Variability of Pathogenic Activity of Entomogenous Fungi (Hyphomycetes) towards the Legume Flower Thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) and their Potentials for Biological Control

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A Thesis submitted to the Postgraduate School, Ahmadu Bello University, Zaria, in Partial Fulfillment of the Requirements for the Award of the Degree of Doctor of Philosophy in Crop Protection

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work conducted at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya under the African Regional Post Graduate Programme in Insect Science (ARPPIS). It has not been presented before in any previous application for a higher degree.

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CERTIFICATION

This thesis entitled "Variability of pathogenic activity of entomogenous fungi (Hyphomycetes) towards the legume flower thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) and their potentials for biological control" by Sunday EKESI meets the regulations governing the degree of Doctor of Philosophy of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation

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TO MY PARENTS FOR THEIR LOVE AND SUPPORT

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ABSTRACT

Twenty-two strains of entomopathogenic fungi were tested in the laboratory to determine their pathogenicity to adult *Megalurothrips sjostedti* (Trybom). All the fungal strains were pathogenic to the insect. *Beauveria bassiana* and *Metarhizium anisopliae* strains caused mortality ranging between 29 to 100% and 54 to 100%, respectively. In contrast, *Verticillium lecanii* and *Paecilomyces fumosoroseus* were less virulent, causing mortality ranging between 29 to 68% and 13%, respectively. The LT_{50} of the most virulent *B. bassiana and M. anisopliae* strains was 2.7 and 2.4 days, respectively. The LC_{50} for the most active *B. bassiana* strain was 7.9×10^6 conidia ml^{-1}. The LC_{50} for the most active strain of *M. anisopliae* was 1.3×10^6 conidia ml^{-1}. Two strains of *B. bassiana* and four strains of *M. anisopliae* were found to be highly pathogenic to *M. sjostedti*, which suggests a potential for their use in biological control of the pest

The effect of temperature on germination, radial growth and pathogenic activity of two strains of *B. bassiana* and four strains of *M. anisopliae* selected during the screening against *M. sjostedti*, was studied in the laboratory. Germination, radial growth and pathogenic activity were low for all strains at 15^0C. Optimum temperature for germination, radial growth and pathogenic activity ranged between 25-30^0C. The fastest growing strain at 25-30^0C was *M. anisopliae* ICIPE 69. Compared to other strains, ICIPE 69 also appeared to have a broad temperature range of pathogenic activity against the pest.

The effect of *M. anisopliae* strain ICIPE 69 was tested against *M. sjostedti* developmental stages. All stages of *M. sjostedti* were susceptible to infection by *M. anisopliae*. However, larval and pupal stages were less susceptible to fungal infection than the adult stage. Mortality in all stages was dose-dependent with the highest mortality occurring at 1×10^8 conidia ml^{-1}. Daily number of pollen emptied in infected thrips was reduced within 24 h in adults but more slowly in larval thrips. Fecundity, egg fertility and longevity in adult surviving larval infection were significantly reduced compared to the control treatment.

The susceptibility of *M. sjostedti* to *M. anisopliae,* strain ICIPE 69, when reared on varieties of cowpea that are susceptible (ICV 2), tolerant (ICV 7), and moderately resistant (ICV 8) to *M. sjostedti* was evaluated at different temperatures in the laboratory. Mortality was significantly higher on the moderately resistant variety at all temperatures compared to the susceptible and tolerant varieties. Correspondingly, lethal time and lethal concentration values were significantly shorter and lower, respectively on the resistant variety compared to the other varieties, thus indicating that the two control methods could be a compatible integrated pest management strategies. The tolerant variety incurred a significantly low level of mortality. Observation on the effects of volatiles and crude extracts of this variety revealed an inhibitory effect on germination and colony forming units of the fungus. This suggest the existence of some antifungal substances in the tolerant variety

Field experiments were conducted at ICIPE Mbita Point Field Station (MPFS), western Kenya, for two seasons to evaluate the potential of the entomopathogenic fungus, *M. anisopliae* for biological control of *M. sjostedti* on cowpea. An Ultra-low volume (ULV) oil/aqueous formulation and a High volume (HV) aqueous formulation of conidia were applied thrice each at two concentrations of 1×10^{11} and 1×10^{13} conidia ha^{-1}. Compared with the controls, both formulations significantly reduced thrips population and plant damage in both seasons. Adult insects collected from fungal treated plots and reared in the laboratory showed high mortality due to mycosis. Flower and pod production was significantly higher in treated plots compared to the control plots. HV formulation was superior to ULV formulation in reducing thrips population and plant damage, and in increasing flower and pod production. During the first season, plots treated with HV formulation at 1×10^{13} conidia ha^{-1} produced yields which were significantly higher than other treatments. No significant difference in grain yield was found between the fungal treated plots and the chemical insecticidal treatment, Lambda-cyhalothrin (Karate® 17.5 EC) during the second

season. The results of these experiments suggest that *M. anisopliae* is a potential candidate for the management of *M. sjostedti* on cowpea.

Field experiments were conducted during two cropping seasons in 1997 to assess the combined effects of intercropping cowpea with maize and application of *M. anisopliae* or synthetic insecticide on the density of *M. sjostedti* and cowpea grain yield. Thrips population density and damage were significantly lower in the intercrop treated with fungus compared to the control. Mortality due to mycosis was significantly higher in cowpea intercrop compared to the monocrop. Flower and pod production were significantly higher within the intercrop treated with *M. anisopliae* than in the monocrop. In both seasons, grain yields in cowpea intercrop treated with *M. anisopliae* were not significantly different from yields within cowpea intercrop treated with Karate. *M. anisopliae* had no adverse effect on the populations of non-target organisms. The results of this study suggest that *M. anisopliae* is a promising candidate for the management of *M. sjostedti*, especially within a cowpea/maize intercropping systems.

Field experiments were conducted for two seasons to evaluate the timing of application of *M. anisopliae* for the control of *M. sjostedti* on cowpea. One application of *M. anisopliae* given at flower bud stage and another at flowering stage did not protect cowpea yield as does chemical insecticide. Instead, one application given at flower bud stage and two applications given at flowering were required to keep *M. sjostedti* density in check through these stages, which are very sensitive to thrips, damage. Studies of persistence showed that *M. anisopliae* remained active in the field for 3-4 days.

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CHAPTER ONE

1 General Introduction

Cowpea, *Vigna unguiculata* (L.) Walp, is an important pod and fodder crop in different parts of the tropics (Quin, 1997). In many places, it provides more than half the plant protein in human diets where it supplements starch staple (Singh and Rachie, 1985). Although cultivated primarily for its edible seeds, direct consumption of cowpea leaves is also widespread in different parts of Africa (Nielsen *et al.*, 1997). Low yields are significant attribute of production estimates where 240-300 kg ha^{-1} is typical in Africa (Singh and Rachie, 1985). This has been attributed largely to heavy pests pressure. Jackai and Adalla (1997) listed at least 20 major insect pests species in the various cowpea-growing regions of the world, the number varying from region to region. Some of the most damaging of all the pest insects include the legume flower thrips, *Megalurothrips sjostedti* (Trybom), legume pod borer, *Maruca vitrata* Fab, a complex of pod and seed suckers of which *Clavigralla tomentosicollis* Stål. is the dominant species and the cowpea weevil, *Callosobruchus maculatus* Fab. the major storage pest.

The legume flower thrips, *M. sjostedti,* is considered as the first major pest attacking the reproductive structures of cowpeas during plant development (Okwakpam, 1967; Taylor, 1969; Nyiira, 1973; Ezueh, 1981). Recorded in the African continent for the first time in 1905 (Trybom, 1908), *M. sjostedti* was first identified as a pest in East Africa (Faure, 1960). Ever since this report, the pest has seriously threatened cowpea production causing yield losses which has been estimated at between 20-100% in various parts of Africa (Singh and Allen, 1980). In Kenya, 94% yield loss has been reported (Ampong-Nyarko *et al.*, 1994).

Chemical pesticides, in practice, are the major control measures of *M. sjostedti* on cowpea (Singh and Jackai, 1985; Singh *et al.*, 1990; Jackai and Adalla, 1997). This increased use of chemicals is undesirable for the environment, especially, since this crop is grown over a wide area.

Moreover, widespread thrips resistance to chemical pesticide has been reported from various part of the world (Royer *et al.*, 1986; Brodsgaard, 1994; Immaraju *et al.*, 1992). Solution to this problems have to be found that will accord with long term public interest, for the potential hazards are too great to allow pests control to become purely a function of chemical pesticides.

Environmental management practices such as intercropping have been reported to reduce population of *M. sjostedti* (Kyamanywa and Tukahirwa, 1988; Kyamanywa *et al.*, 1993). Ampong-Nyarko *et al.* (1994) also showed that yield loss due to *M. sjostedti* can be reduced from 94% to 51% in cowpea/sorghum intercrop receiving chemical treatment. Although there are yield advantages under this condition, 51% yield loss is still enormous and require attention.

Megalurothrips sjostedti is attacked by a number of predators and parasitoids such as *Megaphragma* spp., *Oligosita* spp. and *Orius* spp. The impact of these natural enemies are however, very low and do not contribute significantly to thrips mortality (Kyamanywa *et al.*, 1993; Tamo *et al.*, 1993; 1997), although more recently attention is being drawn to classical biological control based on the introduction of hymenopterous parasitoid, *Ceranisus menes* Walker (Tamo *et al.*, 1997).

Field studies have shown that various species of thrips including *M. sjostedti* are sometimes infected by entomopathogenic fungi, especially of the genera, *Beauveria, Neozygites, Verticillium, Paecilomyces* and *Hirsutella* (Raizada, 1976b; Salifu, 1986; Greenwood and Mill, 1989; Tamo *et al.*, 1993). *Entomophthora* spp are also known to cause epizootic among *Thrips tabaci* Lindeman (Carl, 1976; MacLeod et al., 1976; Samson *et al.*, 1976). In what appears to be a quantitative assessment of the key factors responsible for pest status of *M. sjostedti* on cowpea, Tamo *et al* (1993) reiterated the need to investigate into the possibility of utilizing entomopathogens in the control of *M. sjostedti*. Several precedents exist for the application of myco-insecticides for the control of crop pests (Ferron, 1978; Zimmermann, 1986b; Ferron *et al.*, 1991; Moore and Prior

1993; Lomer *et al.*, 1997). However, very few reports are available in literature on the evaluation of these pathogens for the control of thrips species. Gillespie *et al.* (1983) reported good control of *T. tabaci* by *Verticillium lecanii* (Zimmermann) Viegas. *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are also known to be pathogenic to *T. tabaci* (Gillespie, 1986). Vestergaard *et al.* (1995) reported the susceptibility of *Frankliniella occidentalis* (Pergande) to *V. lecanii* and *M. anisopliae*. Fungi offer a potentially environmentally benign alternative to the ever-increasing pesticide addiction and mounting costs, in addition to severe harm to non-target species and the environment. The environmental safety of biological pesticide is of particular relevance where crops such as cowpeas, which are grown on, a large area is to be treated. The conidia of dry-spored fungi as *Beauveria* and *Metarhizium* are hydrophobic and are miscible in oil. Oil formulations would also be suitable for ultra-low volume application, where the use of water sprays is precluded by their rapid evaporation and the large volume of water required (Prior, 1988; Mills, 1991; Moore and Prior, 1993). This work thus attempt to investigate the possible use of entomopathogenic fungi either as a single component control agents in the field, or part of an integrated pest management programme for the control of *M. sjostedti* on cowpea.

1.1 Objectives of the study

Motivated by the preceding background information, investigation were conducted into the effect of entomopathogenic fungi on *M. sjostedti*. The overall goal of the project was to develop entomopathogenic fungi for the control of *M. sjostedti* on cowpea. This was achieved through the following specific objectives:

1.1.1 To evaluate the pathogenicity of various strains of *Beauveria, Metarhizium, Verticillium* and *Paecilomyces* through bioassay in order to select the most virulent strain that may be considered for control of *M. sjostedti*.

1.1.2 To study the effect of temperature on fungal germination, growth and pathogenic activity to *M. sjostedti*.

1.1.3 To evaluate the susceptibility of various developmental stages of *M. sjostedti* to the selected strain.

1.1.4 To evaluate the effect of fungal infection on fecundity, egg fertility and longevity of adult *M. sjostedti* surviving infection as larvae

1.1.5 To investigate the effect of fungal infection on feeding by larvae and adult *M. sjostedti*

1.1.6 To study the effect of host plants on the susceptibility of *M. sjostedti* to selected strains.

1.1.7 Field evaluation of high volume aqueous formulation and ultra-low volume oil-based formulation of the most virulent strain for the control of *M. sjostedti.*

1.1.8 Assess the effectiveness of the pathogen under intercropping conditions.

1.1.9 To study the impact of field application of the fungal pathogen on some non-target organisms.

1.1.10 Timing of application and persistence of selected strain for the control of *M. sjostedti.*

1.2 Hypotheses

Based on the preceding objectives, the following hypotheses were tested:

1.2.1 Entomopathogenic fungi does not cause mortality (in other words, are not pathogenic) to the legume flower thrips.

1.2.2 All fungal strains germinate, grow and cause equal level of mortality in *M. sjostedti* at all temperatures

1.2.3 Entomopathogenic fungus cause equal level of mortality in all developmental stages of *M. sjostedti.*

1.2.4 Larvae and adults of *M. sjostedti* infected with fungus consume equal amount of food as non-infected ones.

1.2.6 Fecundity, egg fertility and longevity of adult *M. sjostedti* surviving infection as larvae are not significantly different from non-infected adults.

1.2.7 Entomopathogenic fungus cause equal level of mortality in *M. sjostedti* on susceptible, tolerant and moderately resistant varieties of cowpea.

1.2.8 Entomopathogenic fungus cannot cause mortality/reduction in thrips population and damage under field conditions and hence cannot increase cowpea grain yield.

1.2.9 Entomopathogenic fungus caused equal mortality/reduction in thrips population and damage within cowpea monocrop and cowpea intercrop.

1.2.10 There is no significant difference in yield parameters between the monocrop and intercrop treated with entomopathogenic fungus.

1.2.11 The population of non-target beneficial organisms in plots treated with entomopathogenic fungus is not equal to the populations in untreated control plots.

1.2.12 Application of entomopathogenic fungus given at various treatment regimes does not influence thrips population, damage and yield of cowpea.

CHAPTER TWO

2 Literature Review

2.1 Distribution

Megalurothrips sjostedti is widely distributed in Africa, south of the Sahara (Figure 1). It has been recorded from Cameroons, Zaire, Kenya, Nigeria, Rwanda, South Africa and Uganda (Faure, 1960). It also occurs in Ghana, Gambia, Gabon, Comoros Islands and Malta (Faure, 1960).

2.2 Damage by *M. sjostedti*

Heavy flower shedding due to *M. sjostedti* on cowpea was reported by Okwakpam (1967); and Taylor (1969) described damage by the pest as characterized by distortion, malformation and discoloration of floral parts of cowpea. This report remained controversial for almost a decade, however recent studies (Ezueh, 1981; Singh *et al.*, 1990; Tamo *et al.*, 1993; Ampong-Nyarko *et al.*, 1994) have emphasized the importance of *M. sjostedti* as a major pest of cowpea. Early damage by *M. sjostedti* occasionally occur on leaf laminae but the pest mainly concentrate its damage on the terminal leaf buds which in extreme cases can be completely destroyed thereby retarding seedling growth. The pest moves to the flower buds after its formation, which becomes progressively damaged. Damaged flower buds form brown crusts on the main stem and branches of the plant, affecting the reproductive performance of the plants. Plants become stunted, remain green, fail to produce flowers and any pods which set subsequently senesce and dry-up (Ezueh, 1981).

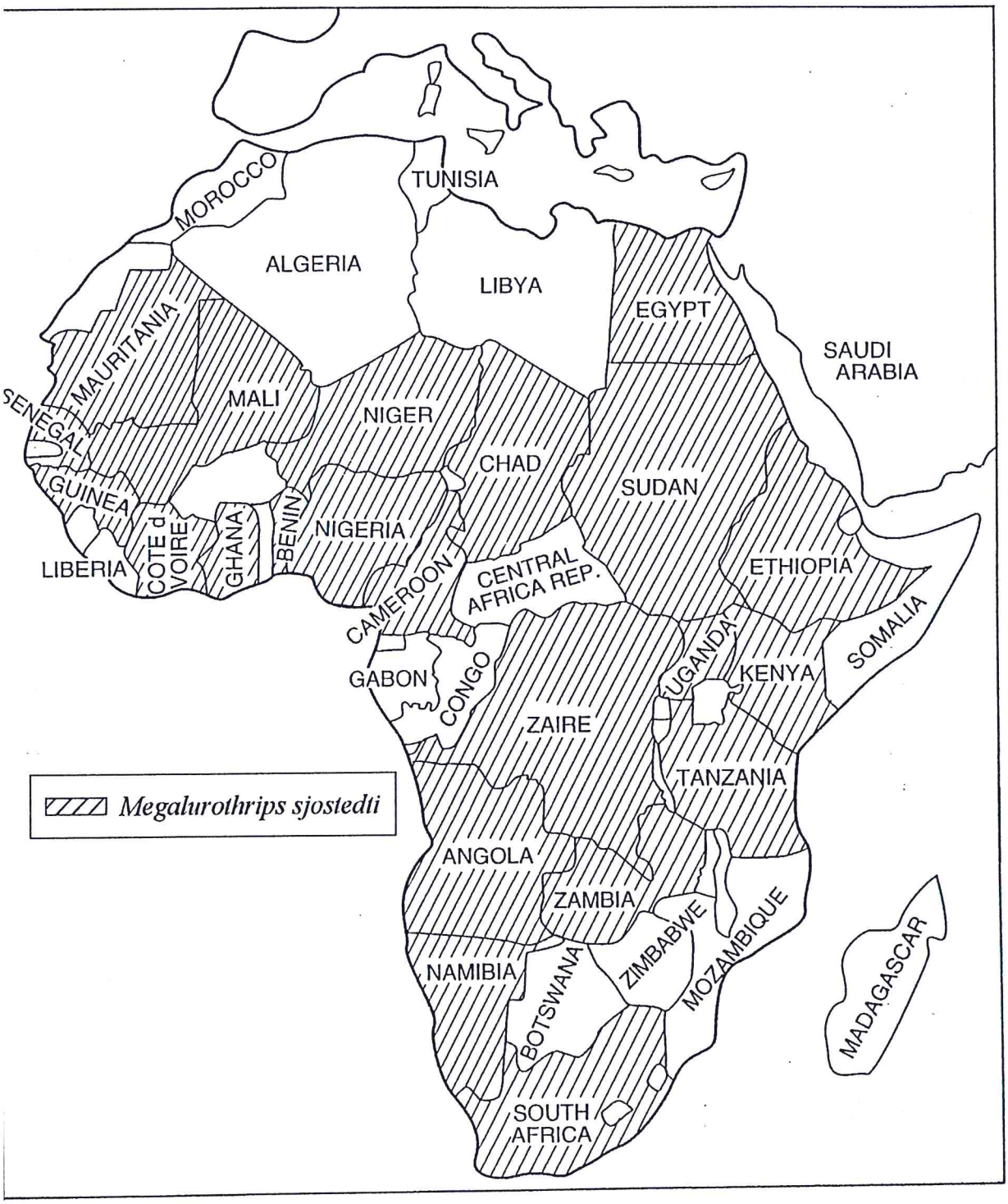

Figure 1. Map of Africa showing the distribution of *Megalurothrips sjostedti*

2.3 Host plants of *M. sjostedti*

It is believed that migrant populations from large number of host plants initiate the infestation of cowpea by *M. sjostedti*. This pest has been recorded from *Phaseolus vulgaris* L., *Crotolaria juncea* L., *Newboldia leavis* Beauv., *Glyricidia* sp., *Thoningia* sp. *Saccharum officinarum* L., *Lycopersicum esculentum* Mill., *Musa sapientum* Chev. (Taylor, 1969). Tamo *et al* (1993) gave an annotated list of alternative host plants of *M. sjostedti*, which fell under three families, namely Mimosaceae, Caesalpinaceae and Leguminoseae. Majority of the host plants belong to the family Leguminoseae which include the following: *Calopogonium mucunoides* Desv., *Centrosema pubescens* Benth, *Clitoria ternata* L., *Crotolaria goreensis* L., *C. macrocalix* L., *C. mucronata* L., *Cajanus cajan* (L.) Huth, *Delonix regia* (Hook), *Dolichos lablab* L., *Desmodium velutinum* Desv., *D. tortuosus* D., *D. salicitolium* D., *Eriosema psoraloides* D., *E. dendroides* D., *Indigofera hirsuta* L., *I. tinctoria* L., *I. dendroides* L., *Lens esculenta* Mill., *Lonchocarpus sericeus* (Poiret), *L. cyanescens* Kunth, *Psophocarpus tetragonolobus* L., *P. palustris* L., *Pterocarpus sartalinoides* L., *Pueraria phaseoloides* Willd., *Tephrosia bracteolata* Pers., *T. candida* Pers., *T. elegans* Pers., *T. linearis* P., *T. purpurea* P., *Vigna campestris* L.

Feeding on the above listed families is confined to flowers and both adult and larval thrips were recovered from all host plants. *M. sjostedti* has also been reported from soybeans and Lima beans where they also damage the flower buds thus reducing the number of flowers (Singh and Taylor, 1978).

2.4 Life cycle

The life cycle of each *M. sjostedti* involves an egg, two or three active larval instars that feed, followed by two relatively inactive pupal instars that probably do not feed, then the adults

(Okwakpam, 1978; Salifu, 1992). Oviposition occurs on floral tissues normally, on petals and calyx tubes occasionally (Okwakpam, 1991). Adults also oviposit on peduncles and young pods (Tamo *et al.*, 1993). Eggs are laid singly in an incision made in plant tissues by the ovipositor. Eggs hatch in 2-3 days. There are 4 or 5 instars between the egg and adult. Customarily, the first two feeding instars are called larvae, and the later, non-feeding ones pupae. There are various objections to this terminology. Some aspects of the unique development of thrips resemble insects with incomplete metamorphosis whose young are called nymphs, and those that display complete metamorphosis whose young are called larvae (Bailey, 1957; Fenemore, 1984). Nevertheless, larvae and pupae will be used in this text since they are understood in the literature on thrips biology. The two larval instars feed on host plant soon after hatching. Okwakpam (1978) reported the occurrence of 3 larval instars, however, (Salifu, 1992) noticed the occurrence of 2 larval stages. Okwakpam based the description of the third stage on bigger size and greater number of eye facets (ommatidia) without records of ecdysis. These criteria according to Salifu are untenable because late second stage larvae can grow to near adult proportion (Lewis, 1973; Salifu, 1992). Looking like a translucent wriggling worm, the first instar larvae emerges from plant tissues through the incision made by the females ovipositor when the eggs was laid. Considerable mortality occurs during emergence as often evidenced by the presence of partially emerged dead larvae on peduncles, leaves and floral tissues. The first instar larva is spindle shaped and nearly colourless with red eyes, but turn yellowish as it feeds on plant tissues. The first instar larvae last 2-3 days. The second instar is more robust and more yellowish than the first instar and last 3-5 days. The entire larval period last 5-7 days and the second instar larvae pupates in soil or litter. At the second moult a prepupae emerges from the cast cuticle, this is an intermediate stage between the larvae and the true pupa. The prepupa do not feed or excrete, and their rate of respiration is retarded. It last for 1-2 days. The prepupa is followed by a single pupal stage which last for 4-6 days. At the final moult the adult

emerges from the pupa. Adult longevity varies from 21-30 days. The entire life cycle is completed in 16-19 (Okwakpam, 1978; Salifu, 1992).

