8.3).

Table 8. 1: Fruit fly species projected changes in climate-envelope area under the two dispersal scenarios.

Species name	Change in climate-envelope area (%)		
	Dispersal	**No dispersal**	**Average**
Bactrocera invadens (Drew, Tsuruta and White)	3.1	-6.9	-1.9
Bactrocera cucurbitae (Coquillett)	-85.8	-99.9	-92.8
Ceratitis capitata (Wiedemann)	-24.6	-74.5	-49.5
Ceratitis anonae (Graham)	-77.8	-97.6	-87.7
Ceratitis cosyra (Walker)	26.4	-30.9	-2.2
Ceratitis fasciventris (Bezzi)	-48.8	-72.3	-60.5
Dacus punctatifrons (Karsch)	-59.1	-96.0	-77.6
Dacus bivittatus (Bigot)	-93.0	-99.9	-96.5
Dacus cilliatus (Loew)	545.5	-47.0	249.3
Trirhithrum coffeae (Bezzi)	-14.1	-54.3	-34.2
Average change in envelope area (%)	**17.2**	**-67.9**	**-25.4**

In the no-dispersal scenario, species' future CEs will drastically decrease in size by an average of 67.9% during the 2050 future period (Table 8.1). In this mode too, the differences among species vulnerability to future climate change were significant (X^2 = 31.038, df = 9, P = 0.0001). The

most climate change vulnerable species are *B. cucurbitae = D. bivittatus>C. anonae>D. punctatifrons*, while the least affected were *B. invadens>D. cilliatus* and *T. coffeae* (Table 8.1; Figure 8.3). *D. bivittatus*, *B. cucurbitae* and *C. anonae* under the no dispersal scenario will only retain marginal niches in WMAF, but the latter two are also predicted to retain isolated patches in the NMFs and Usuk Sandy Loams (USL) zones. In contrast, *D. punctatifrons'* distribution will be marginally possible in the NMF and NWS zones (Figure 8.3).

Generally, the results predicted a 25.4% fruit fly species range decline due to the anticipated climate changes during the 2050 future period (Table 8.1). However, there were significant differences in response (X^2 = 30.97, df = 9, P = 0.0001) among the 10 species, with only one species (*D. cilliatus*) showing higher potential (249.3%) for resilience to the projected climate change (Table 8.1). *B invadens* (-1.9%) and *C. cosyra* (-2.2%) also showed negligible decline in their proportional areas in the intermediate dispersal option (average of both dispersal scenarios) (Table 8.2). Under this option, results showed that the species most at risk were *D. bivittatus>B. cucurbitae>C. anonae >D. punctatifrons,* all with approximate loss of over 75% over the 2050 future period (Table 8.1). Southern and western Uganda will not be attractive to most fruit flies.

Effect of carbon dioxide emission scenarios on future species habitat size

The 10 species showed considerable changes in area of occupancy, with habitat losses higher under the B2 (30.56±22.6%) but not significantly different (t = 0.0451, df = 78, P = 0.653) with A2 (16.38±21.8%) emission scenarios (Figure 8.4 A). *D. cilliatus* registered increased habitat size fairly even in both scenarios, while *C. cosyra* showed reduced and increased habitats under A2 and B2 scenarios, respectively (Figure 8.4B). Species most at risk under the A2 scenario were *B. cucurbitae >D. bivittatus>C. anonae >D. punctatifrons,* all with habitat losses ranging

between 72 and 91%, while in B2, losses raged between 85-96%, with C. *anonae* > *B. cucurbitae* > *D. bivittatus*>*C. fasciventris* being the order of vulnerability over the 2050 future period (Figure 8.4 A). Emission scenarios and dispersal options jointly explained significant variability in habitat changes (F = 3.102, df = 3, 76, R^2 = 10.9%, P = 0.032).