2.5 Natural enemies of thrips

2.5.1 Predators

The soft-bodied, slow moving larval thrips are easy prey for a wide range of general arthropod predators. The majority of these accept a wide range of prey, and thrips may not represent their preferred host in many cases. Some of the more specific thrips are the Aeolothripidae, the anthocorid genera *Orius* and *Montandoniola*, the Cecidomyiid genus *Thripsobremia* and the Sphecid genus *Microstigmus* (Mills, 1991).

Anthocorid bugs, especially in the genus *Orius* attack thrips populations in cowpea (Tamo *et al.*, 1993; 1997), Cotton (Stoltz and Stern, 1978), in soybean (Irwin and Kuhlman, 1979), and in ornamental fig (Cock, 1985). Nymphs and adult bugs move among thrips on plant and pierce victims in either head, thorax or abdomen, often holding down the struggling prey with their forelegs. In addition, the coccinellid, *Scymmus thoracicus* T., is suggested to be able to regulate populations of *Chaetanaphothrips orchidii* (Moulton) on banana (Delattre and Torregrossa, 1978). Most other predators will contribute to the control of thrips populations but are unlikely to be useful biological control agents. In context of glasshouse crops, the phytoseiid mites, *Amblysieus* species are mass-produced for the control of *T. tabaci* (Ramakers, 1983). These mite predators are not specific in their diet and can only be effective in confine places (Mills, 1991).

2.5.2 Parasitoids

Both egg and larval parasitoids attack thrips. Egg parasitoids of the genus *Megaphragma* are some of the smallest known insects and have been recorded from a variety of thrips species (Lewis,

1973). They have been little studied (McMurtry, 1961), and do not appear to contribute significantly to thrips mortality. In contrast, the Eulophid larval parasitoids play a more dominant role with 70-80% parasitism being recorded for *Caranisus* species on bean thrips, pea thrips and onion thrips.

2.5.3 Pathogens

Allanthonematid nematodes have occasionally been found infesting thrips but records are few (Ananthakrishnana, 1984). While these nematodes are internal parasites, they don't cause the death of their host and their impact is confined to a reduction of adult fecundity.

Microsporidial infection has been reported on *Scirtothrips oligochaetus* Par. on cotton (Raizada, 1976a). This is however, the only known record. In addition, there are no known viral (Martignoni and Iwai, 1981) or bacterial diseases of thrips (Mills, 1991).

Many genera of entomopathogenic fungi has been reported on thrips including *Beauveria, Verticillium, Paecilomyces, Hirsutella, Entomophthora* and *Cephalosporium* (Carl, 1976; Raizada, 1976b). *Beauveria brongniartii* (Saccardo) Petch attack cocoa thrips in West Indies and Central America, especially in damp places and seasons, appearing as a white mould on the bodies of adults and larvae, and attaching them lightly to leaves (Lewis, 1973; Callan, 1943). *Beauveria bassiana* infected up to 20% of larvae of *Haplothrips* species in Bulgeria (Lyubenov, 1961) and probably attack thrips that overwinter in the soil. In Surinam, a species of *Cephalosporium* has been reported on living cacao thrips larvae. *Entomophthora sphaerosperma* (Thaxter) Fresenius was recorded on *T. tabaci* in Massachusetts (Charles, 1941) and in Switzerland *Entomophthora* species transform second stage larvae of *T. tabaci* into blackened mummies packed with spores (Stradling, 1968). Two species of Entomophthorales have been described (MacLeod, *et al.*, 1976; Samson *et al.*, 1976) that infect larval host while feeding on foliage and a *Hirsutella* species has recently been

isolated by CIBC from foliage feeding larvae of *Liothrips* species (Greenwood and Mills, 1989). The occurrence of *Verticillium* species and *Entomophthora* species has been reported from *M. sjostedti* (Tamo *et al.*, 1993; Salifu, 1986).

2.6 Control of thrips

2.6.1 Cultural control

Several cultural control practices such as irrigation, mulching, use of coloured sticky trap, mixed cropping and destruction of alternative hosts are used against thrips. Irrigation or flooding can be used to destroy thrips, which spend their pupal stage in the soil. Overhead irrigation has been observed to dislodge thrips from leaves (Bernado, 1991). Mulching watermelon fields with rice straw or hog manure reduces the populations of *T. tabaci* (Rejesus *et al.*, 1986). Yellow and blue sticky traps at the height of 14 cm above the Chrysanthemum plants have been used to trap *F. occidentalis and Megalurothrips usitatus (Bagnall)* (Bansiddhi and Poonchiastri, 1991; Chang, 1990). Intercropping cowpea with maize and/or sorghum reduces damage by *M. sjostedti* (Matteson, 1982; Ezueh and Taylor, 1984; Kyamanywa and Ampofo, 1988; Kyamanywa and Tukahirwa, 1988; Kyamanywa *et al.*, 1993; Ampong-Nyarko *et al.*, 1994). Many harmful thrips have a wide host range including wild plants, and they often survive on weeds growing in and around fields when there is no suitable crop present. So the control of weeds in most crops clearly helps to lessen infestation.

2.6.2 Chemical control

This is the most widely practiced method of control by farmers (Singh and Rachie, 1985). The farmers generally apply the insecticide routinely, both as a preventive and curative measure. The growers also apply the insecticides at higher rates and frequencies (Hirose, 1990). Several types of

chemical compounds ranging from arsenicals to organochlorines, organophosphates, carbamates and pyrethroids have been used to combat thrips (Lewis, 1973; Talekar, 1991; Jackai and Adalla, 1997). Thrips have been found to be key pests in areas where pesticides inputs are high (Hirose, 1990). This suggest that the misuse of insecticides have favoured rapid development of resistance to insecticides, the mechanism of which is, however unknown (Talekar, 1991; Parker *et al.*, 1991).

2.6.3 Biological control

There are 43 species of parasitoids, 169 species of predatory mites and 30 species of predatory thrips in the world reported to be natural enemies of thrips (Lewis, 1973). These predators and parasitoids kill often-large proportions of field populations of thrips but there have been few deliberate attempts to introduce or encourage natural enemies to control pests species.

Four thrips species, *T. tabaci, Gynaikothrips ficorum, Selenothrips rubrocinctus* and *Heliothrips haemorrhoidalis* have been targets for biological control and the natural enemy agents selected for importation have included both predators and parasitoids. The results of importation have not been successful, with only the anthocorid, *Montandoniola moraguesi*, being credited with providing partial control of *G. ficorum* (Cock, 1985). Of the natural enemies that are known to attack thrips, predators are the least specific and of least interest in terms of potentials for biological control (Mills, 1991). Egg parasitoids appear to be infrequent and to have little impact on their host population. Larval parasitoids, such as *Ceranisus* spp. have a very significant impact on their hosts but little is known of host range or habitat preference. Of the known or probable occurring pathogens, the allantonematid nematodes also have little potential for use in biological control (Mills, 1991).

Several fungal genera attack thrips (Raizada, 1976b), however the only known record from *M. sjostedti* is *Entomophthora* and *Verticillium* reported by Salifu (1986) and Tamo *et al* (1993),

respectively. Entomopathogenic fungi can be more readily mass-produced and formulated for application as mycoinsecticides.

2.7 Entomopathogenic fungi

Aristotle was the first to mention that bees suffered disease in his book Historia Animalium (Lipa, 1975). Bassi (1834) however, demonstrated experimentally for the first time that the fungus, *B. bassiana* was the cause of the "white muscardine disease" in silkworm. Burges and Hussey (1971) define "disease as a departure of the insect from a state of health. Two types of insect diseases are recognized (Lipa, 1975; Pionar and Thomas, 1984): (1) The non-infectious disease caused by abiotic factors like temperature, humidity, chemicals, injury etc. and,

(2) infectious disease caused by pathogens such as viruses, bacteria, fungi, nematodes and rickettsia. Many attempts have been made to use entomogenous fungi to control economically important insect pest of crops (Ferron, 1978; Zimmermann, 1986b; Ferron *et al.*, 1991). There are a lot of promising approaches and active interest from large agrochemical companies will increase the development process (Reinecke *et al.*, 1990).

2.7.1 Systematic position of Entomopathogenic fungi

Entomopathogenic fungi are distributed approximately throughout the whole kingdom of fungi, from the Subdivision Mastigomycotina to fungi Imperfecti (Subdivision Deuteromycotina) passing through Ascomycotina and Basidiomycotina (Ferron, 1985). More than 700 species, mostly Deuteromycetes and Entomotophthorales from about 90 genera, are pathogenic to insects (Charnley, 1989).

2.7.1.1 Subdivision Mastigomycotina

2.7.1.1.1 Class Chytridiomycetes: this class is represented by the *Coelomomyces* (Order: Blastocladiales). They are host specific, endoparasites of larvae of mosquitoes, blackflies and chironomids. The main species are *C. dodgei* Couch and Dodge, *C. psorophorae* Couch and *C. punctatus* Couch and Dodge.

2.7.1.1.2 Class Oomycetes: The most important species here is *Lagenidium giganteum* Couch which is an effective fungal pathogen against a wide range of culicine and anopheline mosquitoes (Federici, 1981).

2.7.1.2 Subdivision Zygomycotina

2.7.1.2.1 Class Trichomycetes: This is represented among the entomogenous species by *Smittium morbosum* Sween., pathogen of many mosquito larvae (Sweeney, 1981).

2.7.1.2.2 Class Zygomycetes: The Order, which contain the largest number of arthropod pathogens is the Entomophthorales. Six entomopathogenic genera are recognized (Remaudière and Keller, 1980):

2.7.1.2.2.1 *Massospora*, highly specific to Homoptera Cicadidae.

2.7.1.2.2.2 *Entomophthora*, pathogenic to a wide range of insects. The main species are *E. culicis* (Braun) Fres., *E. crupta* Thaxter., *E. muscae* (Cohn) Fres., *E. planchnoniana* Cornu., *E. thripidium* Per.. and *E. weberi* Fres.

2.7.1.2.2.3 *Neozygotes*, includes species pathogenic to Acari, Homoptera and Thysanoptera. The main species are *N. floridana, N. adjarica, N. fumosa, N. lageniformis, N. parvispora, N. tetranychi* Weiser (Humb.) and *N. turbinata* Humb.

2.7.1.2.2.4 *Conidiobolus*, pathogenic to Arachnids and insects. Some of the main species are *C. coronatus* (Costantin) Batko, *C. batkoi* Batko, *C. adiaeretus* Drechsler, *C. pumilus* Dreschsler, *C. thromboides* Drechsler, *C. grylli* Fres., *C. pseudapiculatus* Keller

2.7.1.2.2.5 *Zoophthora*, parasitic on all orders of insects and include *Z. aphidis* (Hoffman), *Z. occidentalis* (Thaxter) Humber, *Z. anglica* (Petch) Ben-Ze'ev, *Z. phalloides* (Batko) Humber, *Z. phytonomi* (Arthur) Humber and *Z. radicans* (Brefeld) Batko.

2.7.1.2.2.6 *Erynia*, pathogenic to wide range of insects. Some of the main species are *E. curvispora* (Nowakowski) Remaudiere, *E. ovispora* (Nowakowski) Remaudiere and *E. aquatica* (Anderson and Ringo) Humber.

2.7.1.3 Subdivision Ascomycotina

2.7.1.3.1 Class plectomycetes: This is represented by the genus *Ascosphaera* which are responsible for the chalkbrood disease of the honeybee *Apis melifera*, and of the cutting-bees, *Megachile*. The main species are *A. apis* (Maassen) Olive, *A. proliperda* Skou and *A. major* (Proekschl and Zobl) Skou.

2.7.1.3.2 Class Laboulbeniomycetes: These are obligate parasites of a wide range of insects, especially Coleoptera; seem to have little or no effect on the health of their hosts.

2.7.1.3.3 Class Pyrenomycetes: More than 300 species of Cordyceps have been reported. The susceptible insect hosts are Diptera, Orthoptera,, Lepidoptera, Hemiptera and Coleoptera. Very little use of Cordyceps has been made for biological control.

2.7.1.4 Subdivision Basidiomycotina

Contains many species generally considered as symbiotic, growing in association with scale insects.

2.7.1.5 Subdivision Deuteromycotina

This subdivision contains the largest number of entomopathogens. More than 40 genera have been identified but the knowledge of their potentialities is limited to a small number of them: *Aschersonia* (Sphaeropsidiales), *Beauveria, Culicinomyces, Hirsutella, Metarhizium. Nomuraea, Paecilomyces, Tolypocladium and Verticillium* (Moniliales).

2.7.1.5.1 Class Coelomomyces: This is represented by the genus *Aschersonia*, mostly pathogens of whiteflies. The main species are *A. aleyrodis* Webber, *A. goldiana* Saccardo and Ellis, *A. inasperrata* Humber and Rombach, and *A. turbinata* (Berkeley) Petch.

2.7.1.5.2 Class Hyphomycetes: This is represented by the following genera.

2.7.1.5.2.1 *Beauveria.* This is most frequently isolated from dead insects collected from the fields, and is responsible for the white muscardine disease (De Hoog, 1972). Two species are known *B. bassiana* and *B. brongniartii*. The host range of both species is very large, and many experiments have been developed to show their potentialities to control pest populations.

2.7.1.5.2.2 *Metarhizium.* This genus is responsible for the green muscardine disease known on more than 200 species of insects. Two species are known, *M. anisopliae* (Metschnikoff) Sorokin and *M. flavoviride* Gams and Rozspal.

2.7.1.5.2.3 *Nomuraea.* Three species are known. *N. rileyi* (Farlow) Samson, *N. atypicola* (Yasuda) Samson and *N. anemonoides* Hocking are known to be responsible for natural epizootic on noctuids (Humber, 1992).

2.7.1.5.2.4 *Hirsutella.* About 35 species have been described, with most isolated from arthropods and especially from mites and leafhoppers. The best known species is *H. thompsonii* Fisher.

2.7.1.5.2.5 *Paecilomyces.* This genus contains 14 entomogenous species, mainly *P. farinosus* (Holm) Brown and Smith, *P. fumosoroseus* (Wize) Brown and Smith and *P. tenuipes* (Peck) Samson.

2.7.1.5.2.6 *Verticillium.* The species *V. lecanii* (Zimmermann) Viegas is pathogenic for all stages of development of insects, and also of Arachnida, especially scale insects, aphids and whiteflies.

2.7.1.5.2.7 *Culicinomyces.* The species *C. clavosporus* Couch, Romney and Rao is known to be pathogenic to Anopheles mosquitoes (Federici, 1981).

2.8 Modes of infection of Entomopathogenic fungi.

Entomopathogenic fungi differing from bacteria and viruses can infect their host not only through the gut but also through spiracles and particularly through the integument. Roberts and Humber (1981) divided the disease development process of entomopathogenic fungi into 10 steps.

2.8.1 Attachment of the conidium to the cuticle: The failure of a pathogen to adhere to the cuticle is considered a feature of avirulent isolates. Attachment is normally achieved through the secretion of muscilage. However enzymes, lectins, hydrophobic bonding and electrostatic forces also play a role (Boucias *et al.*, 1982). Mucilage associated with some fungi interacts with and modifies epicuticular waxes as indicated by production of imprints of invasive propagules on the cuticle (Wraight *et al.*, 1990). This is caused by action of cuticle degrading enzymes (CDEs) secreted with the muscilage and probably serves in host recognition as well as cementing the pathogen to a suitable substratum (Butt, 1990).

2.8.2 Germination of the conidium on the insect cuticle: A wide range of factors influence spore germination and behaviour; water, ions , fatty acids, nutrients, the biota on the cuticle surface, and the physiological state of the host (Butt, 1990). Free water is mandatory for mycopathogens, if they are to successfully infect their host. Hassan *et al.*, (1989) have shown that soaking conidia of

M. anisopliae accelerates germination and these spores are more virulent towards *Manduca sexta* L. than non-soaked conidia. Cuticular extract of the pea aphid stimulates germ tube formation in aggressive strains of *Conidiobolus obscurus* but not non-aggressive strains. Fatty acids have a profound effect on spore germination and differentiation e.g. conidia of *Erynia variabilis* on water agar produced secondary conidia only in the presence of Oliec acid (Ferron *et al.*, 1991). In general, high relative humidity (> 90%) is needed for germination (Roberts and Campbell, 1977), but the microenvironment on host cuticle and foliage, particularly during periods of dew, frequently afford the proper conditions for germination even when the macroclimate is very dry.

2.8.3 Penetration of the cuticle: The germ tube may penetrate directly into the cuticle, or an appressorium may be formed which attaches firmly to the cuticle, and a narrow infection peg sent into the cuticle (Zacharuk, 1970a-c). Appressorium formation is a prerequisite for infection for most but not all entomopathogens (e.g. *N. rileyi*). They form at the end of short germ tubes, subterminally, or on side branches after extensive growth.

Cuticular invasion involves both enzymatic and physical activities. The enzymes elaborated by germinating conidia have not been identified, but it is known that colonies of entomopathogenic fungi such as *B. bassiana, B. brongniartii, M. anisopliae* and *E. muscae* produce protease, lipases and chinases in liquid and agar media (Gabriel, 1968).

2.8.4 The production of toxins: After crossing the cuticular barrier, the fungus comes up against the cellular defense reactions of the host. Plasmatocytes, normally dispersed in the haemolymph, accumulate around the fungus and gives rise to melanization (Vey *et al.*, 1975). Entomopathogenic fungi overcome the host defense system by producing toxins. Several toxic compounds were isolated and identified from cultures of *Beauveria, Metarhizium, Entomophthora, Paecilomyces* and *Cordyceps*. Cyclic depsipeptides, such as destruxins A,B,C D, and E, desmethyl destruxin B,

cyclodepsipeptide such as beauvericin and Cordycepin were isolated and their chemical structure determined and subsequently synthesized (Roberts, 1969; Suzuki and Tamura, 1972).

2.8.5 Growth of the fungus in the haemocoel: The fungus usually grows in the haemocoel as yeast like hyphal bodies, essentially blastospores, which multiply by budding. In some cases, hyphae rather than hyphal bodies occur (Zacharuk, 1970ab).

2.8.6 Death of the host: The death of the insects marks the end of the parasitic phase of the fungus. This may be preceded by behavioural changes such as tremors, loss of coordination, or climbing to an elevated position (Ferron, 1978).

2.8.7 Growth in the mycelial phase with invasion of virtually all organs of the host: When the insect dies, the fungus proceeds to grow saprohytically and spread through virtually all tissues of the insect. Competition between the fungus and the intestinal bacterial flora occurs. Cadavers are usually transformed into mummies resistant to bacterial decay apparently because of antibiotics produced by the fungus. Cordycepin produced by *Cordyceps* and Oosporein produced by *Beauveria* are known to have antibacterial activity (Mclnness *et al.*, 1974).

2.8.8 Penetration of the hyphae from the interior through the cuticle to the exterior of the insect: If the "mummy" is held under conditions of moderate to low relative humidity, the fungus is conserved in the form of chlamydospores. Other forms of conservation are sporanges in Coelomomycetaceae, resting spores in Entomophthoraceae, and sclerotes in *Cordyceps*. When the atmosphere reaches saturation the mycelium emerges through the integument and develop conidiophores (Ferron, 1978).

2.8.9 Production of infective unit: This normally occur on the exterior of the host insect and is ensured according to the modalities specific to each genus. Each cadaver constitute an infective focus which by multiplying of the quantity of inoculum ensures propagation of the disease.

2.8.10 Dispersion of spores: This can occur by projection as in the case of Entomophthorales; spores are also carried away by air, rain, and even insects and mites (Ferron, 1978).

2.9 Epizootiology

Fungal disease may occur in insect populations at consistent but rather low levels (enzootics) or may flare up into outbreaks which involve large proportions of the population (epizootics) (Hall and Papierok, 1982; Fuxa and Tanada, 1987). The presence and development of the disease depend on a number of environmental factors, abiotic and biotic.

2.9.1 Abiotic factors

2.9.1.1 Temperature: The rapidity of mycelial development and evolution of infection depends on temperature (Kalvish, 1974). In general, optimum values fall between 20^0 and 30^0C (e.g., 23^0C for *B. brongniartii*, 24^0C for *E. obscura* and *E.exitalis*, 25^0C for *M. anisopliae*, 30^0C for *E. virulenta*). Temperature lower than optima distinctly retard the development of mycosis without necessarily affecting the mortality (Ferron, 1978). Investigating the effect of temperature on susceptibility of noctuid larvae to several isolates of *P. fumosoroseus* and *N. rileyi*, Maniania and Fargues (1992) showed that temperature acts neither on the host nor on the pathogen alone but on the pathological interrelation as a whole.

2.9.1.2 Relative Humidity (RH): Relative humidity affects entomopahagous fungal epizootics in terrestrial environment (Ferron *et al.*, 1991). RH was often cited as the key abiotic factor influencing the potential of entomopathogenic fungi. However, recent studies have shown that RH is less critical for infectivity than was previously hypothesized. Ferron (1977) demonstrated that while 92% RH is required for germination of *B. bassiana*, this fungus would infect bean weevils,

Acanthosclides obtectus F., regardless of the ambient RH. Similar findings were obtained with *B. bassiana* on Chinch bug, *Blissus leucopterus* (Say) (Ramoska, 1984).

2.9.1.3 Sunlight: The activity and persistence of fungi in the field are generally influenced by sunlight. The average half-life of conidia of *N. rileyi* and *B. bassiana* on soybean foliage was found to be 2-5 days or more (Ignoffo, 1982; Gardner et al., 1977) due mostly to the detrimental effect of ultraviolet components of terrestrial sunlight (Leach, 1971). Despite this detrimental effect of solar UV radiation on the persistence of fungi, light radiation may stimulate certain stages in the life cycle of entomopathogenic fungi. Glare (1987) showed that light is essential for *N. rileyi* to produce conidia from disease-killed cadavers of *Heliothis* spp.

2.9.2 Biotic factors

2.9.2.1 Pathogen properties and quantity of spores: The pathogen properties that are involved in fungal disease are infectivity, virulence and pathogenicity (Fuxa and Tanada, 1987). Infectivity is the ability to cause infection. Virulence is the disease-producing power of the pathogen, i.e., the ability to invade and injure the hosts tissues. Virulence has been used interchangeably with pathogenicity. The later refers to groups or species of pathogens, whereas, virulence is used in the sense of degree of pathogenicity within a group or species.

Positive correlation between the number of infective spores and mortality by mycosis has been established by many authors (Ferron, 1978), but the influence of sublethal doses has been little studied. In practice, Soviet authors recommend 2-4kg Boverin ha^{-1}, a biological preparation containing 6×10^9 conidia of *B. bassiana* g^{-1} (= $1.2\text{-}2.4 \times 10^{13}$ spores ha^{-1}), against *Leptinotarsa decemlineata* (Say). For soil insect such as *Melolontha melolontha*, Ferron (1978) used about 5×10^{14} conidia of *B. brongniartii* ha^{-1}. In laboratory the disease normally develops after contamination

of insects either directly by spore suspensions (10^6-10^8 spore ml^{-1}), or by mixing the soil with 10^5-10^8 spores g^{-1} or cm^3 soil insects (Burges, 1981).

2.9.2.2 Host insects: All stages of insects development are generally susceptible to fungal infection although, this susceptibility varies within one host species. Since the penetration of the integument is the usual route of invasion by pathogenic fungi, the moulting process plays an important role in insect resistance to fungal infection (Vey and Fargues, 1977). Fungal epizootics generally occur at high host population densities, thus increasing the probability of contact between the pathogen and the hosts as well as between uninfected and infected hosts (Benz, 1987).

2.9.2.3 Host plant: Host plants may interfere with fungal pathogens either directly by itself or indirectly by its influence on the host insect. Hare and Andreadis (1983) described an indirect effect of host plant on fungal pathogen when they found that the Colorado potato beetle, *L. decemlineata* were least susceptible to infection with *B. bassiana* on the plant species most suitable for beetle survival. Host plant susceptibility also declined with age. Zimmermann (1984) found that *M. anisopliae* was less effective in the control of *Otiorhynchus sulcatus* F. on Cyclamen than on other plant species. It was suggested that the fungus was impaired directly by this plant.

2.10 Use of Entomopathogenic fungi for the control of crop pest

The use of entomopathogenic fungi for biological control has been tried for many years with varying success. Some of the species widely used include:

2.10.1 *Beauveria bassiana* and *B. brongniartii (=B. tenella).*

The two species are the most intensively studied of all entomopathogenic fungi. In Russia, *B. bassiana* is formulated as BOVERIN (a microbial preparation containing 2 x 10^9 conidia g^{-1})

for the control of different insect pests mainly the Colorado potato beetle, *L. decemlineata* and *Cydia pomonella* (Ferron, 1981). According to Sikura and Sikura (1983), 90% mortality of Colorado potato beetle is obtained with 3 kg ha^{-1} of BOVERIN, against the first instar, and 6 kg ha^{-1} against the second instar. Beside the Russian BOVERIN, a new preparation, BOVEROL, is presently being used in Czechoslovakia (Dirlbekova *et al.*, 1987). An aqueous suspension of BOVEROL mixed with sublethal doses of Deltamethrin is sprayed at the rate of 300-400 liters ha^{-1} when there are more than 80% of eggs and first instar- or second instar larvae in the potato crop. Without deltamethrin, the effectiveness of BOVEROL varies between 20-60% (Dirlbekova *et al.*, 1987).

In the US foliar sprays (5×10^{13} spores ha^{-1}) of *B. bassiana* applied six times weekly against the Colorado potato beetle on potatoes produced yield equal to those resulting from applications of fenvalerate at 0.227 kg a.i. ha^{-1}. A 3-year US Department of Agriculture pilot test programme initiated in 1983, was discussed by Hajek *et al.*, (1987). The *B. bassiana* preparations used were Wettable Powder formulations produced and formulated by Abbott Laboratories. The preparations were applied at the low rate of 5×10^{12} or at the high rate of 5×10^{13} CFU ha^{-1} in 935 liters ha^{-1}. This treatment was evaluated against chemical applications of Oxamyl at the rate of 154g a.i. ha^{-1}, or of a combination of Fenvalerate and Piperonyl butoxide in a 1:4 ratio [18.4g a.i. Fenvalerate and 73.6g a.i.Piperonyl butoxide ha^{-1}]. Yield showed no significant difference between the biologically treated plots and insecticide treated plots.

More recently, Mycotech Corporation in the US has developed and commercialized a strain of *B. bassiana* registered as Mycocide GHA for the control of grasshoppers, locusts, and mormon cricket, *Anabris simplex* Haldeman (Jaronski and Goettel, 1997).

In Kenya, Maniania (1993a) compared granular and aqueous formulations (one to two applications at 10^{12}-10^{13} conidia ha^{-1}) of *B. bassiana* with Trichlorfon (Dipterex 2.5GR) (applied at

3-4 kg a.i. ha^{-1}) for the control of *Chilo partellus* (Swinhoe). Granular formulation of the pathogen performed better than the insecticide in the control of the pest. Grain yield was also significantly higher in the biologically treated plots than in the insecticide treated plots. Riba (1984) also demonstrated the effectiveness of *B. bassiana* and *B. brongniartii* now commercialized as OSTRINIL and BETEL for the control of *Ostrinia nubilalis* (Hbn) and *Maharnava posticata* L., respectively.

China has developed the practical use of entomopathogenic fungal preparations to the highest standards during the past 10 years. Oil formulations of *B. bassiana* are in common used in 13 provinces of China to reduce outbreaks of the pine caterpillar, *Dendrolimus punctatus*. About 300,000 ha of forest have been treated with the microbial preparation .This product is also widely used against *O. nubilalis* and various species of leafhoppers (Hussey and Tinsley, 1981; Ferron *et al.*, 1991).

2.10.2 *Aschersonia aleyrodis*

A dose of 2×10^8 conidia/plant of this fungus applied as an Ultra low volume spray in a cucumber crop is used to control whiteflies and scales in the Netherlands and UK. This fungus is highly specific and is used together with the parasite *Encarsia formosa* (Ramakers and Samson, 1984). It is also recommended against whiteflies in the USSR under the trade name ASERONIJA (Ferron *et al.*, 1991).

2.10.3 Entomophthorales

The introduction of aphids artificially infected with four species of Entomophthorales significantly reduce *Aphis fabae* populations in beans (Wilding, 1983). Equally, the release of an Israeli strain of

Z.radicans in Australia against the accidentally introduced aphid *Therioaphis trifoli f. maculata* delivered encouraging results (Milner *et al.*, 1982).

2.10.4 *Hirsutella thompsonii*

In Florida, a biological preparation of *H. thompsonii* is used against the citrus mite, *Phyllocoptruta oleivora* (McCoy, 1981). It is marketed in the USA as MYCAR for the control of citrus rust mite. Odindo *et al* (1991) used spore concentration of 1.3×10^{13} conidia ha^{-1} of *H. thompsonii* to control Cassava green mite, *Mononychellus tanajoa* Bondar in Kenya.

2.10.5 *Metarhizium anisopliae*

In Brazil this pathogen is marketed as METAQUINO where it is used on large hectares of land for the control of pasture and sugar cane insect pests such as spittle bugs, *M. posticata* and various cercopids. More than 150,000 ha of sugar cane is treated each year against these pests (Ferron, 1981). In the Pacific Islands and South East Asian countries, *M. anisopliae* has been used successfully for the control of palm rhinoceros beetle, *Oryctes rhinoceros* (Bedford, 1982).

Field trials by the LUBILOSA project has shown considerable promise in grasshopper and Locust control with *Metarhizium flavoviride* (Gams and Rozypal) and a commercial product registered as Green Muscle® is expected in the market by 1998 (Lomer *et al.*, 1997).

2.10.6 *Verticillium lecanii*

The control of white flies and aphids by mean of *V. lecanii* has received increased attention during the past 10 years. It has resulted at least temporarily in commercial preparations of viable conidia of *V. lecanii*, marketed as MYCOTAL and VERTELAC in the UK by Microbial Resource Limited

(Hall, 1981; Ferron *et al.*, 1991). It has also been reported that MYCOTAL registered against aphids, might also be used against thrips (Ferron *et al.*, 1991).

2.10.7 *Nomuraea rileyi*

N. rileyi distributed in the form of dead infected larvae of *Heliothis virescens* in soybean field gave good control of *Plathypena scabra* and *Heliothis zea* in North Carolina (Zimmerman, 1986b).

2.11 Safety of microbial insecticides

Development and use of microbial insecticides has increased attention because of the problems associated with chemical insecticides such as resistance of target insect species, non target toxicity, and environmental persistence. While both chemical and microbial insecticides share safety concerns with respect to toxicity, irritancy and allerginicity, microbial agents are unique in their ability to multiply within a suitable host. *M. anisopliae* has never been reported as infecting humans. Rats, mice and rabbits given this entomopathogen in laboratory by inhalation, oral administration, sub cutaneous injection, intraperitoneal injection and topical administration had no signs of infection or illness (Siegel and Shadduck, 1987, 1990).

Safety tests have been conducted predominantly on *B. bassiana*. Muller-Kogler (1965) reported moderate to severe allergic reactions to spore preparations from scientist working with *B. bassiana* (Heimpel, 1971), however, Ignoffo (1973) reported no deleterious effects in humans repeatedly handling cultures of *B. bassiana*. Multiple reports of *B. bassiana* causing fungal keratitis prompted Ishibashi *et al* (1987) to conduct a series of test comparing the pathogenicity of corneal lesions from *B. bassiana* and *Candida albicans*. Histopathology reveal only a weak pathogenicity by *B. bassiana* for the cornea and no invasion of the anterior chamber of rabbit. To add to this records, Ignoffo (1973) reports indirect evidence of possible toxicity, pathogenicity and

allergenicity in humans; and isolation of species of *Beauveria* from vertebrate tissue. Due to the multiplicity of conflicting reports, Ignoffo states additional testing is needed. Safety tests with *H. thompsonii* have included oral dosing of rats for 21 days with no evidence of toxicity or pathologic lesions (Ignoffo, 1973).

A "safe" fungus, with respect to vertebrates, is essentially one with a restricted host range; fungi with narrow host range pose the least treat to non target organism (Goettel *et al.*, 1990). The honey bee, *A. mellifera* is used in the commercial production of honey and is the most important pollinator of agricultural crops. Naturally, approval of any agricultural pesticide must include some assessment of it potential on honey-bees (Goettel *et al.*, 1990). No candidate fungal control agent has been reported to cause an epizootic among honey-bees (Bailey, 1971) or other important pollinators such as Syrrphids (Weems, 1955).

It has been shown in a number of cases that fungi can be detrimental, either directly or indirectly, to predators and parasitoids. Other studies show that the two may be compatible. Mycoses have been observed in a number of predators and parasitoids (Balazy, 1965; Muller-Kogler, 1965). Little is known about the epizootiology of these mycoses and resultant effect on the control of target species, however, indications are that in nature mycoses in these insects are relatively uncommon and many species seems refractory to infections (Muller-Kogler, 1965). Relatively few studies have dealt with the compatibility of entomopathogenic fungi with natural or introduced species of parasitoids and predators to determine which one can be used most effectively in an integrated manner. The effective coexistence of species of *Hirsutella, Entomophthora* and parasites and predators in the control of citrus pest arthropods has been demonstrated in Florida (Muma, 1955). Ignoffo (1981) showed that natural epizootics of *N. rileyi* in the noctuid, *P. scabra,* did not apparently affect the efficacy of the pest's natural parasite complex. This also appeared to be true for the *N. rileyi, Microplitis croceipes* and *Heliothis* spp

complex in corn fields of central Mississippi (Smith *et al.*, 1976). Non-target organisms may also serve as secondary hosts in which the inoculum is maintained and propagated, thus promoting later infections in the target host population (Fargues and Remaudière, 1979). However, fungi are frequently even more specific under field conditions and especially during epizootics. There are several reports of fungi attacking only one host even though closely related susceptible species are present (Hall and Papierok, 1982). Such resistance is thought to occur as a result of the complex biotic and abiotic interactions which, occur in the field (Fargues and Remaudière, 1979).

CHAPTER THREE

3 General materials and methods

3.1 Insect culture

A culture of *M. sjostedti* was maintained in a controlled environment room at 26 ± 2^{0}C and 60 ± 5% RH under a photoperiod of L:D 12:12 to ensure a regular supply of larvae, pupae and adult insects of the same age. The rearing system was modified from Kirk (1985) and Chang (1988). The initial stock culture was obtained from a field population of *M. sjostedti* infesting ICV 2 cowpea plots at the ICIPE Headquarters, Nairobi, Kenya. Adult female thrips were aspirated from cowpea flowers and transferred to polythene specimen tubes (95 x 30 mm). A microscreen with a mesh size of 192µm was glued over the cut end of the tube which, served as cage. About 20 females were introduced in each cage, which contained the upper 3 cm of cowpea peduncle (variety ICV 2) as the oviposition substrate. Pollen and floral tissues were provided as food. Female thrips were allowed to oviposit for 2 days, after which time the peduncles were removed and transferred to another cage (110 x 15 mm). First instar larvae emerged on surfaces of peduncles within 2-3 days. Larvae were reared on peduncles until they were about to pupate. They were then transferred to screened transparent plastic vials (50 x 100 mm) which were lined with moistened cheesecloth and sandwiched between two layers of filter paper. Peduncles were also provided as food. Pupation occurred on the underside of the first and second layer of filter paper. Adults emerged within 4 to 5 days.

3.2 Fungal culture

All fungal strains tested in the experiments were obtained from the ICIPE culture collection (Table 1) except *Beauveria bassiana* (Balsamo) Vuillemin Technical powder GHA (TP-GHA) and *Paecilomyces fumosoroseus* (Wize) Smith and Brown MYCOTECH 613 (MY 613) which were

donated by Dr. T.J. Poprawski (Beneficial Insect Research Unit, Weslaco, Texas, USA). The fungi were grown on Saboraud dextrose agar (SDA) and maintained at 26 ± 2^0C.

3.3 Plant materials

Three cowpea varieties obtained from the ICIPE Germplasm collection and described by Pathak and Olela (1986) were used during the study period:

1. ICV 2, a single-plant selection from landraces of eastern Kenya. It has a spreading, indeterminate growth habit with small leaves, dark purplish-blue flowers, and green stem and pods. It is highly susceptible to *M. sjostedti* and matures in about 70-75 days.

2. ICV 7, has a semi-erect growth habit with light bluish-purple flowers and green stems and pods. It is tolerant to *M. sjostedti* and matures in 65-70 days.

3. ICV 8, has a semi-erect, determinate growth habit with creamy-white flowers and green stems and pods. It is moderately resistant to *M. sjostedti* and matures in 70 days.

3.4. Study sites

Laboratory studies were conducted at the ICIPE Headquarters, Duduville, and field experiments were conducted at the ICIPE, Mbita Point Field Station (MPFS) on the shores of Lake Victoria, 1200 m above sea level. MPFS station has two rainy seasons: (March-June and October-December). The amount of rainfall varies from year to year. Due to unreliability of rainfall, irrigation was used in some experiments. Temperature during both seasons varies from 25-30^{0}C and in the dry season the temperature rises upto 35^{0}C. The soil type in MPFS stations largely varies from sandy clay to clay and the colour of the top soil varies from dark brown to grayish brown (Rachilo and Wataka, 1980).

CHAPTER FOUR

Pathogenicity of entomopathogenic fungi (Hyphomycetes) to the legume flower thrips, *Megalurothrips sjostedti*

4 Introduction

Various entomopathogenic fungi have been reported from *M. sjostedti* (Salifu, 1986; Tamò *et al.*, 1993; Ekesi and Maniania, unpublished); however, their pathogenicity to this insect has not been studied. The pathogenicity of entomopathogenic fungi to other thripids such as *F. occidentalis* and *T. tabaci* Lindeman have been reported (MacLeod *et al.*, 1976; Samson *et al.*, 1976; Brownbridge *et al.*, 1994; Vestergaard *et al.*, 1995).

The pathogenicity of different strains of hyphomycetous fungi to adult *M. sjostedti* was, therefore, evaluated in the laboratory, in order to select candidate strains that may be considered for management of the pest. In this study, the Null hypothesis that entomopathogenic fungi are not pathogenic to legume flower thrips will be rejected if analysis of variance show significance in mortality between fungal treated insects and untreated control insects; and if mortality caused by mycosis could be confirmed by microscopic examination of hyphae and spores on dead insects.

4.1 Materials and methods

4.1.1 Insect

Megalurothrips sjostedti colony was secured from an infested field of ICV 2 cowpea at ICIPE, Nairobi and maintained in the laboratory according to the rearing methods described in chapter three. 3-day old adults were used in this experiment.

4.1.2 Fungi

Fungal strains tested against *M. sjostedti* are listed in Table 1. The fungi were cultured on Sabouraud dextrose agar (SDA) (Biotec Laboratories, Suffolk, UK) in Petri dishes. Plates were incubated for 2-3 weeks at 26 ± 2^0C.

4.1.3 Bioassay procedure

Conidia were harvested from 21 day-old surface cultures (Plates 1 and 2) by scraping. Inocula were suspended in 20 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing 3 mm glass beads. The conidial suspension was vortexed for 5 minutes to produce a homogenous suspension. Spore concentrations were determined using a Malassez cell-counting chamber (Prolabo, Créteil, France). The viability of conidia was determined by spread-plating 0.1 ml of conidial suspension (titrated to 3×10^6 conidia ml^{-1}) on SDA plates. Two replicated sterile microscope cover slips were placed on each plate. Plates were incubated at 26 ± 2^0C and examined after 24 and 36 hours. Percentage germination was determined from 100 spore counts for each cover slip.

Treatments were performed by direct spray of 10 ml of a given concentration onto insects using the Burgerjon (1956) spray tower (INRA, Montpellier, France). All isolates were tested at a standard concentration of 1×10^8 conidia ml^{-1} corresponding to a surface coverage rate of 1×10^7 conidia cm^{-2}. Insects were immobilized by chilling them for 25 seconds prior to spraying. Control lots were treated with sterile distilled water containing 0.05% Triton X-100 (PSPARK Scientific Ltd., Northampton, UK). Insects were then transferred to rearing cages and fed with ICV 2 cowpea floral tissues daily. Mortality was recorded daily for 7 days and mortality caused by fungi was confirmed by microscopic examination of hyphae and spores on the surface of the insect.

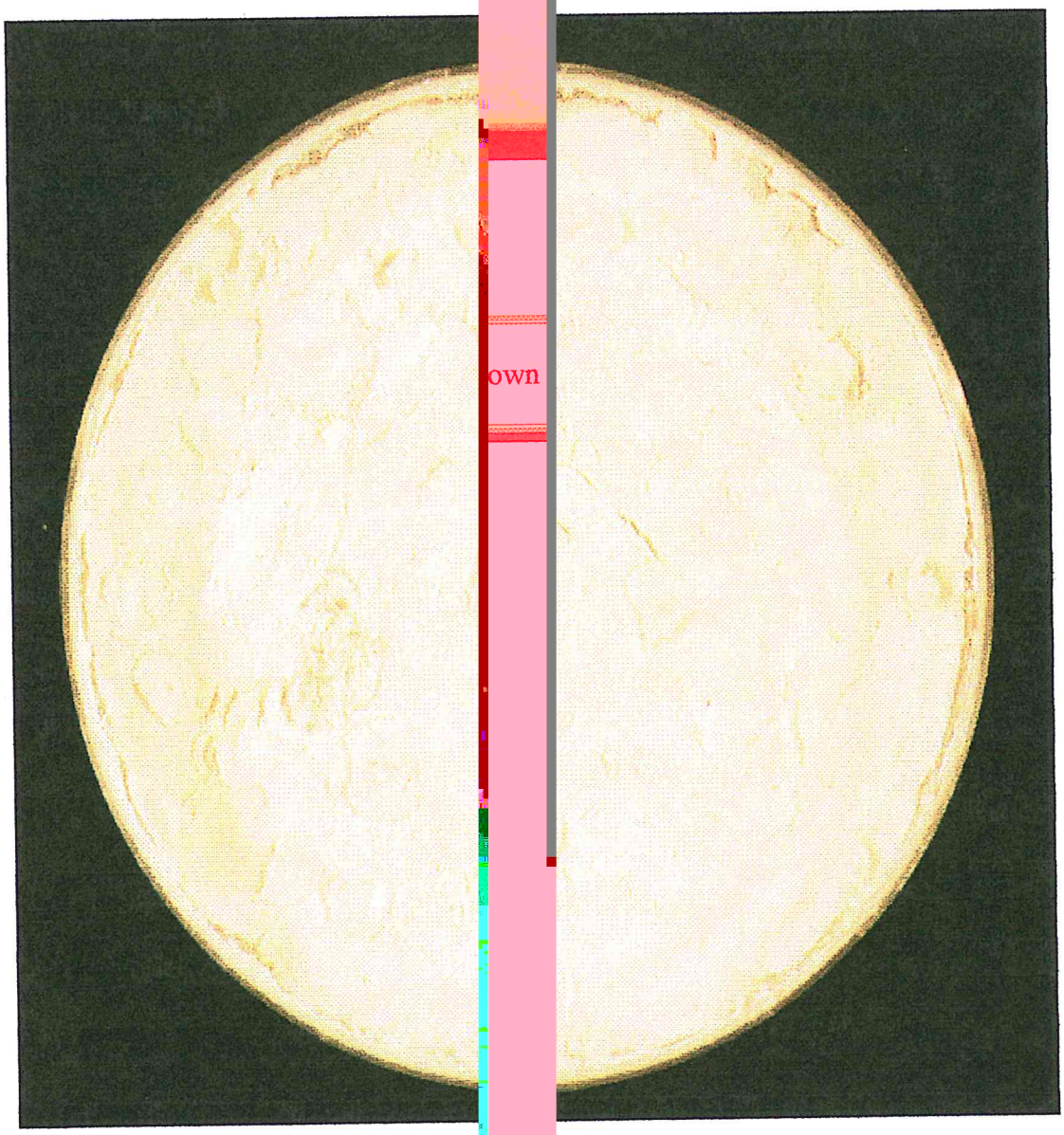

Plate 1. Pure culture of *Beauveria bassiana* g on Saboraud Dextrose Agar X 8

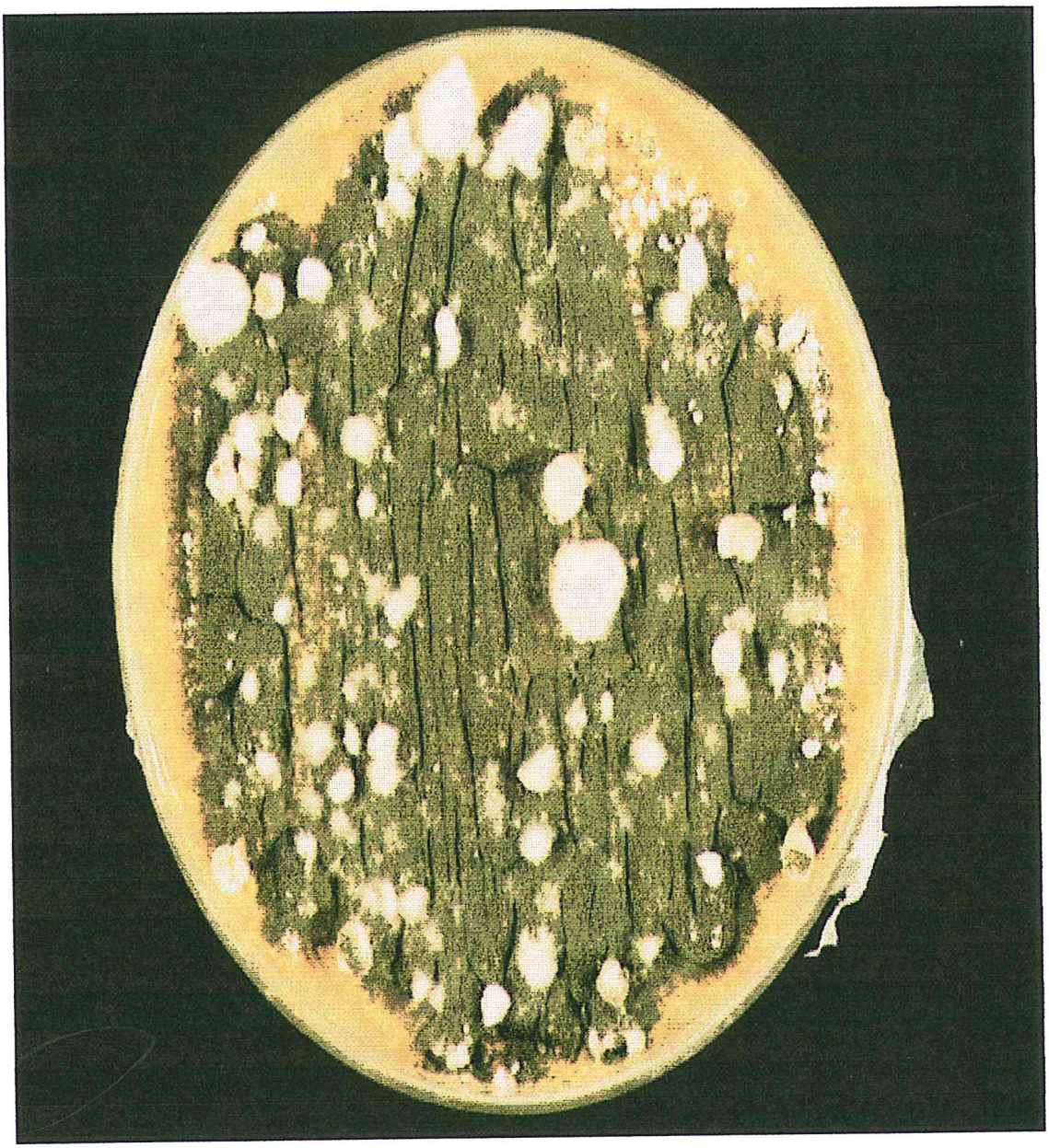

Plate 4. Pure culture of *Metarhizium anisopliae* grown on Saboraud Dextrose Agar X 2

Dose-mortality relationships were calculated for the most pathogenic strains by using 7 doses of inoculum: 1×10^5, 3×10^5, 1×10^6, 3×10^6, 1×10^7, 3×10^7 and 1×10^8 conidia ml^{-1}.