The two climate-change models were qualitatively consistent in predicting distribution effects and future range shifts on fruit fly species. However, CCCMA consistently predicted more extreme changes in size than the HADCM. However, among the species, there were significant differences in the two model predictions for future changes (F = 3.174, df = 19, 60, R^2 = 50.1%, P = 0.000). The highest differences in change magnitude were noted among *C. capitata*, *C. fasciventris*, and *T. coffeae*, with all being consistently more with CCCMA. The only models contradiction in occupancy prediction direction was in *C. cosyra*, CCMA predicted decline, while HADCM an increase.
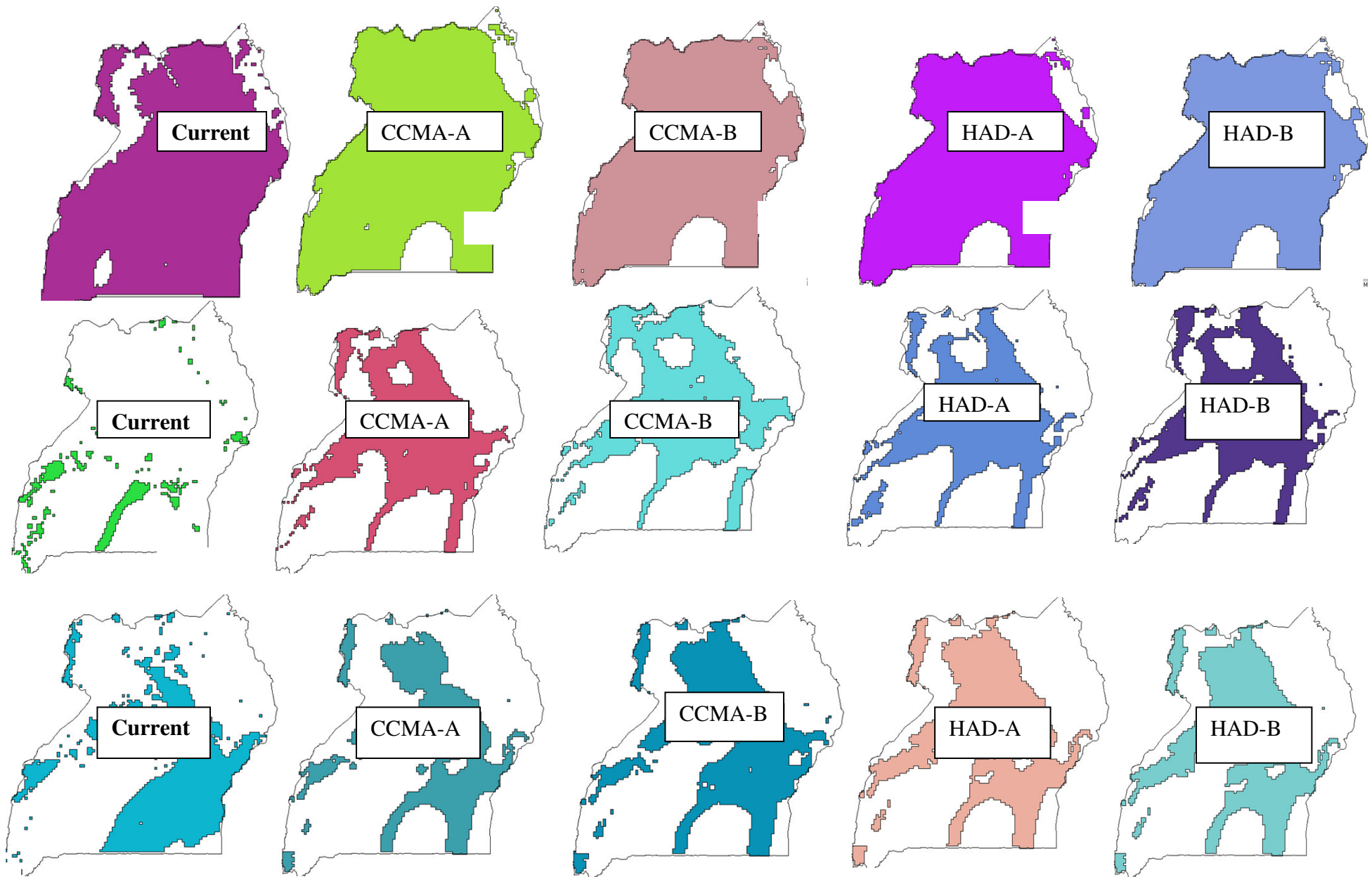
Figure 8. 1 Change in the size of climate envelopes predicted by four climate-change models for the three fruit species with gains from a 1950-2000 baseline to the 2050 future period for the dispersal scenario in which species move entirely into future climatic niches. The species are *B. invadens* (first five in line), *D. cilliatus* (second five in line) and *C. cosyra* (last five in line). Species such as *C. capitate, C. rosa, D. punctatifrons* that did not show gains in the period are not illustrated here