All test insects were maintained in a controlled temperature room at 26 ± 2^0C under a photoperiod of L:D 12:12. Each experiment was replicated four times with 20 insects per treatment.

4.1.4 Statistical Analysis

Percentages of mortality were transformed by arcsine to normalize mean percentages (Gomez and Gomez, 1984) and the angular values were separated by Student-Newman-Keuls (P=0.05) (Sokal and Rohlf, 1981) using the ANOVA procedure of SAS (SAS Institute, 1988). Regression analyses were used to estimate the lethal time to 50% mortality (LT_{50}) and the lethal concentration causing 50% mortality (LC_{50}), using the LIFEREG procedure of SAS.

4.2 Results

Viability tests showed that germination of all strains tested (Table 1) exceeded 80% after 24 h (Table 2), except for *B. bassiana*, strain TP-GHA, and *M. anisopliae*, strain ICIPE 20. The percentage germination of these two strains, however, reached 100% after 36 h. Compared to control insects (Plate 3) all fungal treated insects rapidly underwent external hyphal development and sporulation under moist conditions (Plate 4 and 5). Hyphae initially emerged from intersegmental regions of the killed host and then quickly covered the cadaver with diffuse hyphal growth and sporulation.

Plate 3. Unmycosed adults of *Megalurothrips sjostedti* from control treatment X 16

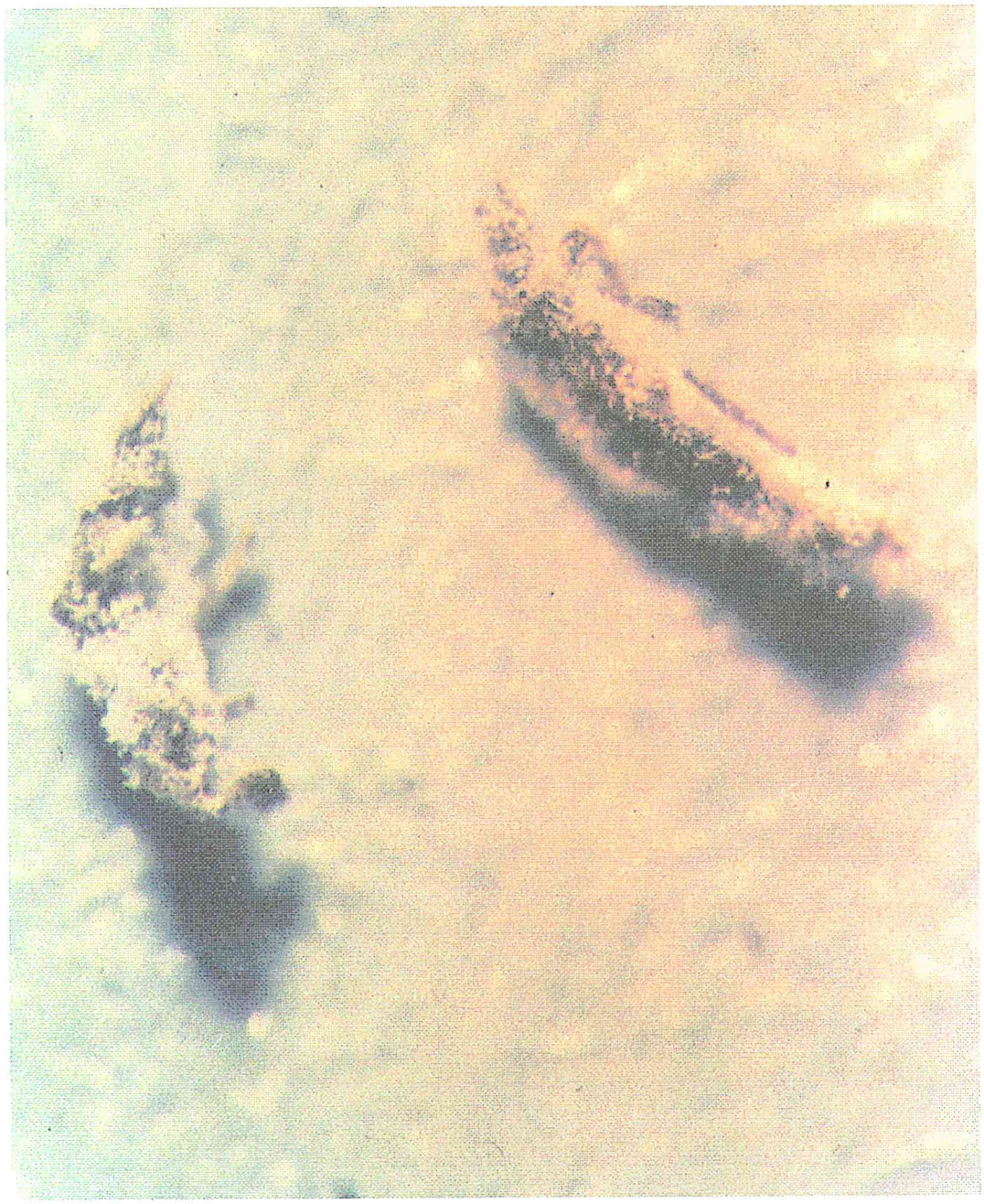

Plate 4. Mycosed adults of *Megalurothrips sjostedti* covered with hyphae and spores of *Beauveria bassiana* X 8

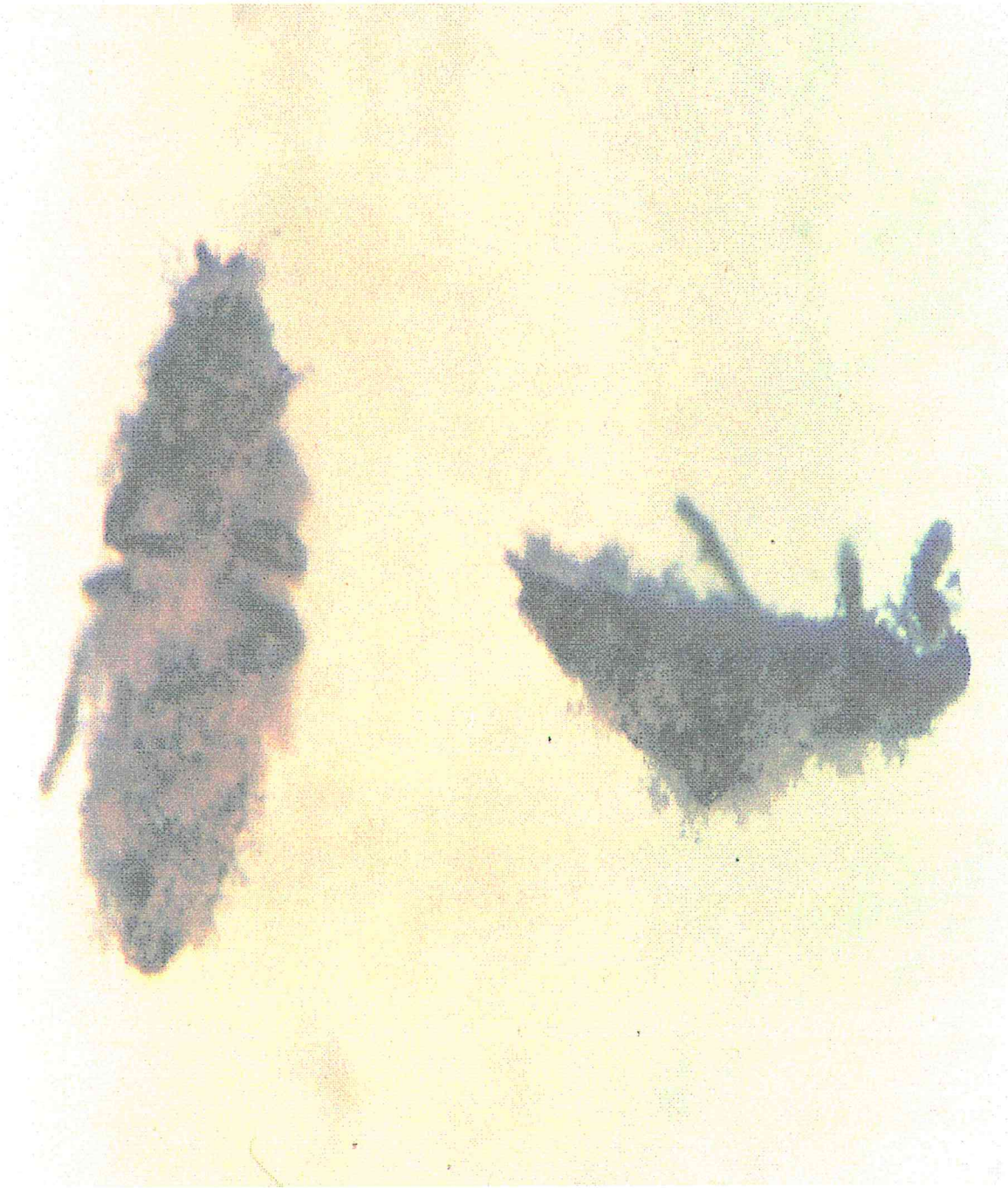

Plate 5. Mycosed adults of *Megalurothrips sjostedti* covered with hyphae and spores of *Metarhizium anisopliae* X 8

Mortality in the controls did not exceed 6.3% and this was significantly lower compared to other treatments (F=34.2; DF=3,69; P=0.05). At the standard concentration of 1 x 10^8 conidia ml^{-1}, all fungal strains tested were pathogenic to adult *M. sjostedti* (rejection of Null hypothesis). Compared with control insects (Plate 3), all strains rapidly underwent external hyphal development and sporulation under moist conditions (Plates 4 and 5). Hyphae initially emerged from intersegmental regions of the killed host and then quickly covered the cadaver with diffuse hyphal growth and sporulation (Plates 4 and 5).

Pathogenicity varied according to fungal strain and fungal species (Table 3). For example, mortalities caused by *B. bassiana* ranged from 29 to 100%. Mortalities caused by *M. anisopliae* ranged from 54 to 100%. *V. lecanii* caused mortalities ranging from 29 to 68% mortality caused by *P. fumosoroseus* was 13%. The lethal time required to achieve 50% mortality also varied according to fungal species and strains within species. At a concentration of 1 x 10^8 conidia ml^{-1}, LT_{50} values for *B. bassiana* strains ranged from 2.7 to 8.4 days (table 3). At a similar concentration, *M. anisopliae* strains had LT_{50} values ranging from 2.4 to 6.8 days (Table 3). Most of the strains had moderate to steep slopes indicating that there was a significant time-mortality relationships for these fungal species. Strain TP-GHA had the shortest LT_{50} (2.7 days) within the strains of *B. bassiana*. It fiducial limit overlapped with the LT_{50} of strain ICIPE 53. Strain ICIPE 69 had the shortest LT_{50} (2.4 days) within the *M. anisopliae* strains. The fiducial limit of this LT_{50}, however, overlapped with those of *M. anisopliae* strains ICIPE 30 and 74 (Table 3).

With regard to dose-mortality relationships (Table 4), *M. anisopliae* strains had lower LC_{50} values, ranging from 1.3 to 1.6 x 10^6 conidia ml^{-1}. *B. bassiana* strains had LC_{50} values ranging from 1.8 to 7.9 x 10^6 conidia ml^{-1}. Among the six strains tested *M. anisopliae* strain, ICIPE 69, had the lowest LC_{50} value of 1.3 x 10^6 conidia ml^{-1} (Table 4).

Table 1. Fungal strains tested against *Megalurothrips sjostedti* with details of origin

Fungal species	Strain	Host species (family)	Locality (country)	Date of isolation
Beauveria bassiana	ICIPE 48	*Diaperasticus erythrocephala* (forficulidae)	Kanyamwakologi (Kenya)	1989
	ICIPE 53	Soil sample	Mbita (Kenya)	1989
	ICIPE 59	Soil sample	Kabello (Kenya)	1990
	ICIPE 77	*Cylas puncticolis* (Curculionidae)	Ungoye (Kenya)	1990
	ICIPE 78	*Temnoschoita nigroplagiata* (Curculionidae)	Ungoye (Kenya)	1990
	ICIPE 82	*Glossina pallidipes* (Diptera)	Nguruman (Kenya)	1995
	ICIPE 83	*Megalurothrips sjostedti* (Thripidae)	Mwae (Kenya)	1996
	TP-GHA	(Chrysomelidae)	Maine (USA)	1980
Metarhizium anisopliae	ICIPE 18	Unknown	Unknown	Unknown
	ICIPE 20	Soil sample	Migori (Kenya)	1989
	ICIPE 30	*Busseola fusca* (Noctuidae)	Kendu Bay (Kenya)	1989
	ICIPE 60	Soil sample	Kabello (Kenya)	1990
	ICIPE 62	Soil sample	Kinshasa (Zaire)	1990
	ICIPE 63	Soil sample	Kinshasa (Zaire)	1990
	ICIPE 66	Soil sample	Kinshasa (Zaire)	1990
	ICIPE 67	Soil sample	Kinshasa (Zaire)	1990

Table 1 continued. Fungal strains tested against *Megalurothrips sjostedti* with details of origin

Fungal species	Strain	Host species (family)	Locality (country)	Date of isolation
	ICIPE 69	Soil sample	Kinshasa (Zaire)	1990
	ICIPE 74	*C. puncticolis* (Curculionidae)	Mbita (Kenya)	1990
	ICIPE 75	*C. puncticolis* (Curculionidae)	Mbita (Kenya)	1990
Verticillium	ICIPE 85	Unknown	(Denmark)	Unknown
lecanii	ICIPE 86	Unknown	(Denmark)	Unknown
Paecilomyces	MY 613	Unknown	(USA)	Unknown
fumosoroseus				

Table 2. Percentage germination of fungal strains tested against *Megalurothrips sjostedti* after 24 h at 26 ± 2^{0}C on SDA.

Fungal species	Strain	% germination ± S.E
B. bassiana	ICIPE 48	92.0 ± 0.4
	ICIPE 53	94.5 ± 0.6
	ICIPE 59	100 ± 0.0
	ICIPE 77	93.0 ± 1.6
	ICIPE 78	90.3 ± 6.3
	ICIPE 82	100 ± 0.0
	ICIPE 83	97.0 ± 10.5
	TP-GHA	68.3 ± 0.5
M. anisopliae	ICIPE 18	100 ± 0.0
	ICIPE 20	72.5 ± 0.5
	ICIPE 30	99.5 ± 0.5
	ICIPE 60	94.3 ± 1.3
	ICIPE 62	100 ± 0.0
	ICIPE 63	91.3 ± 0.5
	ICIPE 66	81.8 ± 2.1
	ICIPE 67	96.5 ± 1.6
	ICIPE 69	100 ± 0.0
	ICIPE 74	100 ± 0.0
	ICIPE 75	92.8 ± 0.3
V. lecanii	ICIPE 85	95.8 ± 8.3
	ICIPE 86	83.3 ± 0.3
P. fumosoroseus	MY 613	93.0 ± 0.5

Table 3. Pathogenicity of entomopathogenic fungi to adult *Megalurothrips sjostedti*: % mortality rates and LT_{50} values of different strains at the concentration of 1 x 10^8 conidia ml^{-1} at 7 days post treatment.

Fungal species	Strain	% Mortality[a] ± S.E.	LT_{50} in days (95% Fiducial limit)	Slope	*t* test[b] on slope	Intercept
Beauveria bassiana	ICIPE 48	70.0 ± 4.1c	5.5 (5.3-5.9)	0.32	ns	-1.26
	ICIPE 53	100 ± 0.0a	2.9 (2.8-3.0)	0.78	*	-1.79
	ICIPE 59	28.8 ± 6.3e	7.9 (7.6-8.1)	0.69	*	-4.99
	ICIPE 74	3.8 ± 2.5d	8.1 (7.8-8.5)	0.26	ns	-1.60
	ICIPE 78	31.3 ± 4.8e	8.4 (8.0-8.6)	0.36	ns	-2.53
	ICIPE 82	43.8 ± 2.4d	7.5 (7.3-7.7)	0.60	*	-4.02

Table 3 continued. Pathogenicity of entomopathogenic fungi to adult *Megalurothrips sjostedti*: % mortality rates and LT_{50} values of different strains at the concentration of 1 x 10^8 conidia ml^{-1} at 7 days post treatment.

Fungal species	Strain	% Mortality[a] ± S.E.	LT_{50} in days (95% Fiducial limit)	Slope	*t* test[b] on slope	Intercept
	ICIPE 83	71.1 ± 4.2c	5.4 (5.2-5.5)	0.46	*	-1.98
	TP-GHA	100 ± 0.0a	2.7 (2.5-3.0)	0.55	*	-0.96
Metarhizium anisopliae	ICIPE 18	62.5 ± 2.9c	6.1 (5.8-6.2)	0.41	ns	-2.00
	ICIPE 20	53.8 ± 2.5d	6.8 (6.6-7.1)	0.32	ns	-1.67
	ICIPE 30	100 ± 0.0a	2.5 (2.4-2.6)	0.69	*	-1.44
	ICIPE 60	62.5 ± 2.9c	6.1 (5.9-6.2)	0.41	ns	-2.00

Table 3 continued. Pathogenicity of entomopathogenic fungi to adult *Megalurothrips sjostedti*: % mortality rates and LT_{50} values of different strains at the concentration of 1 x 10^8 conidia ml^{-1} at 7 days post treatment.

Fungal species	Strain	% Mortality[a] ± S.E.	LT_{50} in days (95% Fiducial limit)	*t* test[b] Slope	on slope	Intercept
Metarhizium anisopliae	ICIPE 62	93.8 ± 9.5b	3.3 (3.1-3.5)	0.53	*	-1.27
	ICIPE 63	93.8 ± 9.5b	3.4 (3.2-3.6)	0.68	*	-1.79
	ICIPE 66	100 ± 0.0a	2.7 (2.5-2.8)	0.69	*	-1.36
	ICIPE 67	85.0 ± 9.1b	4.2 (4.0-4.4)	0.52	*	-1.68
	ICIPE 69	100 ± 0.0a	2.4 (2.3-2.6)	0.69	*	-1.15
	ICIPE 74	100 ± 0.0a	2.5 (2.2-2.6)	0.93	*	-1.75

Table 3 continued. Pathogenicity of entomopathogenic fungi to adult *Megalurothrips sjostedti*: % mortality rates and LT_{50} values of different strains at the concentration of 1 x 10^8 conidia ml^{-1} at 7 days post treatment.

Fungal species	Strain	% Mortality[a] ± S.E.	LT_{50} in days (95% Fiducial limit)	Slope	*t* test[b] on slope	Intercept
Metarhizium anisopliae	ICIPE 75	91.3 ± 4.9b	3.3 (3.0-3.4)	0.49	*	-1.16
Verticillium lecanii	ICIPE 85	67.5 ± 5.0e	8.4 (8.1-8.7)	0.36	ns	-2.53
	ICIPE 86	28.8 ± 4.8e	9.5 (9.3-9.8)	0.25	ns	-1.88
Paecilomyces Fumosoroseus	MY 613	12.5 ± 2.7f	8.7 (8.5-8.9)	0.35	ns	-6.00

[a]Within-column means followed by the same letters are not significantly different by Student-Newman-Keuls (*P*=0.05). Means were angularly transformed before analysis but values represent detransformed means. [b]t Test on slope of the regression equation; *, significantly different from zero (*P*=0.05); ns, do not differ significantly .

Table 4. LC_{50} values for the most active fungal strains against adult *Megalurothrips sjostedti* at 7 days post treatment.

Fungal species	Strains	LC_{50} conidia ml^{-1} (95% Fiducial limit)	Slope	*t* Test on slope[a]	Intercept	x^2 test of linearity
Beauveria	ICIPE 53	7.9×10^6 $(6.8\text{-}8.9)10^6$	0.37	*	-1.44	10.235
bassiana	TP-GHA	1.8×10^6 $(1.6\text{-}2.0)10^6$	0.52	*	-1.98	15.642
Metarhizium	ICIPE 30	1.5×10^6 $(1.4\text{-}1.6)10^6$	0.74	*	-1.45	18.076
anisopliae	ICIPE 66	1.6×10^6 $(1.5\text{-}1.8)10^6$	0.79	*	-1.28	18.173
	ICIPE 69	1.3×10^6 $(1.2\text{-}1.4)10^6$	0.58	*	-1.44	15.943
	ICIPE 74	1.5×10^6 $(1.2\text{-}1.8)10^6$	0.24	ns	0.95	5.085

[a]t Test on slope of the regression equation; *, significantly different from zero (P=0.05); ns, not significantly different.

4.3 Discussion

The use of entomopathogenic fungi for the control of agricultural pests has long been recognized (Ferron, 1978; Zimmerman, 1986b; Ferron *et al.*, 1991). However, the success in the use of entomopathogenic fungi as mycoinsecticides largely depends on strain selection (Soper and Ward, 1981). Therefore, screening and selection of the most pathogenic strains for further field evaluation against *M. sjostedti* were the main objectives of this study. Results of the bioassays indicate that all the fungal strains were pathogenic to *M. sjostedti*, but there was considerable variation in the pathogenicity both between species and strains within species. Within-strain differences in virulence was more pronounced for *B. bassiana* than for *M. anisopliae*. *Beauveria bassiana* and *M. anisopliae* are best known for their wide geographical distribution and broad host range (Hall and Papierok, 1982). Interspecific and intraspecific variations in pathogenic activity of entomopathogenic fungi observed in our studies are similar to those reported for other insects (Maniania and Fargues, 1984; Poprawski *et al.*, 1985; Feng and Johnson, 1990). In general, strains of *M. anisopliae* caused more rapid death than strains of *B. bassiana, V. lecanii* and *P. fumosoroseus*. This corroborates the observations of Vestergaard *et al.* (1995) on *Frankliniella occidentalis*. Fast kill could be attributed to the effect of depsipeptide toxins (destruxins) produced by *M. anisopliae* (Gillespie and Claydon, 1989).

It is generally believed that strains of entomopathogenic fungi are more pathogenic to the species of insect from which they were isolated. This is true in the case of scarabaeid beetles where a strict adaptation of strains of *M. anisopliae* to their original host has been reported (Ferron *et al.*, 1972). However, *B. bassiana* ICIPE 83 isolated from *M. sjostedti* was only moderately pathogenic to its original host, causing 71% mortality. Several authors have also reported that *N. rileyi* and *P. fumosoroseus*, pathogenic to noctuid larvae, are less aggressive when tested on their original host (Ignoffo *et al.*, 1976; Boucias *et al.*, 1982; Maniania and Fargues, 1984). *B. bassiana* ICIPE 53

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CHAPTER FIVE

Effect of temperature on germination, radial growth and pathogenic activity of *Metarhizium anisopliae* and *Beauveria bassiana* to the legume flower thrips, *Megalurothrips sjostedti*

5 Introductio

Temperature is one of the environmental factors that influences fungal growth and disease development in insects (Roberts and Campbell, 1977; Benz, 1987). Temperature-dependent growth and infectivity has been demonstrated for many hyphomycetous fungi, including *B. bassiana* and *M. anisopliae* (Walstad *et al.*, 1970; Doberski, 1981; Soares *et al.*, 1983; Carruthers *et al.*, 1985; Fargues *et al.*, 1992; Vestergaard *et al.*, 1995). Variability in temperature-dependence has been reported by many workers (Roberts and Campbell, 1977; Soares, *et al.*, 1983; Fargues *et al.*, 1992; Maniania and Fargues, 1992)

Increased interest in the use of insect pathogens within pest management programmes necessitates the selection of fungal pathogens tolerant to the temperature range found in the ecosystem involved (Ferron *et al.*, 1991). For example, in order to develop entomogenous fungi as biological control agents of the elm bark beetle *Scotylus scotylus* F., and the black vine weevil, *Otiorhynchus sulcatus* F., both inhabiting temperate regions, Doberski (1981) and Soares *et al.* (1983) selected strains with pathogenic activity below 15^0C.

Field studies have shown that active populations of legume flower thrips can be found at temperatures as low as 15^0C and as high as 30^0C (Alghali, 1991; Ekesi, unpublished data). Alghali (1991), however, reported a mean temperature range of 27-29^0C for highest thrips populations in the field. Identifying a fungal strain with a broad temperature range is therefore necessary for a

rational approach to the management of *M. sjostedti*. The present study, aimed at selecting strain with broad temperature range, examined the effect of temperature on germination, radial growth and pathogenic activity of six strains of entomopathogenic fungi to *M. sjostedti*. The Null hypothesis that all fungal strains germinate, grow and cause equal level of mortality in *M. sjostedti* at 15, 20, 25, 30 and 35^{0}C will be rejected if analysis of variance and/or mean separation show significant differences in germination, growth and mortality among the strains.

5.1 Materials and methods

5.1.1 Insect

Test insects originated from the same colony as described in chapter three. 3-day old adults were used for the various experiments.

5.1.2 Fungi

The test fungi are strains reported in chapter four to be highly pathogenic to adult *M. sjostedti* and they include *B. bassiana* ICIPE 48 and TP-GHA, and *M. anisopliae*, strain ICIPE 30, 66, 69 and 74. Fresh inocula of the fungi were maintained on SDA plates and incubated for 2-3 weeks at 26 ± 2^{0}C.

5.1.3 Effect of temperature on germination

The effect of temperature on germination was tested by spread-plating 0.1 ml of conidial suspension titrated to 3 x 10^6 conidia ml^{-1} on SDA plates in Petri dishes. Sterile microscope cover slips were placed on each plate. Inoculated plates were sealed with Parafilm M and incubated at 15, 20, 25, 30, and 35^{0}C in complete darkness. Percentage germination was determined for 100 spores

counts for each plate at X 40 magnification 24 h post-inoculation. The experiment was replicated 4 times.

5.1.4 Effect of temperature on radial growth

A conidial suspension of 1 x 10^7 spores ml^{-1} was spread-plated on SDA plates. Plates were then incubated at 26 ± 2^0C for 3 days in order to obtain mycelial mats. Mycelial mats were cut from culture plates into round agar plugs using a 8-mm-diameter cork borer (Rapilly, 1968). Each agar plug (ca.5 mm thick) was then transferred onto the center of a fresh SDA plate. Plates were sealed with Parafilm M and incubated in complete darkness at 15, 20, 25, 30 and 35^{0}C. Radial growth was recorded daily for 12 days by measuring the diameter of growth. The experiment was replicated 4 times.

5.1.5 Effect of temperature on pathogenic activity

Ten ml of conidial suspensions were directly sprayed onto insects using a Burgerjon's (1956) spray tower. All strains were tested at a standard concentration of 1 x 10^8 conidia ml^{-1}. Control lots were treated with sterile distilled water containing 0.05% Triton X-100. Insects were immobilized by chilling them for 25 seconds prior to spraying. Following treatment, each replicate of 20 insects was placed in a rearing cage (95 x 30 mm) containing floral tissues and pollen. Each treatment consisted of four rearing cages which were incubated at different temperatures: 15, 20, 25, and 30^{0}C, respectively. Mortality was recorded daily for 14 days. Dead insects were transferred to petri dish lined with damp filter paper. Mortality due to fungi was confirmed by microscopic examination of hyphae and spores on the surface of the dead body.

5.1.6 Statistical analysis

Germination and growth data were subjected to analysis of variance (ANOVA) for a completely randomized design using the ANOVA procedure of SAS (SAS Institute, 1990). Growth was expressed as relative value (%) in relation to maximum speed of growth (G_{max} = 100%) (Fargues *et al.*, 1992) for each strain at different temperatures before analysis. Percentage mortality was submitted to Abbott (1925) formula to correct for natural mortality. Percentages were then transformed by arcsin to standardize mean percentages (Gomez and Gomez, 1984) before analysis. Angular values were separated by the Student-Newman-Keuls test (P=0.05) using the ANOVA Procedure of SAS. Regression analyses were used to estimate the lethal time to 50% mortality (LT_{50}) using the LIFEREG Procedure of SAS. LT50 values were then subjected to ANOVA and means were separated by SNK.

5.2 Results

5.2.1 Effect of temperature on germination

There was no significant difference (P>0.05) in germination between fungal strains at 15^{0}C, 20^{0}C and 30^{0}C (acceptance of Null hypothesis). At 25^{0}C, significant difference (P<0.05) occurred among the fungal strains (rejection of Null hypothesis). Germination at 15^0 and 35^{0}C was generally low for all the strains. The optimum temperature for germination for all strains was 25^{0}C, except for strain TP-GHA (Figure 2).

5.2.2 Effect of temperature on growth

Growth occurred at all temperatures but was slower at 15^0 and 35^0C as compared to 20, 25 and 30^0C (Figure 3). There was no significant difference (P >0.05) between fungal strains at 15^0C and 35^0C(acceptance of Null hypothesis). Significant differences (P<0.05) in growth was, however, observed at 20^0C, 25^0C and 30^0C (rejection of Null hypothesis). Comparison of mean radial growth rates day^{-1} showed that ICIPE 66 was superior to other strains at 15^0C (Table 5). There was no significant difference (P>0.05) among strains at 20^0C and at 35^0C. At 25 and 30^0C, mean growth rate day^{-1} of strain ICIPE 69 was significantly higher (P<0.05) as compared to other strains (Table 5).

5.2.3 Effect of temperature on pathogenic activity

Mortality in the controls was generally low, less than 3%, except at 30^0C where 13% mortality was observed. The six fungal strains tested were pathogenic to the insect at all temperatures, but mortality varied with fungal strain and temperature (Table 6). At 15^0 and 20^0C, there were significant differences (P<0.05) in mortality between fungal strains (rejection of Null hypothesis). Strain ICIPE 66 was the most virulent of all the strains at 15^0C and 20^0C, followed by ICIPE 69 (Table 6). Similarly, there were significant differences (P<0.05) in mortality between strains at 25 and 30^0C, respectively.(rejection of Null hypothesis). *Beauveria bassiana* ICIPE 53 and *M.anisopliae* ICIPE 69 and 74 were the most active strains at 25^0C, while at 30^0C, *M.anisopliae* ICIPE 30, 69, 74 and *B.bassiana* TP-GHA caused 100% mortality of the test insects (Table 6).

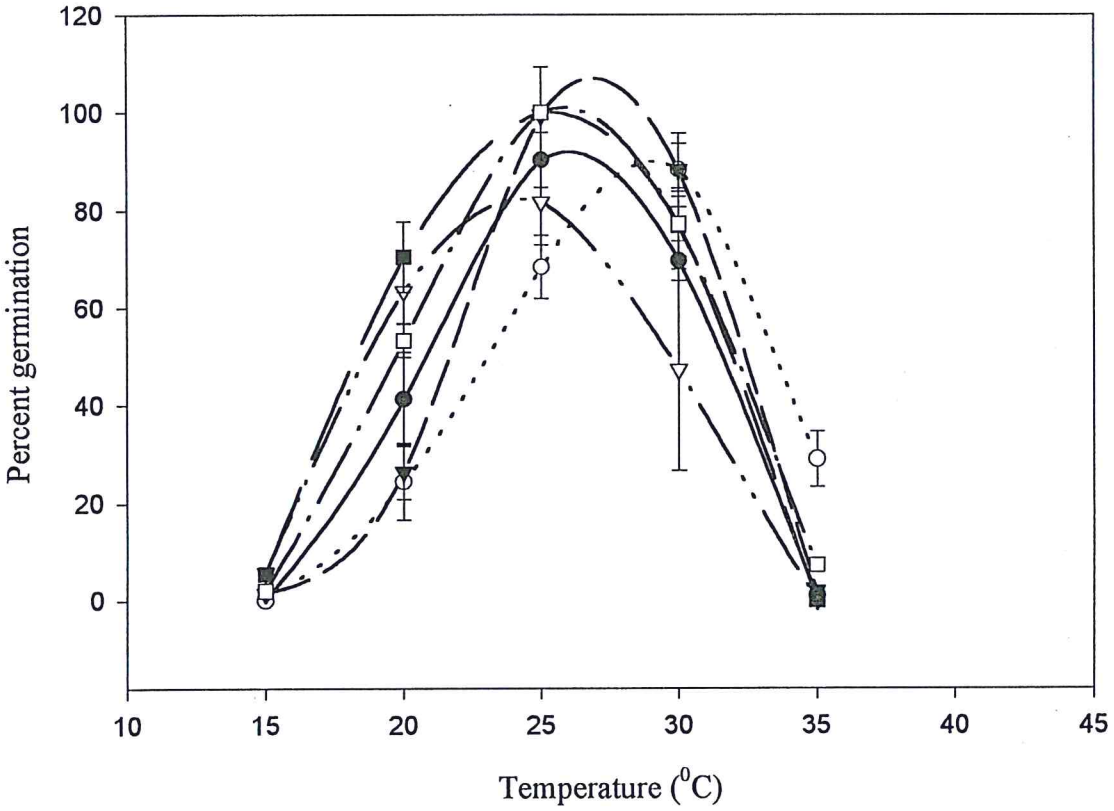

Figure 2. Effect of temperature on germination of two strains of *Beauveria bassiana* and four strains of *Metarhizium anisopliae*

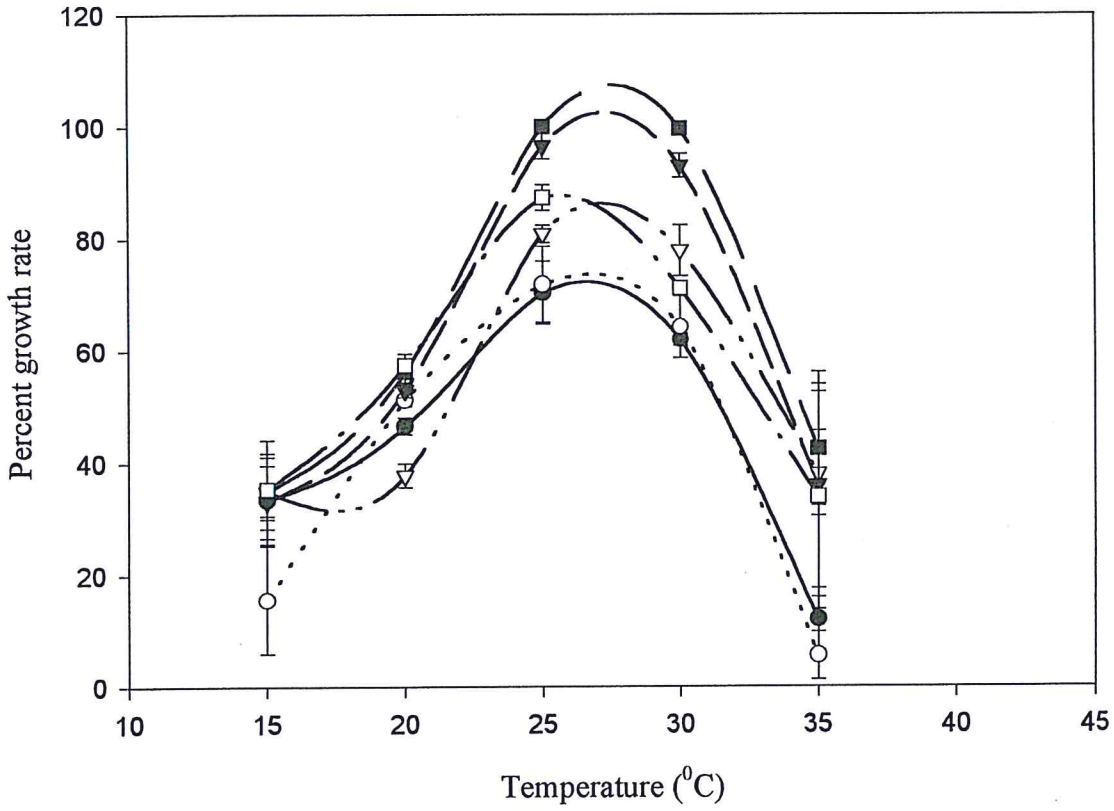

Figure 3. Effect of temperature on radial growth of two strains of *Beauveria bassiana* and four strains of *Metarhizium anisopliae*

Table 5: The growth rate day^{-1} of different strains of *Beauveria bassiana* and *Metarhizium anisopliae* cultured on SDA medium under varying temperatures

Fungal		Mean ± S.E. growth rate (mm day^{-1})				
		Temperature				
species	Strain	15^{0}C	20^{0}C	25^{0}C	30^{0}C	35^{0}C
Beauveria	ICIPE 53	1.72 ± 0.19b	2.41 ± 0.28a	3.14 ± 0.24c	2.76 ± 0.14bc	1.56 ± 0.11a
bassiana	TP-GHA	1.40 ± 0.12b	2.51 ± 0.22a	3.41 ± 0.16c	3.21 ± 0.02b	1.91 ± 0.12a
Metarhizium	ICIPE 30	1.71 ± 0.15b	2.62 ± 0.06a	4.92 ± 0.03b	4.75 ± 0.04b	1.83 ± 0.03a
anisopliae	ICIPE 66	2.53 ± 0.24a	2.56 ± 0.11a	4.01 ± 0.02b	3.91 ± 0.17b	1.14 ± 0.37a
	ICIPE 69	1.82 ± 0.12b	2.92 ± 0.23a	5.32 ± 0.21a	5.11 ± 0.12a	1.10 ± 0.41a
	ICIPE 74	1.87 ± 0.34b	2.63 ± 0.19a	4.43 ± 0.13b	3.72 ± 0.04b	1.52 ± 0.17a

Means within a column followed by the letter do not differ significantly by Student-Newman-Keuls test (P=0.05).

Tab

Met

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Cor

Bea

bass

Met

anis

Mea

Nev

repr

Table 7: Lethal time to 50% mortality of strains of *Beauveria bassiana* and *Metarhizium anisopliae* on adult thrips, *Megalurothrips sjostedti*

Fungal species	Strain	LT_{50} ± SE in days			
		15°C	20°C	25°C	30°C
Beauveria bassiana	ICIPE 53	16.4 ± 2.1a	6.1 ± 0.4b	2.8 ± 0.3a	4.0 ± 0.3b
	TP-GHA	14.8 ± 3.1ab	8.4 ± 1.2a	3.0 ± 0.5a	2.5 ± 0.1c
Metarhizium anisopliae	ICIPE 30	11.6 ± 1.7c	6.4 ± 1.3b	2.9 ± 0.2a	2.8 ± 0.7c
	ICIPE 66	8.1 ± 1.6d	5.9 ± 1.9b	2.9 ± 0.8a	9.4 ± 3.4a
	ICIPE 69	8.9 ± 4.2d	4.6 ± 1.4c	2.9 ± 0.3a	2.9 ± 0.3c
	ICIPE 74	15.4 ± 1.2b	5.4 ± 0.6b	3.0 ± 1.2a	3.4 ± 0.3bc

Means within a column followed by the same letter do not differ significantly by Student Newman-Keuls test (P=0.05).

LT_{50} values ranged from 8.1-16.4 days at 15^{0}C, from 4.6-8.4 at 20^{0}C, from 2.8-3.0 days at 25^{0}C, and from 2.5-9.4 days at 30^{0}C (Table 7). There was no significant difference in lethal time to 50% among strains at 25^{0}C. LT_{50} values, however, differed significantly among fungal strains at 15, 20, and 30^{0}C (Table 7).

5.3 Discussion

Spore germination and mycelial growth of both *M. anisopliae* and *B. bassiana* strains were observed at all temperatures; but the optimum temperature occurred at 25^{0}C for most of the strains except *B.bassiana*, strain TP-GHA. These findings are in accordance with those obtained by many workers with hyphomycetous fungi (Walstad *et al.*, 1970; Roberts and Campbell, 1977; Hall and Papierok, 1982). As reported by Walstad *et al.* (1970), germination by *B.bassiana* and *M.anisopliae* is reduced at the upper temperature limit of 35^{0}C. Fargues *et al.* (1992) reported that fungal strains originating from tropical regions were more thermo-tolerant than strains from temperate climates. Fungal strains tested in the present study originated from tropical Africa, except *B.bassiana* strain TP-GHA, which originated from USA.

Metarhizium anisopliae strains ICIPE 30 and ICIPE 69 had similar germination profiles despite their different origins: ICIPE 30 was isolated from *Busseolla fusca* Fuller in Kenya from a semi-arid area, while ICIPE 69 was isolated from a soil sample taken in Kinshasa, Democratic Republic of Congo which has a hot, humid climate. On the other hand, *M. anisopliae* strain ICIPE 66 which was isolated from the same site as ICIPE 69 showed significant differences in terms of radial growth and pathogenic activity at different temperatures. These differences underline the importance of studying the interaction between pathogen/host/environmental factors in order to select potential candidate(s) for biological control.

Mean growth rate day^{-1} was within the range of 1-2 mm at 15 and 35^{0}C, and 2-5 mm at 25-30^{0}C. Optimum temperature for growth for all strains therefore appeared to lie between 25-

30^{0}C, which was consistent with germination studies. Mean growth rate day^{-1} at the different temperatures studied was within the range reported by Fargues *et al.* (1992). These authors reported an optimal growth temperature of 25^{0}C for tropical strains of entomopathogenic hyphomycetes. *Metarhizium anisopliae* strain ICIPE 69 was the fastest growing strain at the optimum temperature of between 25-30^{0}C as evidenced by its mean growth rate day^{-1}.

Fungal strains were most effective in terms of pathogenicity to *M. sjostedti* at 25 and 30^{0}C. Temperature of 20^{0}C caused significant decrease in the development rate of fungal infections but did not significantly affect percentage mortality caused by the fungi. Disease development progressed at the slowest at 15^{0}C. Ferron (1978) reported that temperature lower than optimal retards development of mycosis without necessarily affecting the total mortality. Infection of *M. sjostedti* with *B. bassiana* and *M. anisopliae* showed that mortality increased as temperature increased and corroborated previous reports. Vestergaard *et al.* (1995) reported that the western flower thrips, *F. occidentalis* was susceptible to *M. anisopliae* at a temperature range of 18-26^{0}C, but that mortality was higher at upper temperatures of 23 and 26^{0}C compared to 18 and 20^{0}C. Rath and Yip (1989) showed that virulence of *M. anisopliae* to the subterranean scarab, *Adoryphorus couloni* Burmeister increased as temperature was raised from 15 to 25^{0}C. Doberski (1981) showed that infections in elm bark beetle *S. scolytus* by *M. anisopliae* did not occur at 6^{0}C but mortality increased sharply between 15 to 25^{0}C. Mortality was higher at 25 and 30^{0}C and corresponded to optimum temperatures for germination and mycelial growth. A correlation between optimum temperature for fungal growth and fungal infection has been reported by several workers (Latch and Kain, 1983; Doberski, 1981; Maniania and Fargues, 1992).

At the lower temperature limit of 15^{0}C, *M.anisopliae* strain ICIPE 66 was able to achieve moderate mortality with the shortest LT_{50} value. This strain, however, performed poorly at the upper temperature limit of 30^{0}C. On the other hand, *B. bassiana* strain TP-GHA was most effective at the upper temperature limit than at the lower temperatures. *Metarhizium anisopliae*

strain ICIPE 69 showed pathogenic activity over a broad range of temperatures. From the ecological standpoint it appears that this strain will infect and kill *M. sjostedti* at temperatures ranging from 15^0 to 30^0C. Although low temperatures generally retarded fungal infection in *M. sjostedti*, it is known that insects generally do not live at a constant temperature in nature but are subject to diurnal and seasonal fluctuations in temperature (Saunders, 1982). This suggests that low temperatures which, normally prevail at night, will retard infection, but this will later increase when temperature increases during the daytime.