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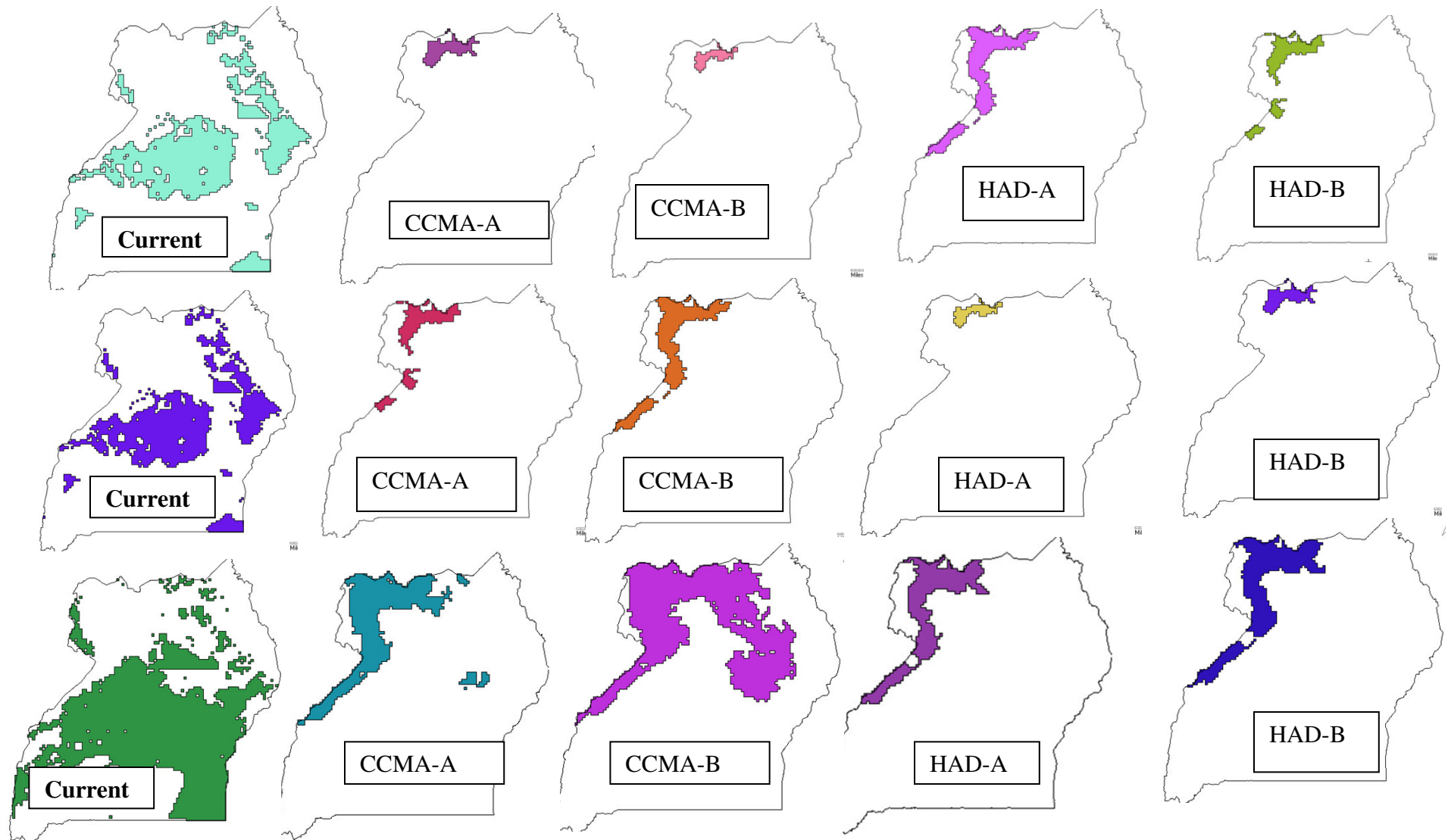
Figure 8. 2 Change in the size of climate envelopes predicted by four climate-change models for the three fruit species with losses from a 1950-2000 baseline to the 2050 future period for the dispersal scenario in which species move entirely into future climatic niches. The species are *D. bivittatus* (first five in line), *B. cucurbitae* (second five in line) and *C. anonae* (last five in line). Species that did not show losses in the period are not illustrated here.

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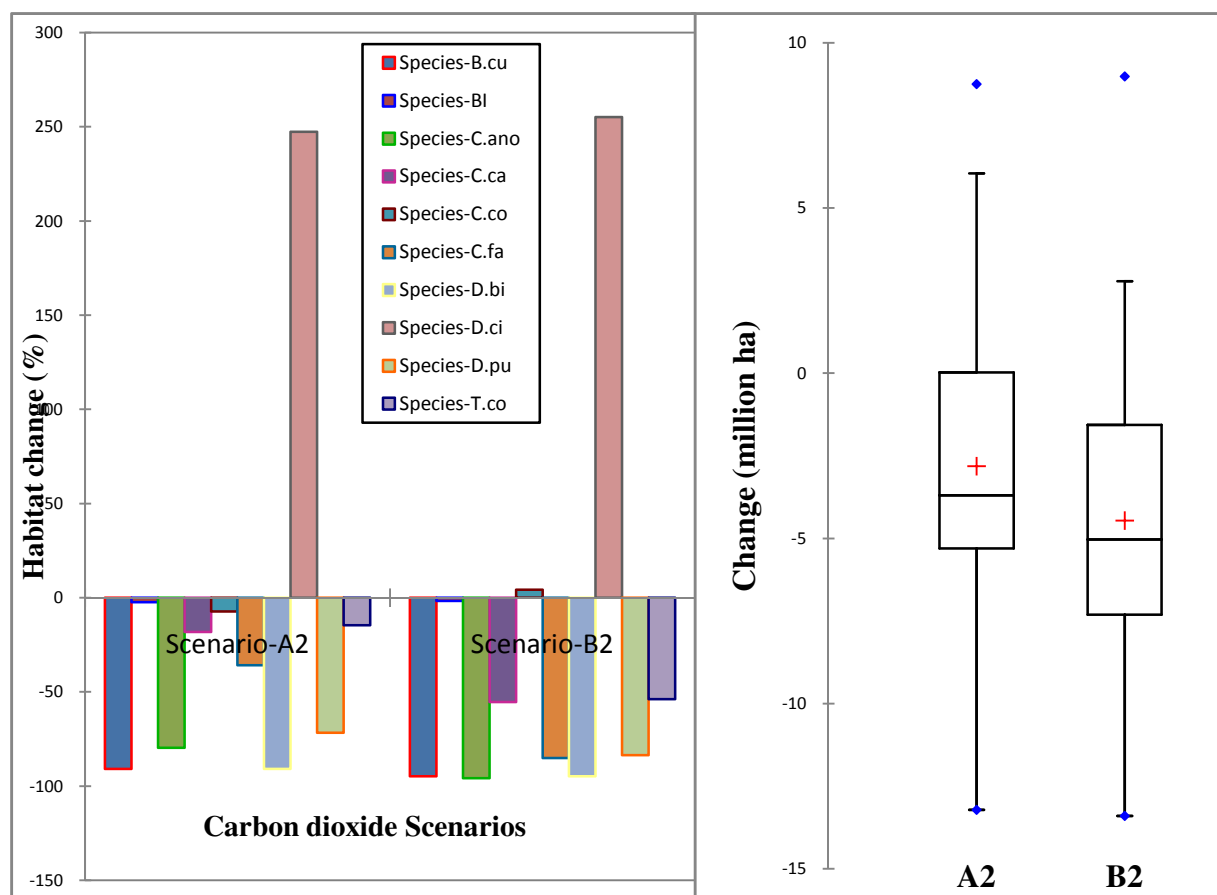
Figure 8. 3: Proportional changes in habitat size of predictions under the two carbon dioxide emission scenarios for the 10 fruit fly species (A) and box plots for carbon dioxide scenarios (B) from a 1950-2000 baseline to the 2050 future period. Box plots show median, mean, 25th and 75th percentiles, and 10th and 90th percentiles. Abbreviations are: B-cu, *B. cucurbitae*; C.ano, *C. anonae*;D. bi, *D. bivittatus*;C.fa*, C. fasciventris*;BI*, B. invadens*; D.ci*., D. cilliatus;* T.co*., T. coffeae;* D.pu., *D. punctatifrons;* C.co*., C. cosyra;* C.ca*., C. capitata.*