Laboratory studies which simulate varying field conditions can provide quantitative data that may be useful to predict field results (Hall, 1982). The optimum temperature of between 25-30^0C for germination, growth and pathogenic activity of the fungi to the pest reported here compared favourably with the optimum temperature of 27-29^0C for high thrips numbers under field conditions (Alghali, 1991). This implies that the insects would be susceptible to infection by the pathogens at the temperatures at which *M. sjostedti* are active in the field.

The broad temperature range of activity of *M. anisopliae,* strain ICIPE 69, suggests that this strain have good potential for management of *M. sjostedti* in the field. It was therefore, selected from among the 6 strains tested for further studies aimed at developing an effective pest management option for *M. sjostedti*.

CHAPTER SIX

Susceptibility of different developmental stages of *Megalurothrips sjostedti* to *Metarhizium anisopliae*, strain ICIPE 69 and the effect of infection on feeding and on fecundity, egg fertility and longevity of adults surviving infection as larvae.

6 Introduction

The susceptibility of insects to fungal pathogens vary with developmental stages (Ferron, 1985). For example, it has been reported that the susceptibility of whiteflies to *Aschersonia aleyrodis* decreases with age, with the early stages being much more susceptible to infection than the late stages (Fransen *et al.*, 1987), whereas the early stages of *Heliothis* spp. have been reported to be less susceptible to *N. rileyi* than late stages (Mohamed *et al.* 1977). Fargues and Rodriguez-Reuda (1980) also reported that sixth instar of *Spodoptera littoralis* Biosduval was less susceptible to *N. rileyi* than other instars. Crops are often infested with mixtures of different stages of insect. Understanding which stage is most susceptible to infection is important for the development of management tactics.

Various authors have shown that infection by fungal pathogen can result in reduced feeding in insect pests (Fargues and Rodriguez-Reuda, 1980; Mohamed *et al.*, 1982; Thorvilson *et al.*, 1985; Moore *et al.*, 1992; Seyoum *et al.*, 1994; Thomas *et al.*, 1997)). Cases of equal assimilation of food between fungal treated and untreated insects are also known for some lepidopteran insect pests (Tyrrell, 1990). Although various authors have measured the feeding rate in thrips (Kirk, 1997a), no report on the effect of fungal infection on feeding in thrips is available in literature.

Insect fecundity, egg fertility and longevity is affected by a variety of intrinsic and extrinsic factors, including temperature (Teulon and Penman, 1991), food quality (Trichilo and Leigh, 1988)

and pathogens (Nnakumusana, 1985; Fargues et al., 1991). Although fecundity and longevity is a critical aspect of thrips population dynamics (Kirk, 1997b), there is no published information on the effect of pathogens on reproduction and longevity of thrips. A reduction in fecundity, egg fertility and longevity caused by mycosis would be expected to have a significant effect on the dynamics of *M. sjostedti* population. Information on these parameters will also be useful in the development of predictive epizootiological models and successful biological control programmes. The objectives of this study were to (1) elucidate which stage of *M. sjostedti* is most susceptible to infection by *M. anisopliae* (2) the effect of fungal infection on feeding by larvae and adult *M. sjostedti,* and (3) investigate the effect of *M. anisopliae* on fecundity, egg fertility and longevity of *M. sjostedti* adults surviving infection as second instar larvae. Based on these objectives, the Null hypotheses laid down are (1) *M. anisopliae* cause equal level of mortality in all developmental stages of *M. sjostedti* (2) Larvae and adults of *M. sjostedti* infected with *M. anisopliae* consume equal amount of food as non-infected control insects and (3) the fecundity, egg fertility and longevity of adult *M. sjostedti* surviving infection as larvae are not significantly different from non-infected adults.

6.1 Material and methods

6.1.1 Insect

The various developmental stages used in this experiment were obtained from *M. sjostedti* culture reared under the conditions described in chapter three.

6.1.2 Fungus

The fungus used in this experiment was *M. anisopliae*, strain ICIPE 69 which was reported in previous chapter to be highly pathogenic to adult *M. sjostedti* at a broad temperature range. The fungus was grown for 2-3 weeks on SDA plates and maintained at 26 ± 2^0C.

6.1.3 Preparation of conidial suspension

Conidia were harvested from surface cultures by scraping. Spores were suspended in 20 ml sterile distilled water containing 0.05% Triton X-100 in glass bottles containing 3 mm glass beads. Bottles were stoppered and vortex for 5 min to produce a homogeneous conidial suspension. Conidia were then quantified with a Malassez cell-counting chamber following serial dilutions in sterile distilled water. Viability of conidia were determined by spread-plating 0.1 ml of conidial suspension (titrated to 3 x 10^6 conidia ml^{-1}) on four SDA plates. Sterile cover slip were placed on each plate and incubated at 26 ± 2^0C. Percentage germination was examined after 24 h from 100-spore counts on each plate.

6.1.4 Inoculation of different developmental stages

Megalurothrips sjostedti was treated with *M. anisopliae* at the following stages: second instar larva, pupa and adult. Because rearing was scheduled, thrips belonging to different developmental stages could be treated simultaneously with conidial suspensions. Three concentrations of conidia were tested against each stage by directly spraying 10 ml of a given concentration of spores onto the stages using the Burgerjon's (1956) spray tower. Concentrations of 1 x 10^6, 1 x 10^7 and 1 x 10^8 conidia ml 1 were used resulting in surface coverage rates of 1 x 10^5, 1 x 10^6 and 1 x 10^7 conidia cm^{-2}, respectively. Control lots were treated with sterile distilled water containing 0.05% Triton X-100. The experiment consisted of 4 replicates of 20 insects for each concentration at each developmental stage. After treatment, larvae and adults were introduced individually into 20 mm diameter escape-proof cells. The cells were similar to the pollen-feeding cells described by Kirk (1987) for measuring the feeding rate of various species of thrips. Pupae were kept individually in a 60 x 30 mm rearing cage. Prior to the introduction of larvae and adults into the feeding cells, pollen was sprinkled evenly on the floor of the cells, without clumping, so that they could be seen and

counted later. Fresh pollen grains taken from flower buds, which were about to open, were used throughout the experimental period. In preliminary trials, larval and adult thrips were found to consume 452 ± 21 and 883 ± 15 grains, respectively, within 12 to 24 h. The amount of grains sprayed $cell^{-1}$ was therefore, within the range of 550 grains $cell^{-1}$ for larvae and 950 grains $cell^{-1}$ for adult. Cells containing thrips were placed in a humid transparent plastic container and kept at 26 ± 2^0C under a photoperiod of L: D 12: 12. Cages containing pupal stage were kept in complete darkness but at similar temperature as above. Mortality was recorded daily for 7 days. The criteria for scoring mycoses were (1) death of larvae and adults accompanied with fungal sporulation on the cadavers and (2) failure of pupae to develop accompanied with fungal sporulation on colonized puparia.

6.1.5 Effect of *M. anisopliae* infection on feeding by larvae and adult *M. sjostedti*

To assess the effect of fungal infection on feeding rate, the number of pollen grains consumed $thrips^{-1}$ day^{-1} was recorded using the method described by Kirk (1987). Cells were removed from the plastic containers and thrips immobilized by chilling for few seconds. The insects were then removed from the cells without disturbing the pollen. The grains on the floor of the cell were then stained with 0.1% cotton blue in lactophenol. A cover slip was then gently lowered unto the cell and viewed under the microscope. Under the light microscope (x 25), full grains that have not been fed on (i.e. cytoplasm) appear dark blue, while empty grains that have been fed on (i.e. without cytoplasm) appear very light blue and often shrunken. The recorded number of empty grains provided the number of pollen grains consumed after 12 h of infection and on a daily basis.

6.1.6 Fecundity, egg fertility and longevity of adu rviving infection as larvae

Female thrips which had survived larval infection f e experiment above and which had been previous paired with surviving male thrips were pla ividually in an oviposition cage similar to those described by Ekesi *et al* (1998). One end of ge was covered with a stretched Parafilm membrane (PM). A drop of distilled water was de d on the stretched PM and covered with another layer of stretched PM. Eggs were deposi tween the double layer of stretched PM. Thrips were fed on pollen and egg production was ded daily. Eggs were allowed to hatch by exposing the top layer of the double PM at the eye" stage of egg development and their viability was recorded during a 10 day period. longevity was recorded as the time from emergence from pupae until death of the insect. were 4 replicates of 8 female thrips per replicate.

6.1.7 Statistical analysis

The recorded percentages of mortality and egg nability were normalized through angular transformation after correcting for Abbott (192 rmula. Mortality rates, pollen consumed, fecundity, egg hatchability and longevity were sep d across stages and concentrations with the Student-Newman-Keuls (P=0.05) test, using the VA procedure of SAS. Regression analyses of mortality data were used to estimate LT_{50}s LD_{50}s for each stage using the LIFEREG procedure of SAS.

6.2 Results

6.2.1 Susceptibility of different *M. sjostedti* dev nental stages to *M. anisopliae*

In viability test, more than 87% of spores ger d. Control mortality for larvae, pupae and adults were 3, 7, and 9%, respectively. Data in T show the mortality caused by *M.*

anisopliae, strain, ICIPE 69 at different developmental stages of *M. sjostedti*. There was a significant difference ($P<0.05$) in mortality among the developmental stages at all concentrations (rejection of Null hypothesis). Larval mortality and pupal mortality were significantly lower ($P<0.05$) compared with the adult mortality. LT_{50} values at 1 x 10^8 conidia ml^{-1} and LC_{50} values were significantly shorter and lower ($P<0.05$), respectively in adult stage compared with the larval and pupal stages (Table 8). Observations were made on test insects 2-3 days post-inoculation. Fungal treated insects exhibited two unusual types of behaviour: leg twitching and abdominal arching. This behavoiur was not observed in control insects.

6.2.2 Effect of *M. anisopliae* infection on feeding by larvae and adult *M. sjostedti*

Data on daily and cummulative number of pollen consumed by *M. sjostedti* is shown in Tables 9-10. At lower concentrations (1.0 x 10^6 and 1.0 x 10^7 conidia ml^{-1}) of inoculum, there was no significant difference in daily number of pollen consumed by larval thrips in the treatments compared to the control (Table 9) (acceptance of Null hypothesis). However, at the highest concentration, significant reduction ($P<0.05$) in food consumption by larvae by about 47% was observed 3 days after treatment (DAT) and by about 76% reduction at 4 (Table 9) (rejection of Null hypothesis). The cumulative number of pollen consumed by larvae at highest concentration was also significantly different compared to the other treatments by days 3-4 (Table 9).

Daily pollen consumption by adults showed no significant difference ($P>0.05$) among treatments at 12 h after infection (Table 10). However, marked reduction in daily pollen consumption occurred 24 h after treatment (day 1), especially at the highest concentration with about 53% reduction in pollen consumption (rejection of Null hypothesis). This trend continued through days 2-7 after treatment (Table 10). In the 1.0 x 10^7 and 1.0 x 10^8 treatments, some insects ceased feeding 24-48 h before death.

Table 8. Effect of *Metarhizium anisopliae* on the developmental stages of *Megalurothrips sjostedti*

Developmental	Conidial concentration (1 x 10^x conidia ml^{-1})				LT_{50}	LC_{50}
stages	Control	10^6	10^7	10^8	(days)	(conidia ml^{-1})
Larva	3.1 ± 0.2bD	15.2 ± 1.6bC	20.3 ± 2.5cB	25.7 ± 1.8cA	9.5 ± 1.2a	1.4 x 10^9 (± 0.3)10^9a
Pupa	6.9 ± 0.4aD	20.5 ± 3.4bC	38.2 ± 1.9bB	45.6 ± 5.3bA	7.2 ± 1.3b	1.5 x 10^8 (± 0.5)10^8b
Adult	8.6 ± 0.2aD	64.1 ± 7.5aC	72.7 ± 5.8aB	100 ± 0.0aA	3.1 ± 0.4c	1.3 x 10^6 (± 0.1)10^6c

Mortality due to mycosis at 7 days post-inoculation. Means within-column followed by the same lower case letter and within-row followed by the same upper case letter are not significantly different by Student-Newman-Keuls test (P=0.05).

Table 9. Effect of *Metarhizium anisopliae* on feeding by second instar larva of *Megalurothrips sjostedti:* Mean daily and cummulative number of pollen emptied larva^{-1}

	Pollen emptied larva^{-1}				
	Days after treatment				
Treatment	12 h	1	2	3	4
Control	391.1 ± 18.3a	441.3 ± 31.1a	449.5 ± 21.3a	387.4 ± 14.1a	451.3 ± 23.7a
1 x 10^6	365.7 ± 21.3a	421.7 ± 17.8a	381.7 ± 18.7a	361.5 ± 21.3a	365.3 ± 18.7a
1 x 10^7	451.1 ± 33.4a	365.1 ± 19.2a	341.3 ± 21.3a	302.7 ± 11.5a	351.4 ± 15.5a
1 x 10^8	423.1 ± 22.7a	361.7 ± 21.4a	351.4 ± 11.3a	205.1 ± 13.7b	107.4 ± 10.5b

Table 9 continued. Effect of *Metarhizium anisopliae* on feeding by second instar larva of *Megalurothrips sjostedti:* Mean daily and cummulative number of pollen emptied larva^{-1}

	Pollen emptied larva^{-1}				
	Days after treatment				
Treatment	12 h	1	2	3	4
Cumulative					
Control	391.1 ± 18.3a	832.4 ± 21.1a	1281.9 ± 35.2a	1669.3 ± 41.3a	2120.6 ± 38.6a
1 x 10^6	365.7 ± 21.3a	787.4 ± 28.4a	1169.1 ± 29.3a	1530.6 ± 37.1a	1895.9 ± 31.3a
1 x 10^7	451.1 ± 33.4a	861.2 ± 34.1a	1157.5 ± 31.1a	1460.2 ± 34.2a	1811.6 ± 35.3a
1 x 10^8	423.1 ± 22.7a	784.8 ± 21.3a	1136.2 ± 24.6a	1341.3 ± 27.1b	1448.3 ± 21.2b

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

Table 10. Effect of *Metarhizium anisopliae* on feeding by adult *Megalurothrips sjostedti:* Mean daily and cummulative number of pollen emptied adult^{-1}

	Pollen emptied adult^{-1}							
	Days after treatment							
Treatment	12 h	1	2	3	4	5	6	7
Control	797.3 ± 21.3a	863.5 ± 19.5a	881.7 ± 25.4a	885.3 ± 11.7a	781.3 ± 23.7a	753.4 ± 27.5a	804.7 ± 27.5a	873.1 ± 32.5a
1 x 10^6	865.1 ± 30.5a	701.3 ± 10.7a	618.1 ± 21.3b	401.5 ± 18.3b	311.4 ± 16.3b	225.3 ± 10.8b	143.4 ± 11.3b	134.3 ± 12.4b
1 x 10^7	784.5 ± 18.5a	511.7 ± 14.5b	334.1 ± 15.3c	154.7 ± 11.4c	101.3 ± 11.1c	84.3 ± 9.6c	36.5 ± 4.7c	0.0 ± 0.0c
1 x 10^8	821.7 ± 25.3a	403.5 ± 10.1b	201.3 ± 11.6c	95.7 ± 8.4d	21.3 ± 4.7d	0.0 ± 0.0d	0.0 ± 0.0d	All insects dead

Table 10 continued. Effect of *Metarhizium anisopliae* on feeding by adult *Megalurothrips sjostedti:* Mean daily and cummulative number of pollen emptied adult^{-1}

	Pollen emptied adult^{-1}							
	Days after treatment							
Treatment	12 h	1	2	3	4	5	6	7
Cumulative								
Control	797.3 ± 21.3a	1660.8 ± 25.6a	2542.5 ± 30.2a	3427.8 ± 28.7a	4209.1 ± 34.2a	4962.5 ± 37.3a	5767.2 ± 35.1a	6664.3 ± 27.9a
1 x 10^6	865.1 ± 30.5a	1566.4 ± 21.3a	2184.5 ± 25.5b	2586.0 ± 31.1b	2897.4 ± 41.3b	3122.7 ± 28.1b	3266.1 ± 21.7b	3400.4 ± 34.5b
1 x 10^7	784.5 ± 18.5a	1296.2 ± 22.5b	1630.3 ± 26.7b	1785.0 ± 25.7c	1886.3 ± 19.7c	1970.6 ± 27.1c	2007.1 ± 23.1c	2007.1 ± 22.1c
1 x 10^8	821.7 ± 25.3a	1225.2 ± 26.4b	1426.5 ± 18.7c	1522.2 ± 23.1d	1543.2 ± 21.1d	1543.2 ± 21.1d	1543.2 ± 21.1d	

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test

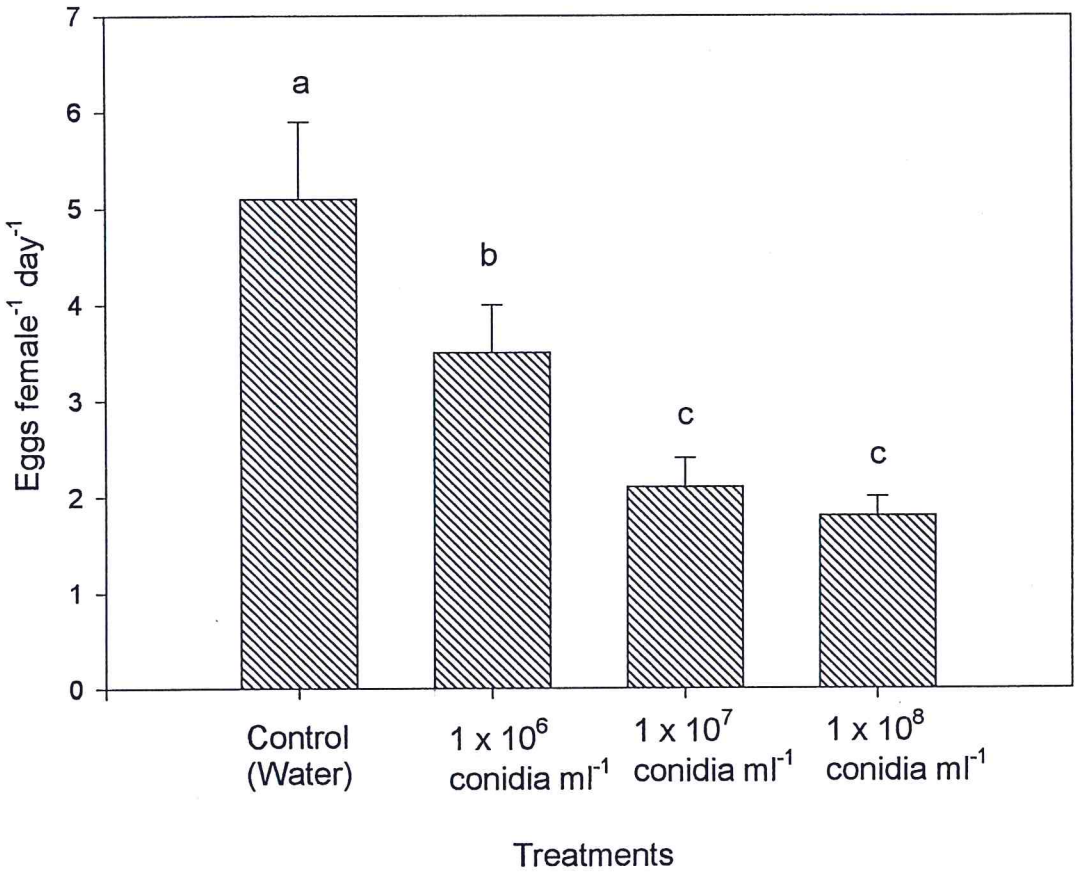

Figure 4. Daily egg production by *Megalurothrips sjostedti* surviving infection by *Metarhizium anisopliae* as larva. Bars superscripted with the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

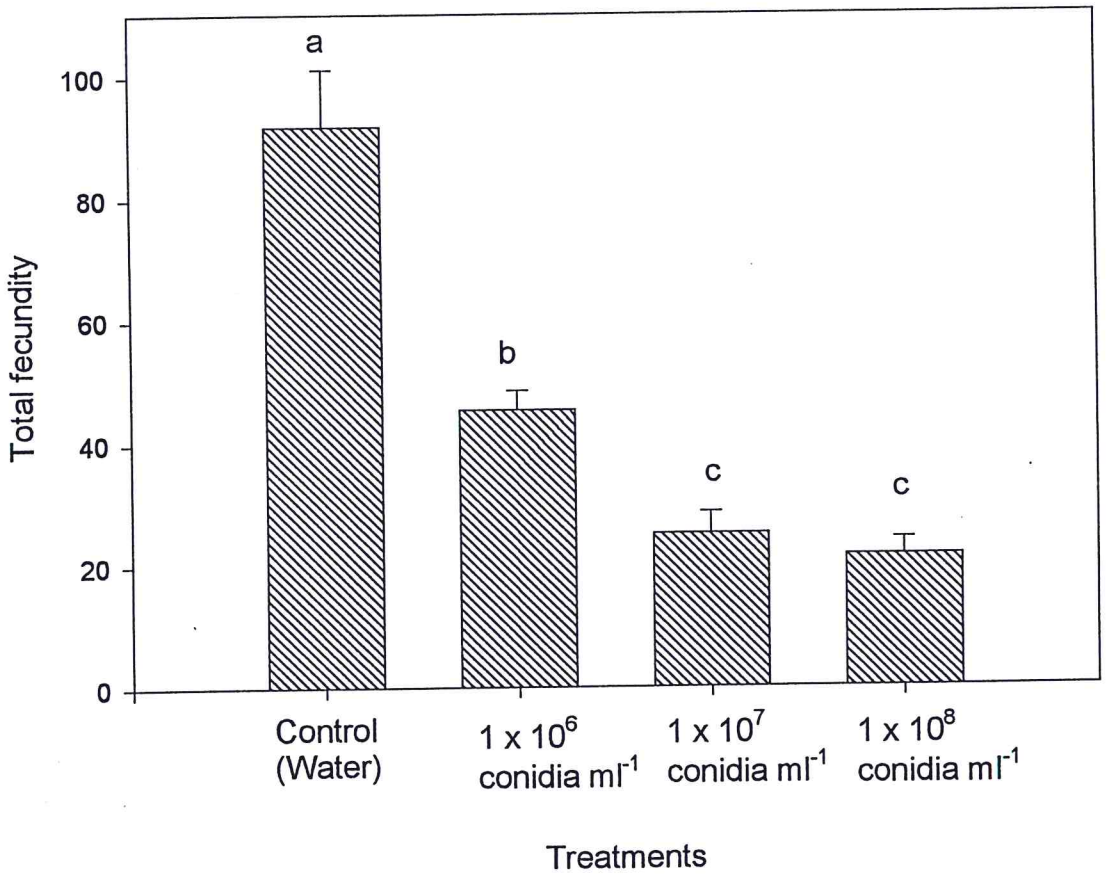

Figure 5. Total total fecundity (total number of eggs laid female^{-1}) by *Megalurothrips sjostedti* surviving infection by *Metarhizium anisopliae*as second instar larva. Bars superscripted with the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

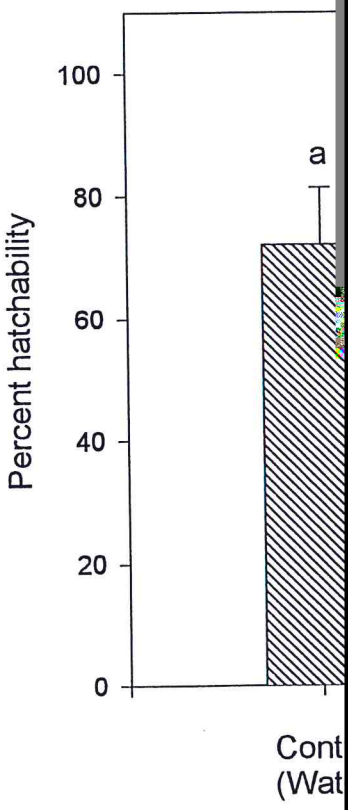

Figure 6. Egg fertility of *Me*

as second instar la

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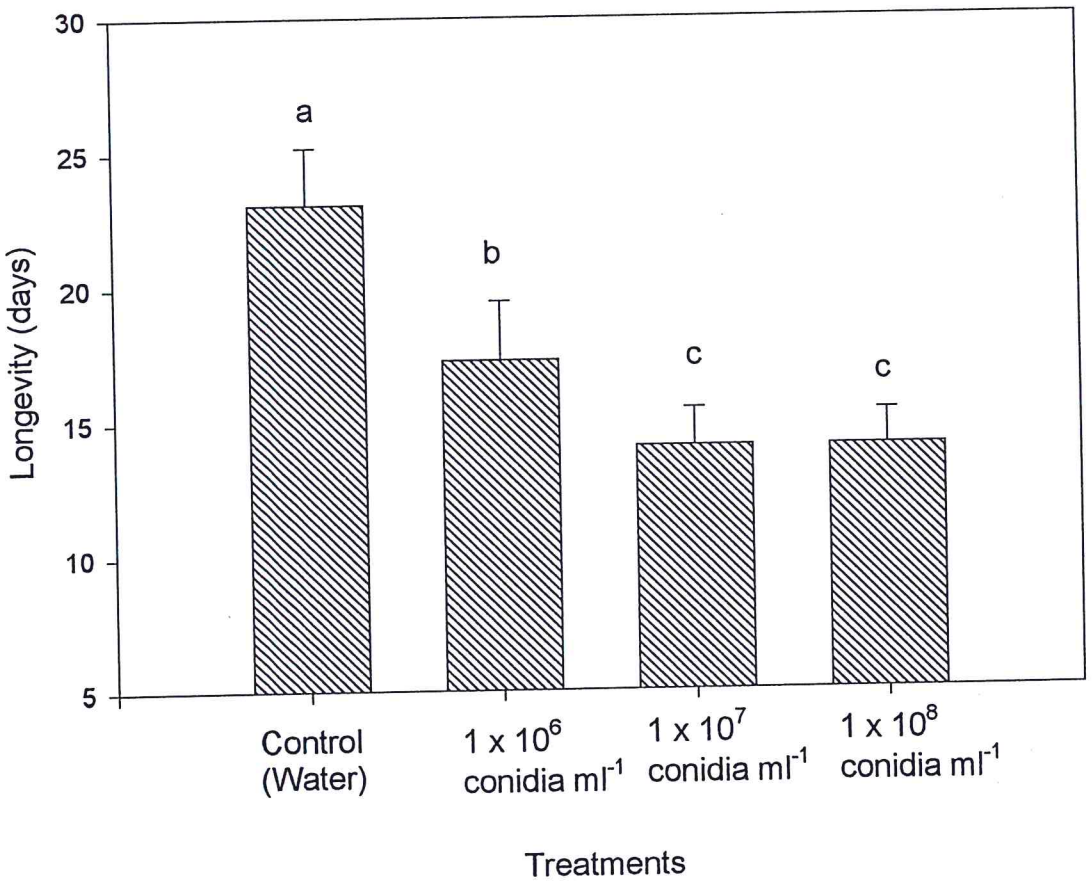


Figure 7. Longevity of adult *Megalurothrips sjostedti* surviving infection by *Metarhizium anisopliae* as second instar larva. Bars superscripted with the same letter are not significantly different by Student-Newman-Keuls (*P*=0.05) test.

When presented as accumulated number of pollen emptied adult^{-1}, the results clearly indicate a significant reduction in the total number of pollen consumed thrips^{-1} in insects treated with the fungus compared with control. At the two highest doses of conidia, pollen consumption to death at 5-6 days was less than 3 days of feeding by control insects. In the 1 x 10^6 treatment, the number of pollen emptied to termination of experiment at 7 days was equivalent to 3 days of feeding in the control treatment (Table 10).

6.2.3 Effect of *M. anisopliae* infection on fecundity, egg fertility and longevity in adult surviving infection as larvae

Adult thrips surviving larval infection produced significantly (P<0.05) fewer eggs female^{-1} day^{-1} compared to the control (rejection of Null hypothesis). Correspondingly, the total fecundity (total eggs produced female^{-1}) were significantly (P<0.05) lower in treated insects compared to the control (Fig. 5) (rejection of Null hypothesis). Egg hatchability and longevity decreased significantly (P<0.05) in adult surviving larval infection compared to the control (Figs. 6 and 7) (rejection of Null hypothesis).

6.3 Discussion

The different developmental stages of *M. sjostedti* varied in their susceptibility to infection by *M. anisopliae*. Larval and pupal stages were less susceptible to infection than the adult stage. This observation concur with those of Vestergaard *et al.* (1995) who reported that larval and pupal stages of the western flower thrips, *F. occidentalis* were more resistant to infection by *M. anisopliae* than adults. This differential susceptibility at various life stages can be ascribed to interaction between the insect integument being penetrated by the fungus and ecdysis of larval and pupal stages. Ecdysis has been reported to be important in the mechanisms of insect resistance to fungal infection, particularly when the time interval between successive ecdysis is short (Vey and

Fargues, 1977). Germinated and ungerminated conidia have been observed on the exuviae of *F. occidentalis* larvae following infection with *M. anisopliae* (Vestergaard *et al.*, 1995). In *M. sjostedti*, the development from second larval stage to prepupal stage takes between 3-5 days (Salifu, 1992; Ekesi *et al.*, 1998). This time interval is presumably too short for propagules to germinate and penetrate the cuticle, hence the low mortality observed. Developmental interval between pupal and adult stages takes about 4-6 days, and adult longevity varies from 21-30 days. During this time interval, *M. anisopliae* evidently had ample time to germinate and penetrate the cuticle of the host, thus high mortality at these stages. Germlings of *M. anisopliae* are also known to respond to physical and chemical cues (Butt, 1992) and it has been shown that some germlings will only form appresoria on rigid, hydrophobic substrate (Leger *et al.*, 1989). It therefore, appears that physiochemical cues for penetration of cuticle differ among the developmental stages. For example, the cuticle of adult *F. occidentalis* can be breached by spores of *M. anisopliae* within 36-48 h, but the fungus takes longer time to breach the cuticle of the larvae and the conidia is shed with the exuvium (Vestergaard *et al.*, 1995).

Mortality in all stages was dose-dependent with the highest mortality occurring at 1×10^8 conidia ml^{-1}. Similar dose-mortality response at different developmental stages have been reported by several authors (Feng *et al.*, 1985; Poprawski *et al.*, 1985; Fransen *et al.*, 1987). The abberrant behaviour of leg twitching and abdomenal arching observed in larval and adult stages at 3-4 days post inoculation have been reported for other thrips species (Vestergaard *et al.*, 1995). This could be attributed to toxins produced by *M. anisopliae* following successful invasion of the host (Gillespie and Claydon, 1989).

The length of time taken to kill an insect is one disadvantage of fungal biopesticides. However, Moore and Prior (1993) opined that delay in kill could be of less significance if the debilitating effect of infection can reduce an insect's capacity to harm crop. *M. sjostedti* is a pest because of the damage it causes on cowpea plant. Reduction in feeding should therefore, be of

practical importance in the management of *M. sjostedti*. In this study, adult *M. sjostedti* treated with *M. anisopliae* showed remarkable reduction in the amount of pollen consumed by the pest. This trend was however only evident in larvae at the highest dose probably due to the factors outlined earlier. A number of studies have shown that fungal pathogens could affect insect feeding rates. Studies by Moore *et al.* (1992) and Seyoum *et al.* (1994), and Thomas *et al.* (1997) have all reported significant reductions in feeding as early as 1-4 days after treatment with *Metarhizium flavoviride* (Gams and Rozypal), in the desert locust, *Schistocerca gregaria* (For.) and the variegated grasshopper, *Zonocerus variegatus* (L.), respectively. *Beauveria bassiana* infection in the Colorado potato beetle, *L. decemlineata* has been observed to cause a decrease in food consumption three days after treatment (Fargues *et al.*, 1994). Infection by *N. rileyi* can cause reduced feeding two days after treatment in *Heliothis zea* (F.) (Mohamed *et al.*, 1982), five days after treatment in *H. virescens* (F.) (Mohamed, 1982) and six days after treatment in *Plathypena scabra* (F.) (Thorvilson *et al.*, 1985). Results obtained in this study therefore agree with previous investigations. Reduction of feeding may be attributed to production of secondary metabolites and mechanical disruption of tissues by mycelial growth. Vey and Quiot (1989) suggested that secondary metabolites produced by *M. anisopliae* act on insect tissues including the midgut and this could be responsible for loss of appetite. The reduction in feeding due to infection may offset the slow speed of kill of *M. anisopliae* compared to conventional insecticides and may be important in field control.

In microbial control of insect pests, secondary effects of pathogen infection in addition to mortality on target generations may play an important role in the management of the pest (Falcon, 1985). The results of this study show that *M. anisopliae* induced a significant reduction in fecundity, egg fertility and longevity of adult *M. sjostedti* surviving infection as larvae. Pollen was the only source of food provided during the experimental period. Pollen feeding have been shown to increase growth rate and decrease mortality of various species of thrips (Kirk, 1997a). Egg

production in insects is affected by the amount of protein in an insect diet (Engelman, 1984) and pollen, which is high in protein (Mound *et al.*, 1980) is an important requirement of egg production in thrips (Trichilo and Leigh, 1988; Teulon and Penman, 1991; Kirk, 1997b). The results obtained, therefore, indicate true differences in egg production as affected by fungal infection. Sikura *et al.* (1972) reported that *B. bassiana* in adult Colorado potato beetle surviving infection as larvae, induce histological and cytological injuries to ovaries thus preventing follicle development or causing their degeneration, thereby reducing fecundity. Fargues *et al.* (1991) showed that *B. bassiana* infection of larval Colorado potato beetle markedly reduced fecundity of adult survivors at 22^{0}C but fecundity of survivors was not affected by fungal infection at 25^{0}C. N'Doye (1976) also reported that egg fertility of *Chilo supressalis* Walker adults surviving infection as larvae by *B. bassiana* was reduced. Finally, Nnakumusana (1985) showed that *Apergillus* infections significantly reduced the longevity, fecundity and egg fertility of different species of mosquitoes dependent on the larval instar that was infected.

In conclusion, this study has shown that *M. anisopliae*, strain ICIPE 69, is most effective against adult *M. sjostedti*. Good control achieved at this stage is encouraging for field extrapolation of the results. The adult stage is probably the most damaging stage of *M. sjostedti* because of its high longevity and feeding rate. Reduction in adult population could mean a reduction in the rate of population build up of the insect as fewer progeny is produced and possibly less number of generations per season. Although, *M. anisopliae* caused low mortality in larvae of *M. sjostedti*, the ability of the fungus to reduce fecundity, egg fertility and longevity in adults surviving infection as larvae also suggest that significant reduction in thrips population in the next generation can also be achieved under field condition. The rapid reduction in feeding at the adult stage observed in this study also show that the pest status of infected *M. sjostedti* can be markedly reduced 24 h after treatment with the fungus even though mortality may not be observed until two days after

treatment. Field testing of *M. anisopliae*, strain ICIPE 69, will ultimately determine its place in *M. sjostedti* management programme.

CHAPTER SEVEN

Susceptibility of *Megalurothrips sjostedti* to *Metarhizium anisopliae* on different varieties of cowpea

7 Introduction

Biological control including the use of entomopathogenic fungi is an attractive integrated pest management (IPM) component because it helps to reduce the use of chemical pesticides (Bull, 1982). The use of resistant varieties is another important component of IPM strategy, which is generally assumed to be compatible with other control measures (Duffey & Bloem, 1986). In previous studies, we demonstrated the pathogenicity of several strains of entomopathogenic fungi to *M. sjostedti* and selected *M. anisopliae* strain ICIPE 69, as the most pathogenic to *M. sjostedti* at a broad temperature range of 15-30^{0}C (Ekesi *et al.*, 1998).

A number of cowpea varieties with known level of resistance to *M. sjostedti* have been developed by International Centre of Insect Physiology and Ecology (ICIPE) and International Institute of Tropical Agriculture (IITA) (Pathak & Olela, 1986; Jackai & Adalla, 1997). Currently, little is known on the compatibility of host plant resistance with other *M. sjostedti* management options for cowpea (Jackai & Adalla, 1997). However, studies in other systems show that the use of host plant resistance could be compatible with the use of biological control agents (Bong *et al.*, 1991; Hare, 1992; Meade & Hare, 1994) although exceptions to this observations do exist (Felton *et al.*, 1987). Beside other abiotic factors, the efficacy of both entomopathogenic fungi and host plant resistance is also affected by temperature (Benz, 1987; Smith, 1989). In this study, the susceptibility of *M. sjostedti* to *M. anisopliae* when reared on a susceptible, tolerant and moderately resistant varieties of cowpea at different temperatures was examined, with the aim of determining the compatibility of the fungus with host plant resistance for *M. sjostedti* management. In this study, the hypothesis that

entomopathogenic fungus caused equal level of mortality in adult *M. sjostedti* on a susceptible, tolerant and resistant varieties of cowpea will be rejected if analysis of variance and/or mean separation show significant difference in mortality among the varieties.

7.1 Material and methods

7.1.1 Plants

The plants used in the experiments were grown in the field and utilized at flowering. The cowpea varieties were ICV 2, ICV 7 and ICV 8 and they are known to be susceptible, tolerant and moderately resistant, respectively, to *M. sjostedti* (Pathak & Olela, 1986; Ekesi *et al.*, 1998).

7.1.2 Insect culture

The insects used originated from the same colony as described in chapter three. Three-day old adults were used for the various experiments.

7.1.3 Fungus

The fungus used was *M. anisopliae*, strain ICIPE 69, and came from the ICIPE culture collection. The fungus was maintained on Sabouraud dextrose agar (SDA) in petri dishes at 26 $\pm$ 2^{0}C. Conidia were harvested from three weeks old sporulating cultures by scraping. Inocula were suspended in 20 ml sterile distilled water containing 0.05% Triton X-100 in 25 ml glass bottles containing glass beads (3 mm). The bottles were vortexed for 5 min to produce a homogenous conidial suspension. Spore suspensions were then adjusted to different concentrations using the Malassez-cell-counting-chamber. Viability of the conidia were determined by spread-plating 0.1 ml of 3 x 10^6 conidia ml^{-1} onto 6 SDA plates. Sterile

microscope cover slips were then placed on the plates and incubated in the dark at 26 ± 2^{0}C. Percentage germination was examined after 24 h from 100-spore counts on each plate.

7.1.4 Bioassay procedures

7.1.4.1 Inoculation of insect and substrate

Two sets of bioassays were conducted. The first one consisted of direct spray of insects with conidia and the second one consisted of spraying floral tissues and exposing the insects to the treated substrate. In both bioassays, 10 ml of the following concentrations: 1.0 x 10^5, 1.0 x 10^6 and 1.0 x 10^7 conidia ml^{-1} were sprayed onto insects or floral tissues of each of the three varieties using a Burgerjon's (1956) spray tower. Controls consisted of insects or floral tissues treated with sterile distilled water containing 0.05% Triton X-100. Treated insects were then returned to rearing cages containing surface sterilized floral tissues and pollen of each of the three varieties. In the second bioassay, insects were exposed to treated floral tissues of the different varieties which also served as a source of food for 24 h. After this time period, the treated plant materials were removed and replaced by surface sterilized floral tissues and pollen of the different varieties daily. In both bioassays, 3 replicates of 20 adult insects were used for each spore concentration on each plant variety. Each treatment combination was incubated at 15, 20, 25 and 30^{0}C for each bioassay. Mortality was recorded daily for 14 days. Dead insects were placed on petri dishes lined with moistened filter paper. Mortality due to the fungus was confirmed by microscopic examination of hyphae and spores on the surface of the insect.

7.1.4.2 Experiment on antifungal effect of cowpea varieties

The mortality of insects feeding on the tolerant variety was found to be reduced in both bioassays. An attempt was therefore made to investigate the effect of airborne volatiles and crude extracts of the three varieties on the viability and growth of the fungus. Seeds of the three varieties of cowpea were sown in 0.85 litre plastic pots filled with soil. Planting was scheduled in such a way that flowering of all varieties occurred simultaneously. Three sets of experiments were carried out.

In the first experiment, 5 flowers were excised from each of the 3 varieties and arranged at equal distance in a plastic petri dish (90 cm). Conidial suspension was prepared as described earlier, and 10 ml of the suspension (titrated to 1.0×10^8 conidia ml^{-1}) was dispensed onto the flowers through the Burgerjon spray tower. The viability of the spores on the flowers was tested after 12 h following exposure to the flowers. In control the treatment, spores were not exposed to flowers. Treated flowers were suspended in 10 ml of sterile distilled water in 25 ml glass bottles containing glass beads and vortexed for 5 min to dislodge conidia from the flowers. Germination test followed the protocol described earlier. The experiment was replicated six times.

In the second experiment, the effect of airborne volatiles of the three varieties on the germination of the fungus was tested. A glass chamber apparatus and a trap designed for trapping airborne volatiles (Lwande *et al.*, 1989) (Fig. 8) was used for this experiment.

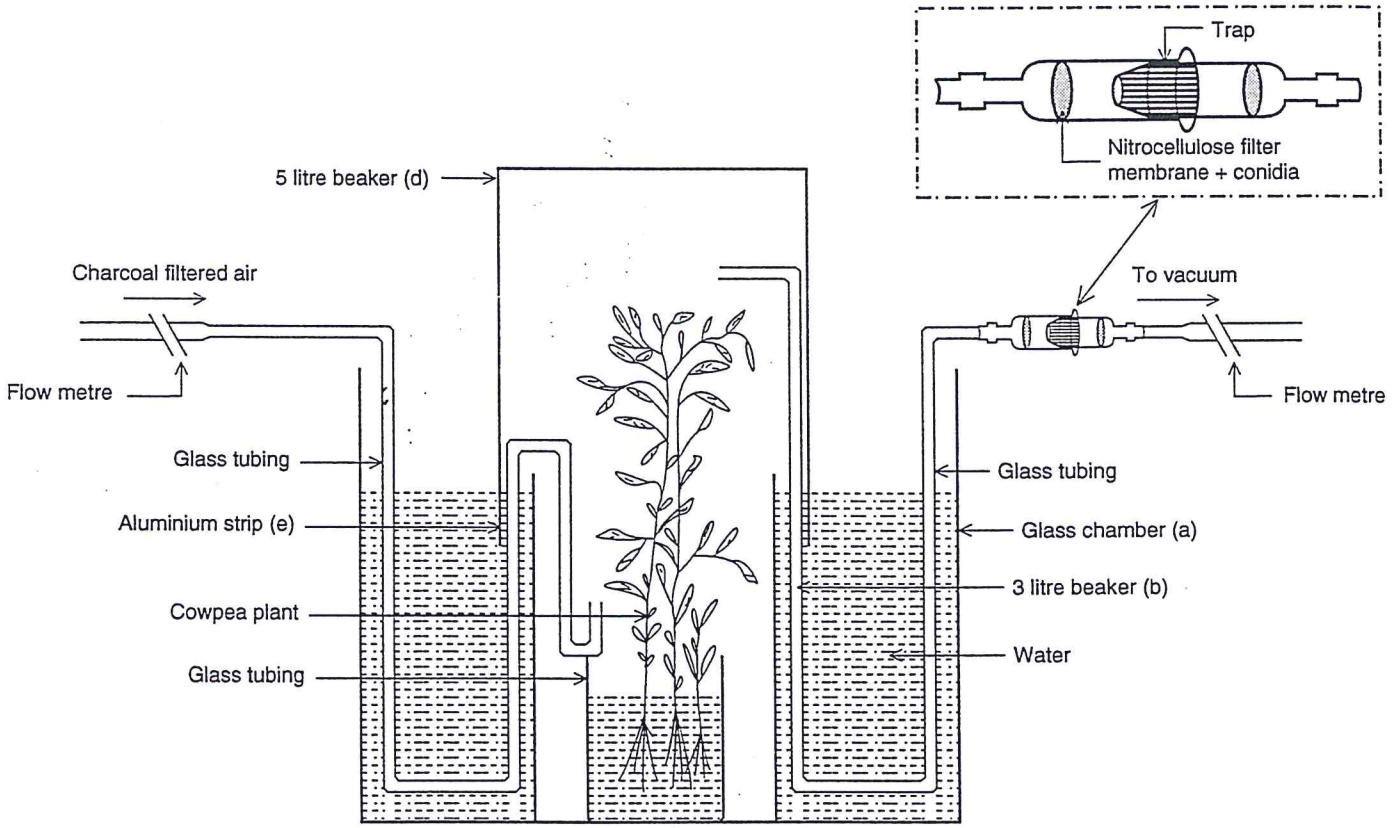


Figure 8. Schematic diagram of glass chamber used for passing airborne volatiles of cowpea through spores of *Metarhizium anisopliae.*

Conidial suspension was prepared as described earlier and titrated to 2.0 x 10^6 conidia ml^{-1}. The spores were retained on a nitrocellulose filter membrane (diam. 47 mm, pore size 0.45 µm, Sigma Chemicals, Poole, UK) by pouring the suspension (10 ml) through a filter holder unit under aspirator vacuum (Maniania, 1994). The filter paper containing the conidia was then transferred to a petri dish and allowed to dry for 15 min in a laminar flow cabinet. After drying, two filter membranes were placed inside the glass trap. Four plants (at 50% flowering) were uprooted from each of the varieties and immediately immersed in a beaker containing tap water, and transferred into the chamber. Charcoal filtered air was drawn over the cowpea plant in the glass chamber and subsequently through the trap carrying the filter papers with conidia. Air was sucked through the trap using a vacuum pump at a flow rate of 80 ml m^{-1}. In the controls, charcoal filtered air was passed through the system without cowpea plants. The experiment was allowed to run for 12 h after which the system was dismantled. The filter papers were removed from the trap and resuspended in 10 ml sterile distilled water containing 0.05% Triton X-100 and vortexed for 5 min to removed the conidia from the membrane. Spores were then tested for germination and colony forming unit (CFU). Germination tests were carried out as described earlier. CFU was tested by spread plating 0.1 ml of conidia (titrated to 1.0 x 10^4 conidia ml^{-1}) on SDA medium. Plates were incubated in the dark at 26 ± 2^0C for 4 days after which the number of CFU was recorded. The experiments were replicated as above.