Potential climate-induced changes in fly assemblages in the three mango growing zones

Species richness was predicted to generally decrease from the current 10 species by 2050, but the decrease significantly differed across the three zones (X^2 = 11.7, df = 2, P = 0.003). The highest decrease was predicted in WMHF (4.5 species, 55%), while the least was in the NMF (8.8 species, 12%) under dispersal mode, while a similar trend was predicted for the non-dispersal mode: WMHF > LVC > NMF (Table 8.2). Indeed dispersal modes differed significantly (V =

21.000, df = 1, P = 0.034), with the full-dispersal predicting higher mean richness (5.7±2) compared to no dispersal mode (4.4±1). Amongst the models the order of species decline followed the order: HAD-B > CCMA-A =CCMA-B > HAD-A (Table 8.2).

Table 8. 2: Predicted species richness and turnover under the four models and two dispersal scenarios in the three main mango-growing regions under.

Model	WMHF		LVC		NMF	
Dispersal	Richness	Turnover (%)	Richness	Turnover (%)	Richness	Turnover (%)
CCMA-A	4	60	3	70	8	20
CCMA-B	4	60	3	70	8	20
HAD-A	6	40	6	40	10	0
HAD-B	4	60	3	70	9	10
Mean	**4.5**	**55**	**3.8**	**62.5**	**8.8**	**12.5**
No dispersal						
CCMA-A	4	60	3	70	5	50
CCMA-B	4	60	3	70	5	50
HAD-A	6	40	6	40	7	30
HAD-B	3	70	2	80	5	50
Mean	**4.3**	**57.5**	**3.5**	**65.0**	**5.5**	**45.0**

Similarly, species turnover across the three zones showed declines in species composition, and the difference among zones was significant (X^2 = 11.34, df = 2, P = 0.003) (Table 8.2). Post-hoc analysis showed that the LVC (63.75±5.3%) had higher potential turnover than WMHF (56.25±3.8%) and LVC (28.75±6.9%), the former two were not significantly different. Turnover under the two dispersal modes differed significantly (P = 0.031), but converse to the species richness. The full-dispersal predicted less mean turnover (43.33±7.3%) than the no dispersal mode (55.83± 4.3%). Amongst the models the predicted order of species turnover followed exactly the same order as that under species richness: HAD-B > CCMA-A =CCMA-B > HAD-A (Table 8.2).

The change in species turnover and richness is projected to vary according to the position along the latitudinal south-north gradient (Figure 8.4). This indicated that species richness and turnover

would have the largest increase and decrease in the southern and northern habitats, respectively, and hence the predicted relatively higher suitability in the NMF, due to the potential migration to suitable climate habitat. Occupancy in the LVC, WMHF and the greater southern Uganda is predicted as unlikely, as the sites showed poor future colonization by most species. Predicted species turnover values for the LVC (63.75±5.3%) and WMHF (56.25±3.8%) indicated that assemblages in these zones would be highly modified due to the potential local extinction of species, which on the contrary would not be the case with the potentially suitable NMF assemblages.
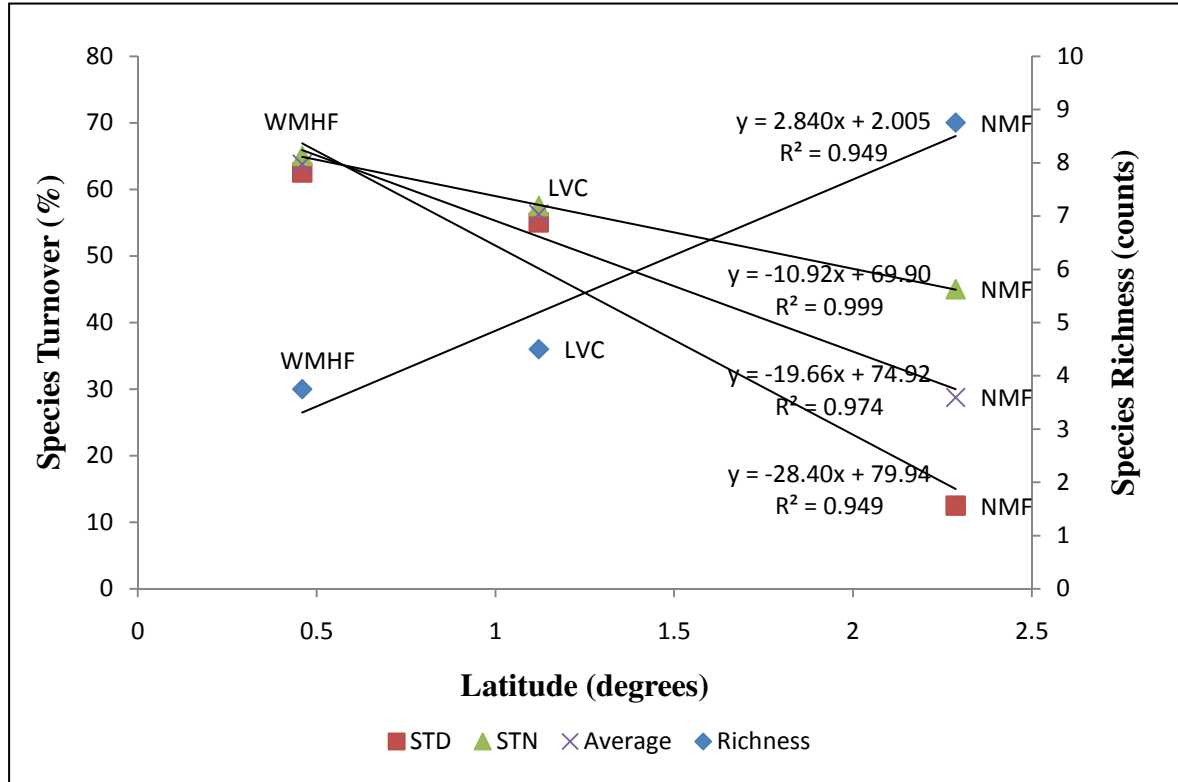
Figure 8. 4: Predicted modifications of fruit fly assemblages and turnover along the latitudinal gradient for the 2050 future period under the dispersal (STD) and no dispersal (STN) scenarios. Regression equations and the associated coefficients of determinations and probability values from fitted models are shown.

8.4 Discussion

Climate change and future distribution of fruit fly species

This study provided a first insight into the potential local-level distribution patterns and range shifts under future climate change for the 10 selected species. Generally, the results predicted declining CE sizes in the future climates, which is in agreement with other studies that have predicted range shifts among tephritid fruit flies with climate change (FAO, 2008). The three species most at risk (*Dacus bivittatus >Bactrocera cucurbitae >Ceratitis anonae)* have relatively narrow CE parameter ranges, and very specific climatic requirements that could limit their future adaptation (McKenney *et al.,* 2007). Currently, these species have very limited distributions in southern, western and northern Uganda, and mostly prefer habitats in central Uganda. This specificity in CE sizes of the three species suggests that their future distribution will be hampered critically by climate variability, particularly their temperatures ranges that are rarely found under the 2050 future climate scenarios. This is the converse for *B. invadens* and *C. cosyra.*

Of particular concern is the Ethiopian fruit fly (*D. cilliatus*), whose range is projected to increase by up to 250% of the current areas. The future proliferation of this species is probably due to the fact that it is known to prefer drier conditions characteristic of Ethiopia, hence projected warming trends should be suitable. Under the A2 emissions scenario, predicted CEs were smaller and more northerly than CEs predicted under the B2 scenario, as also reported by McKenney *et al.,* (2007). In the A2 scenario, the human population is larger and greenhouse gas emissions are higher than in the B2 scenario (Nakicenovic, 2000).

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Effect of dispersal on future climate envelope size and location

Susceptibility to climate changes significantly differed under the two dispersal scenarios (Table 8.1). In the full-dispersal scenario, there was an overall average increase of 17.2%, while the no-dispersal scenario, recorded a drastic decrease in size by an average of 67.9%. The species whose range showed increases and shifts demonstrated capacity to shift their distributions in response to future climate change, while those whose range declined (e.g *D. bivittatus*) were incapable of such shifts. The range declining species may not be able to survive in the future climate niches due to the unprecedented rapidity of projected climatic changes (Schloss *et al.,* 2012). Hence those species may experience reductions in range size due to limited dispersal abilities. Because climate change will likely beat the response capacity of many fruit flies, tephritid vulnerability to climate change may even be more extensive than anticipated.

The dispersal hypothesis makes several assumptions: successful reproduction occurs at the youngest age biologically possible and that offsprings in every generation survive dispersal age and successfully disperse; and that, suitable habitat and climates for establishment, survival, and reproduction will exist between regions of current and future climate and that individuals will disperse directly toward the closest suitable grid cell (Schloss *et al.,* 2012). These assumptions could have had implications on the estimates of the percentages of species that will actually reach and colonize suitable climate habitats, as reality shows that dispersal has high associated mortality (Waser *et al.*, 1994; Sutherland *et al.,* 2000). Particularly, dispersal paths in invertebrates are generally limited by their small size, and this might inhibit their ability to keep pace with climate change. It is therefore most probable that most fruit fly species will not be able

to disperse over long distances, making them more vulnerable to warming trends, with the exception of *D. cilliatus*.

The fact that the models did not consider topography, rivers, roads, and other dispersal barriers, which may impede movement, it is possible that the dispersal option overestimates the percentage of fruit flies that will keep pace with climate change (McKenney *et al.,* 2007). It may therefore be logical to infer that dispersal mode predictions in this study represent the upper limit of species' movement potential rather than a likely dispersal scenario. The relatively higher estimate of species vulnerability to climate change under the no dispersal, unlike the full dispersal scenario is probably because the latter does not incorporate landscape permeability, which the former does (Schloss *et al.,* 2012). Landscape permeability is the degree to which the landscape facilitates animal movement (Bissonette and Adair, 2008).

In addition to climate variables, the relationship between fruit flies and their larval plant-hosts greatly determines the choice of suitable habitats (Scheirs *et al*., 2004). Therefore, the availability of appropriate hosts between some species' ranges and regions of future suitable climate will increase the percentage of species that are able to keep pace with climate change by offering oviposition options along the dispersal trajectories. Where more permeable landscapes with larval hosts and appropriate soil condition for pupae development do not occur, dispersal may be less successful due to the potential for decreased survival and reproduction in less suitable habitats. This offers an opportunity for the design of future management strategies. Managing alternative hosts for the migratory fruit fly species and incorporating species-specific

habitat barriers along the dispersal routes would realistically curtail species migration to suitable climates.

While the results have higher levels of confidence, the uncertainties in bioclimatic modelling could have caused over- or under-estimates in these results (Pearson and Dawson, 2003; Dormann, 2007; Schloss *et al.,* 2012). For example, false assumptions of climatic intolerance due to modeling only the realized niche, and therefore ignoring species interactions and other non climatic determinants of range boundaries, may increase the projected distance a species needs to travel to suitable climate and cause overestimates of the percentages of species unable to track climate change (Schloss *et al.,* 2012). In addition, correlative models have inherent uncertainties associated with their inability to project the suitability of novel climates and to account for evolution. Future fruit fly range expansions may occur from other species populations that are already closer to regions of future suitable climate or from other pest species that were not considered in this study. Incorporating fruit, vegetation and soil (aridity index) as predictor variables might result in more interesting results. For instance, when future distribution of the three most vulnerable species in this study were assessed using temperature, rainfall and aridity index (Precipitation over Potential Evaporation, P/PE), they all showed extinction.

Potential climate-induced changes in fly assemblages in the three mango zones

The results showed that species richness was predicted to decrease significantly differently across zones, with the WMHF recording the highest decrease, while the least was in the NMF. Similarly, species turnover across the three zones was significantly different, with the order converse to richness. Species increased along the south- northern gradient, while turnover

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declined significantly too. This would propose increased diversity northwards as a result of favorable habitat suitability.

In the tropics, many species lack the ability to keep pace with climate change despite forecasts for less dramatic changes in climate. In tropical regions, species generally have more restricted physiological tolerances and hence are expected to be more sensitive to climate change (Tewksbury *et al.,* 2008), which explains the projected declines (25%) in occupancy suitability for the majority of the species. The observed higher percentage (90%) of species unable to reach suitable climates may be the result of lower latitudinal gradients in climate in Uganda and/or higher sensitivity to climatic changes due to narrower climatic niches of the fruit fly species. Indeed, with the exception of *D. cilliatus*, the majorities of the species in this study are least able to keep pace with climate change and are most likely to experience climate-change–induced range reductions.

The results suggest that the Ceratitis genera, which constitutes most of the native species is equally threatened by climate change. Further assessment of the potential vulnerabilities of native species in this genus to climate change will enable management efforts to focus on species that are less vulnerable to climate change by maximizing their alternative biotic and abiotic constraints, which will reduce their resilience.

8.5   Conclusions

This study reports for the first time the potential impacts of climate change on the climatic ranges of 10 fruit fly species in Uganda. The results indicate that most species are vulnerable and will

likely be unable to keep pace with climate change, with habitat losses averaging 25.4% by 2050 future period. The findings showed that fruit fly climate change resilience varied significantly and followed the order: *Dacus cilliatus > Bactrocera invadens > Ceratitis cosyra > Trirhithrum coffeae > Ceratitis capitata > Ceratitis fasciventris > Dacus punctatifrons > Ceratitis anonae > Bactrocera cucurbitae > Dacus bivittatus.* The first three species pose a serious management challenge, while for dispersal-limited species (e.g., *Dacus bivittatus, Bactrocera cucurbitae* and *Ceratitis anonae*); increasing non-climatic stressors will help to manage these species in face of changing environments.

Future habitats are predicted to shift northwards of Uganda, mainly to the Northern Moist Farmlands. Species increased along the south-northern gradient of Uganda, while turnover declined significantly too. This would propose increased diversity northwards of Uganda as a result of favorable habitat suitability. The most likely outcome of the future distributions will be determined not only by climate but also by a hierarchy of factors such as dispersal ability, biotic interactions, genetic adaptation, and abiotic factors, and will probably be somewhere between full- and no dispersal scenarios, particularly for latitudinal shifts where the lack of fertile soil and alternative hosts in northern Uganda likely to limit the north-ward migration of many species.

CHAPTER NINE: GENERAL DISCUSSION AND CONCLUSIONS

9.1 Discussion

9.1.1 Species richness of pest fruit flies is high but dominated by *B. invadens*

Across the three zones, there are at least ten tephritid fruit fly species; but *B. invadens* is the most abundant, and was observed to be displacing the other fruit fly fauna. The observed significant difference in zonal faunal composition can be attributed to their (zones) inherent differences in interactions of environmental conditions, host composition and farming systems. Although the NMFs had climatically suitable conditions, it recorded lesser alternative hosts for fruit flies, which was converse to the LVC and the WMHF. In addition, the longer distances between agro ecological zones, than between respective orchards in a zone might increase dispersal limitation, and therefore lead to different species pool adapted to local conditions (Summerville *et al.,* 2003, 2006). The dominance of *B. invadens* over the other species has been widely reported and can be attributed to competition efficiency (Ekesi *et al*., 2009), and reproduction and food resource distribution (Kiesecker *et al.,* 2001). The LVC farming system offers opportunities for resource distribution, which avoids clumping, converse to the NMFs, which might also explain the difference in displacement pressures between the two zones.

Differences in farm management practices across the three zones could also explain the low infestation in the intensively managed orchards in WMHF as opposed to the poorly managed NMF orchards. The results have also helped to partly refute the earlier assumption that because of its polyphagous nature, *B. invadens* does not necessarily conform to the expected positive relationship between adult oviposition preference and offspring performance. Results demonstrated differences in preference and performance among the fruit species, but the

relationship was poor among mango varieties. It is therefore possible that the differences in preference and performance observed among the fruit species may generate the selective pressures necessary for the evolution of these behaviors, which calls for further studies.

The ability of *B. invadens* to undergo rapid phenotypic variability noted in this study is likely to affect the efficiency of common control methods, and these results should help to recast fruit fly management strategies. The results also offer preliminary evidence of higher levels of genetic variation in natural populations of *B. invadens*, which allows alternative phenotypes in response to host, and geographical variations. This may afford the different populations to adapt and survive in difficult conditions such as the stress caused by control practices and subsequently cause resistance among populations. However, at this stage, it is possible that the recorded fine-scale intraspecific population phenotypic variations may simply be evidence of phenotypic divergences within species (intraspecific) rather than interspecific differences (Schutze *et al.,* 2012). Such differences may be irresolvable using techniques such as molecular analysis, hence the current lack of molecular markers to discriminate between these eco- and host types.

9.1.2 Fruit flies have a range of hosts but tropical almonds, guava and mangoes supersede

Commercial and noncommercial fruit fly hosts was recorded in the study, albeit with significant variability in preference and infestation levels among types, varieties and zones. Variations in fruit type and variety infestation among the zones could indicate that fruits have specific adaptations in their ecological requirements, which ultimately determine their (fruit) susceptibility in their respective environments (Dukas and Bernays, 2000). The long held perception that local mango varieties are more tolerant to fruit fly infestation than the exotics has

been to a great extent refuted in this study. The relatively higher preference of the flies for the local selection and Kagogwa varieties observed in this study may be attributed to an increase in performance on these varieties or due to experience or learning, noted among phytophagous insects. The Uganda country wide distribution of local selections and Kagogwa varieties might have favored adaptation, in addition to the favorable physical and chemical attributes of these fruits that render them susceptible to infestation (Szentesi and Jermy 1990; Dukas and Bernays, 2000).

9.1.3 Temperature and rainfall were strong determinants of fruit fly ecological niche

The most suitable niches encompass areas around Central and mid north zones, while the western, northeastern and areas around Albert Nile were characterized as marginal. These suitable areas also coincide with the best combinations of the precipitation and temperature, the most important abiotic constraints. The predicted current and future niches offer the optimum bioclimatic tolerance limits for their respective fruit fly species. As fruit hosts are assumed to be critical determinants in the presence or absence of most species (Rubio and Acosta, 2010), the predicted suitable niches need to be matched with the right climatic factors (temperature and rainfall). Consequently, current and future potential distributions of fruit flies will be determined not only by climate but also by a hierarchy of factors such as dispersal ability, biotic interactions, genetic adaptation, and abiotic factors. The Ethiopian fruit fly (*D. cilliatus*), *B. invadens* and *D. cilliatus* whose future potential ranges are projected to increase should be of great concern and their effective management will call for climate adaptive strategies.

9.2   Conclusions

In Uganda, a diversity of pest fruit flies infests fruits, with the alien *Bactrocera invadens* being predominat. It is therefore urgent that an integrated fruit fly management programme is implemented to guard the benefits of the horticulture industry. The study noted significant variability in fruit host species and mango variety infestation, with tropical almonds, guavas, and mangoes being the most favorable fruit species among the wide spectrum of fruit hosts. Mango varieties varied in their susceptibility to fruit fly infestation within and across zones, hence making definite conclusions of the most and least susceptible varieties difficult. However, early and late season maturing mango varieties were more susceptible. Significant phenotypic discrimination among host and AEZ by *B. invadens* populations was evident, with very high degree of population classification, indicating that geographic and host-associated adaptation could produce phenotypic variations that can lead to ecotype and host populations. Precipitation and temperature significantly shaped fruit fly niches and critically determined distribution suitability across the country. Central and mid north zones provided the most suitable niches, while the western, north-eastern and areas around Albert Nile were characterized as marginal. Future potential fruit fly species ranges were projected to decline by 2050 albeit differently among species, while significant range shifts mainly to the northern moist farmlands were also likely.

9.3   Management implications of the findings of these studies

1. Fruit fly management in Uganda will have to employ a range of methods/tools because of the widespread nature of the problem, both spatially and temporally. The methods/tools should be those that are specifically affect the species present. Control efforts will have to engage deeply mango/ fruit farmers to assist in the deployment and monitoring of

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controls because fruit trees in Uganda are generally scattered and most fruits are not grown in orchards

2. Control may have to adopt an area-wide pest management strategy because this study has demonstrated that Uganda has a very wide range of plants (both cultivated and non-domesticated) susceptible to fruit fly infestation and capable of sustaining fruit fly populations. However, the potential effectiveness of such a study will require further evaluation. Additionally, every part of the country is able to host the pest species irrespective of prevailing agro-ecological conditions both now and in the future. Climate modelling studies suggest some species may become less frequent/ abundant but others will remain important and expand their geographical ranges. Hence the need to institute within Uganda a viable fruit fly management regime.

3. Uganda's potential/ability to export fruit or process it into other value-added products will remain compromised because of the prevailing fruit fly challenge. Meaning that potential revenues and jobs arising from export trade pathways will not be realized until fruit flies are adequately handled.

9.4   Knowledge Gaps and Recommendation for Future Research

The scale of the debate on the ecology of fruit flies is therefore extensive and multifaceted even at the Uganda country level, as has been suggested by these findings. To generate achievable management strategies and horticulture industry development targets, there is need for more case studies in Uganda to allow further assessment of local dimensions of the subject. Among the knowledge gaps that future studies can focus on are:

1. This study concentrated mainly in three agro ecological zones (LVC, WMHF and NMF). Therefore, further studies should improve the knowledge of ecological aspects of

Tephritid fruit flies in the different agro ecological and edapho-climatic conditions of Uganda.

2. Expand the oviposition preference study to other plant hosts and/or other important fruit fly species to evaluate the consistency of the findings. Also, to refine the study on mango varieties because logistical limitations in the current study prevented the collection of data on some parameters and not all varieties were included in the study.

3. Research to confirm whether the observed host and geographic phenotypic differences have genetic and behavioral confirmatory basis is recommended.

4. There is need for widespread monitoring surveys to ground-truth the fruit fly distribution suitability across Uganda. Such information will help evaluate modeling performance and also reduce apparent commission errors. Such efforts should also include edaphic and host plant data in building fruit fly species models.

5. Evaluate the potential of an AWPM strategy to effectively manage fruit flies in a highly heterogeneous and fragmented environment/landscape as typified by Uganda's agro-systems.

6. The higher incidence of fruit fly damage in NMF orchards may perhaps be explained by the much lower availability of alternative plant host species in this AEZ that would otherwise help to reduce the pest pressure in the orchards. A longer term ecological analysis of infestation pressure, population dynamics and host range in the region would perhaps give more insight. Across Uganda a Knowledge Attitudes and Practices (KAP) study would also give information on how farmers are dealing with the fruit fly challenge and give insight into how the farmers can be engaged further on management aspects.

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Final Message

All of us should be worried about fruit flies as serious pests, because of their diversity; direct larval feeding on the host fruit (economic loss); long list of host plants; flexibility in life history strategies, including rapid adaptation to new habitats; efficient habitat utilization; ability to rapidly develop host and geographic races (particularly true for Bactocera species); and short generation time (rapid multiplication)… **(Brian E. Isabirye, 2015)**

The Road

"Though the road's been rocky it sure feels good to me." ― *Bob Marley*

Clockwise: Catherine, Brian, Emma, Pretty, Anne, Rai…

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