In the third experiment, crude extracts of the three varieties were tested for their effect on germination and growth of the fungus. Sequential extraction of whole cowpea plants at 50% flowering was done by immersion in hexane, followed by chloroform and then methanol for 3 days at room temperature. The crude extract was then filtered through Whatman No.1 filter paper (Whatman, Kent, UK) under aspirator vacuum (Hoefer Scientific Instruments, San

Francisco, USA) and concentrated to complete dryness at 40^{0}C in a rotary evaporator (Brinkmann Instruments, Damstadt, Germany). The extracts were then stored in a refrigerator at 4^{0}C until needed. The chloroform and methanolic extracts showed no antifungal activity and were discarded. The hexane extract was dissolved in 0.05% Silwet L-77® (Loveland Industries, Greeley, USA) to obtain concentrations equal to 50, 100, 150, 200 and 250 parts per million (ppm); which are within the range of allelochemicals reported for cowpea (Carnovale *et al.*, 1990; Lattanzio *et al.*, 1990; Lattanzio *et al.*, 1992). Each concentration was vortexed in 30 ml glass bottles containing glass beads (5 mm) for at least 10 minutes to ensure that the extract disolved completely. 0.5 ml of conidial suspension titrated to 1.0 x 10^7 conidia ml^{-1} was spread-plated on SDA plates. This was left to stand for 1 h after which one sterile filter paper disk (6 mm diam. Whatman No. 1) was dipped into the prepared extracts and placed in the center of the plate. In the controls, filter paper disks were dipped in sterile distilled water containing 0.05% Silwet. The plates were held at 26 ± 2^{0}C in complete darkness for five days, after which the diameters of the zone of inhibition of fungal growth were measured. The experiments were replicated six times.

7.1.5 Statistical analysis

Percentage mortality was submitted to Abbott (1925) formula to correct for natural mortality and then transformed by arcsin to normalize the variance before analysis. Treatment effect were measured at each concentration through comparison of mean percentage mortality on the three varieties at different temperatures by Student-Newman-Keuls (P=0.05) test using the ANOVA procedure of SAS (SAS Institute, 1989). A three-way ANOVA was also applied on mortality data to assess the interaction between temperature/host plant/fungal concentration. Regression analyses were used to estimate LT_{50} (for the most effective conidial concentration) and LC_{50} on different plant variety at different temperatures using the LIFEREG procedure of

SAS. LT_{50} LC_{50}, germination and growth data were subjected to ANOVA for a completely randomized design and means were separated by SNK.

7.2 Results

7.2.1 Effect of cowpea varieties and temperature on the susceptibility of *M. sjostedti* to *M. anisopliae*

In viability test, conidial germination was more than 93%. In the bioassays where insects were directly treated, control mortalities at each temperature were: 15^{0}C (18%, 1%, and 5%), 20^{0}C (20%, 0%, and 4%), 25^{0}C (21%, 4%, and 6%), 30^{0}C (25%, 15%, and 12%) on the moderately resistant, tolerant and susceptible varieties, respectively. In the bioassays where floral tissues were treated and insects were exposed for 24 h to the treated substrate, the moderately resistant, tolerant and susceptible varieties incurred the following respective mortalities: 15^{0}C (16%, 3%, and 3%), 20^{0}C (22%, 4% and 7%), 25^{0}C (20%, 5%, and 4%) and 30^{0}C (24%, 6%, and 5%).

In both bioassays, mortality was significantly higher (P<0.05) on the moderately resistant variety at all concentrations of inoculum and at all temperatures compared to the other two varieties (Tables 11 and 12) (rejection of Null hypothesis). Mortality generally increased with increasing concentrations and temperatures on the resistant and susceptible varieties. Mortality on the tolerant variety was significantly lower (P<0.05) at all concentrations and at all temperatures tested in both experiments (Tables 11 and 12). There was no consistent dose level differences on the tolerant variety. There was a significant temperature x inoculum concentration and variety x inoculum concentration interaction in the two experiments (Table 13). No other interactions among the three factors were significant.

There was a significant difference (P<0.05) in LT_{50} values among the varieties in the first bioassay where insects were exposed to direct spray of the fungus with the resistant

variety having the shortest lethal time at all temperatures (Table 14). Similar differences ($P<0.05$) was observed among the varieties at all temperatures in the bioassay in which floral tissues were treated and insects exposed to the substrate for 24 h (Table 14). LC_{50}s were significantly lower ($P<0.05$) on the moderately resistant variety compared to the other two varieties in both experiments (Table 15). LT_{50} and LC_{50} values generally decreased with increasing temperatures on all varieties, except on the tolerant variety. The tolerant variety had the longest LT_{50} and the highest LC_{50} values in both bioassays (Tables 14 and 15).

7.2.2 Antifungal effect of cowpea on *M. anisopliae*

Exposure of conidia of *M. anisopliae,* strain ICIPE 69,to flowers of the tolerant variety caused significant reduction ($P<0.05$) in germination and CFU compared to the other varieties, respectively (Figures 9 and 10). Germination of conidia exposed to volatiles from the three cowpea varieties were significantly lower ($P<0.05$) in the tolerant variety compared to the other varieties (Figure 9). Similarly, colony forming units were greatly reduced when spores were exposed to volatiles of the tolerant variety (Figure 10).

Hexane crude extract of the tolerant variety inhibited growth of *M. anisopliae* at concentrations of 100-250 ppm. Inhibition was higher at concentrations of 200 and 250 ppm 5 days following treatment (Table 16). Crude extract of the susceptible and moderately resistant varieties had no effect on the growth of *M. anisopliae,* strain ICIPE 69.

Table 11. Mortality of adult *Megalurothrips sjostedti* (% mortality ± SE) directly treated with different concentrations of *Metarhizium anisopliae* on three genotypes of cowpea under four temperature regimes

		% mortality ± S.E.			
		Temperature			
Concentration (conidia ml^{-1})	Cowpea variety	15°C	20°C	25°C	30°C
1×10^5	Resistant	46.7 ± 6.0a	45.0 ± 5.8a	46.7 ± 3.3a	60.1 ± 5.8a
	Tolerant	6.1 ± 2.9c	11.7 ± 7.4c	17.0 ± 2.7c	13.1 ± 4.2c
	Susceptible	18.3 ± 1.7b	28.7 ± 7.4b	33.4 ± 8.7b	38.4 ± 7.2b
1×10^6	Resistant	58.3 ± 10.7a	66.7 ± 11.7a	70.2 ± 15.8a	78.3 ± 16.1a
	Tolerant	11.2 ± 5.6c	10.3 ± 3.3c	23.1 ± 3.8c	27.3 ± 8.4c
	Susceptible	28.3 ± 1.9b	35.0 ± 2.8b	48.3 ± 16.7b	63.3 ± 9.6b
1×10^7	Resistant	65.0 ± 2.9a	75.0 ± 12.3a	95.4 ± 12.8a	100.0 ± 0.0a
	Tolerant	18.3 ± 5.0c	13.3 ± 9.7c	20.5 ± 11.7c	15.7 ± 4.4c
	Susceptible	45.0 ± 2.9b	50.1 ± 12.1b	63.3 ± 11.4b	71.7 ± 10.3b

Means were angularly transformed before analysis but values represent detransformed means. For each concentration, means within a column bearing the same letters are not significantly different by Student-Newman-Keuls (P=0.05) test.

Table 12. Mortality of adult *Megalurothrips sjostedti* (% mortality ± SE) exposed to floral tissues treated with different concentrations of *Metarhizium anisopliae* on three genotypes of cowpea under four temperature regimes

		% mortality ± S.E.			
		Temperature			
Concentration (conidia ml^{-1})	Cowpea variety	15^0C	20^0C	25^0C	30^0C
1×10^5	Resistant	38.2 ± 5.4a	42.1 ± 7.8a	44.3 ± 8.4a	53.5 ± 5.4a
	Tolerant	4.4 ± 1.2c	8.3 ± 1.4c	9.7 ± 3.1c	6.2 ± 2.7c
	Susceptible	15.1 ± 2.3b	20.1 ± 7.6b	29.7 ± 5.6b	33.3 ± 6.4b
1×10^6	Resistant	51.6 ± 10.7a	60.3 ± 12.1a	61.4 ± 12.1a	70.1 ± 10.3a
	Tolerant	10.1 ± 6.3c	7.7 ± 1.8c	19.3 ± 6.1c	13.4 ± 3.7c
	Susceptible	21.7 ± 7.4b	30.4 ± 3.6b	41.4 ± 8.6b	58.7 ± 9.4b
1×10^7	Resistant	60.3 ± 5.5a	71.6 ± 16.8a	83.3 ± 17.4a	89.5 ± 14.1a
	Tolerant	15.1 ± 3.7c	14.4 ± 6.3c	16.5 ± 7.3c	13.6 ± 5.7c
	Susceptible	34.6 ± 7.6b	47.1 ± 8.5b	53.4 ± 10.1b	65.8 ± 9.4b

Means were angularly transformed before analysis but values represent detransformed means. For each concentration, means within a column bearing the same letters are not significantly different by Student-Newman-Keuls (P=0.05) test.

Table 13. Analysis of variance tables for interaction in mortality in *Megalurothrips sjostedti* between temperature, variety and *Metarhizium anisopliae*[a]

Factor	Insect inoculation			Floral tissues inoculation		
	F	DF	P	F	DF	P
Temperature	79.33	3,96	0.0001	61.45	3,96	0.0003
Variety	13.90	2,96	0.0001	10.81	2,96	0.0001
M. anisopliae	126.51	3,96	0.0001	104.51	3,96	0.0001
Temperature * Variety	0.86	6,96	0.5306	0.92	6,96	0.7412
Temperature * *M. anisopliae*	11.05	9,96	0.0011	8.74	9,96	0.0001
Variety * *M. anisopliae*	2.15	6,96	0.0041	3.41	6,96	0.0052
Temperature * Variety * *M. anisopliae*	0.24	18,96	0.6300	0.31	18,96	0.5407

[a]Means were angularly transformed before analysis

Table 14. Effect of cowpea varieties and temperature on the susceptibility of adult *Megalurothrips sjostedti* to *Metarhizium anisopliae* (LT_{50} ± S.E) at concentration of 1 x 10^7 conidia ml^{-1}

Cowpea variety	Temperature 15^{0}C	20^{0}C	25^{0}C	30^{0}C
Insect inoculation				
Resistant	9.2 ± 2.2c	8.1 ± 1.7c	3.0 ± 0.7c	3.0 ± 0.4c
Tolerant	22.5 ± 3.3a	23.3 ± 5.7a	19.3 ± 7.2a	18.0 ± 4.3a
Susceptible	15.2 ± 4.3b	11.6 ± 2.3b	6.1 ± 1.6b	4.5 ± 0.5b
Floral tissue inoculation				
Resistant	9.7 ± 2.3c	8.4 ± 1.5c	3.2 ± 0.7c	3.3 ± 0.5c
Tolerant	23.8 ± 3.1a	23.7 ± 5.1a	19.9 ± 7.1a	18.7 ± 4.3a
Susceptible	16.3 ± 4.2b	12.5 ± 2.4b	6.7 ± 1.4b	5.1 ± 0.6b

LT_{50} values within column followed by the same letter do not differ significantly by Student-Newman-Keuls (P=0.05) test.

Table 15. LC_{50} ± S.E of *Metarhizium anisopliae* applied on adult *Megalurothrips sjostedti* on cowpea varieties at four different temperature regimes

Cowpea variety	Temperature 15°C	20°C	25°C	30°C
Insect inoculation				
Resistant	$1.8 \times 10^5 \pm 1.8 \times 10^4$c	$1.5 \times 10^5 \pm 9.0 \times 10^4$c	$1.1 \times 10^5 \pm 0.5 \times 10^5$c	$6.1 \times 10^4 \pm 0.1 \times 10^4$c
Tolerant	$4.5 \times 10^{10} \pm 7.1 \times 10^9$a	$1.7 \times 10^{10} \pm 0.8 \times 10^{10}$a	$3.2 \times 10^9 \pm 9.5 \times 10^8$a	$1.6 \times 10^9 \pm 1.4 \times 10^9$a
Susceptible	$2.0 \times 10^8 \pm 1.1 \times 10^8$b	$1.1 \times 10^7 \pm 8.2 \times 10^6$b	$2.0 \times 10^6 \pm 0.3 \times 10^6$b	$6.8 \times 10^5 \pm 1.4 \times 10^4$b
Floral tissue inoculation				
Resistant	$2.0 \times 10^5 \pm 6.5 \times 10^4$c	$1.7 \times 10^5 \pm 7.9 \times 10^4$c	$1.3 \times 10^5 \pm 8.7 \times 10^4$c	$6.8 \times 10^4 \pm 0.7 \times 10^4$c
Tolerant	$4.8 \times 10^{10} \pm 8.7 \times 10^9$a	$1.8 \times 10^{10} \pm 6.4 \times 10^9$a	$3.2 \times 10^9 \pm 6.4 \times 10^8$a	$1.7 \times 10^9 \pm 8.6 \times 10^8$a
Susceptible	$2.3 \times 10^8 \pm 8.4 \times 10^7$b	$1.3 \times 10^7 \pm 8.1 \times 10^6$b	$2.3 \times 10^6 \pm 0.1 \times 10^6$b	$7.7 \times 10^5 \pm 0.2 \times 10^5$b

For each bioassay, LC_{50} values within column followed by the same letter are not significantly different by Student-Newman-Keuls' (P=0.05) test.

Table 16. *In vitro* effect of hexane crude extract of cowpea variety tolerant to *Megalurothrips sjostedti on* development of *Metarhizium anisopliae*.

Concentration (ppm)	Diameters (mm) of zones inhibited
Control	0.0 ± 0.0d
50	0.0 ± 0.0d
100	13.2 ± 0.7c
150	18.7 ± 0.9b
200	20.5 ± 1.6a
250	22.1 ± 1.8a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

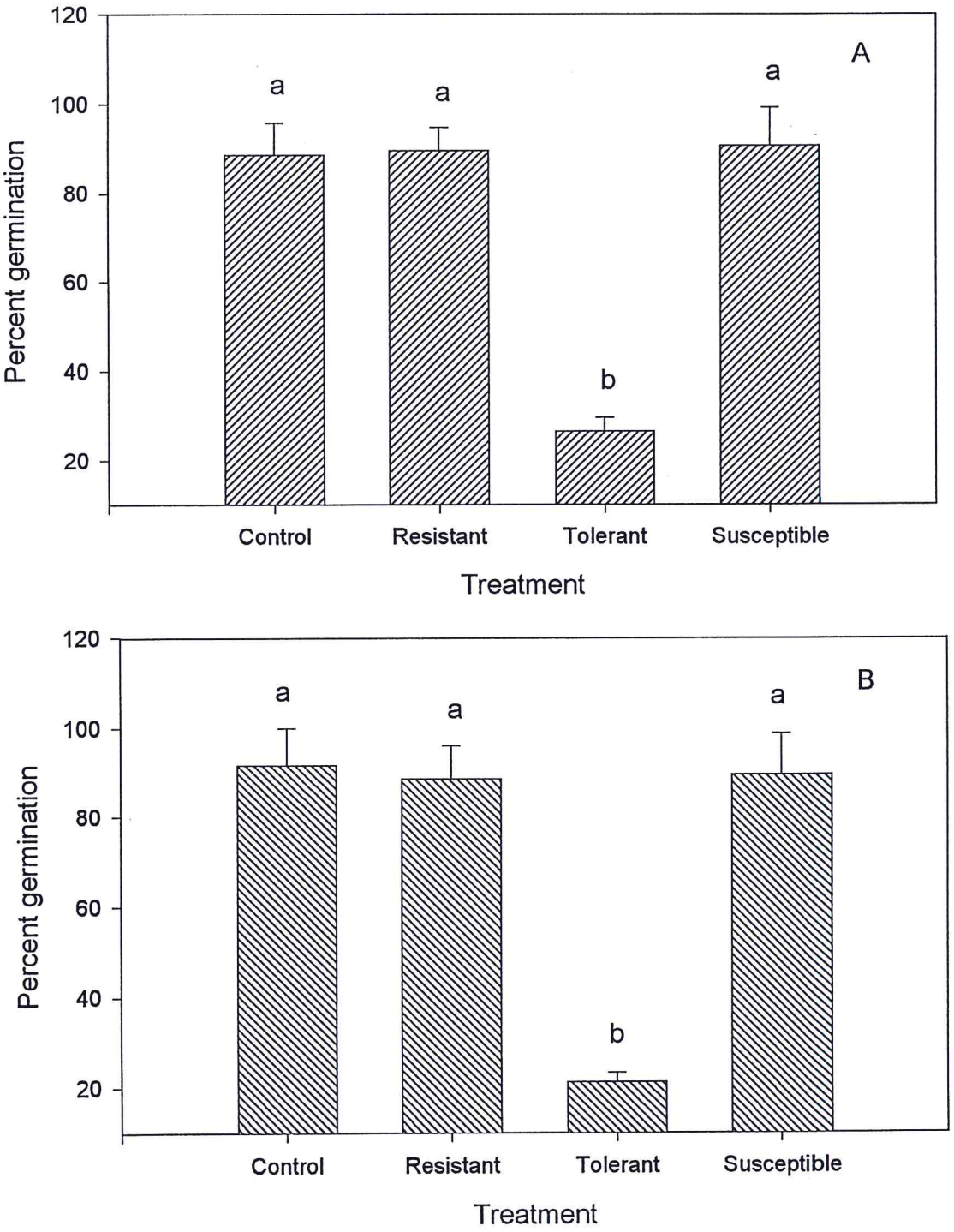


Figure 9. Germination of *Metarhizium anisopliae* exposed to volatiles (A) and directly on flowers (B) of three varieties of cowpea. Bars superscripted with the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

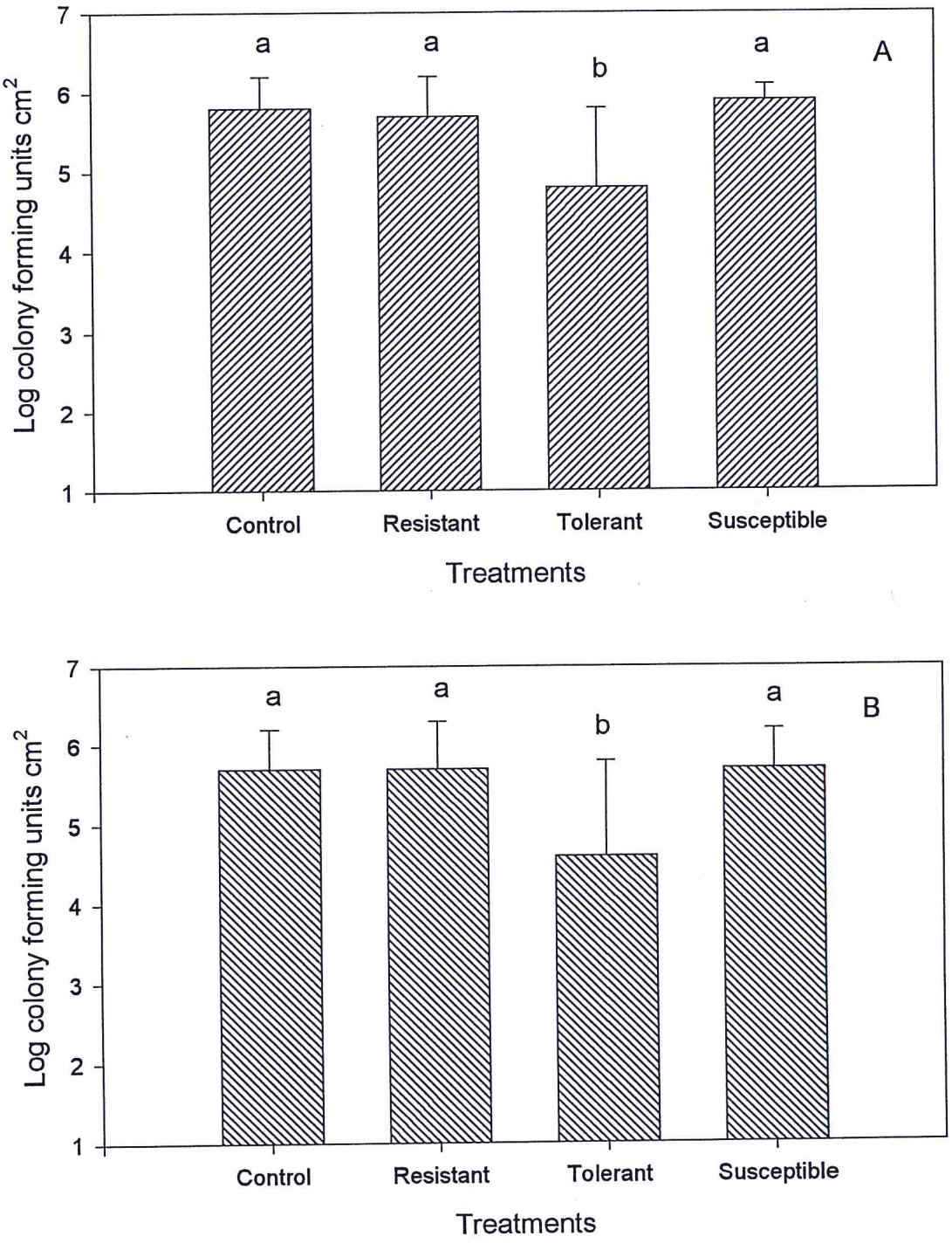


Figure 10. Colony forming units of *Metarhizium anisopliae* exposed to volatiles (A) and directly on flowers (B) of three cowpea varieties. Bars superscripted with the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

7.3 Discussion

The results of this study show that cowpea varieties could affect the susceptibility of *M. sjostedti* to *M. anisopliae*. Mortality of the legume flower thrips was higher on the moderately resistant variety at all concentrations of inoculum and at all temperatures compared to the other varieties. The level of significance obtained for the Variety/*M. anisopliae* concentration interaction further indicates that treatment effects of the fungus differed among the three cowpea varieties. Lethal time and lethal concentration values on the resistant variety were shorter and lower, respectively, compared to the susceptible and tolerant varieties. These results suggest a faster kill of thrips on the resistant variety with a low concentration of inoculum compared to the tolerant and susceptible varieties. Cowpea varieties that are resistant to *M. sjostedti* have been found to affect this insect by extending their developmental period, and reducing their body weights and reproductive potentials (Ekesi *et al.*, 1998). These effects could have increased the susceptibility of *M. sjostedti* to the fungal pathogen due to the physiological and metabolic stress imposed on them as a result of feeding upon a suboptimal host (Butt and Brownbridge, 1997). Hare and Andreadis (1983) reported that larvae of the Colorado potato beetle, *L. decemlineata* were more susceptible to *B. bassiana* when reared on their less suitable solanaceous hosts. Olieviera (1981 cited by van Emden, 1987) also found that the effect of *Nomuraea rileyi* (Farlow) Samson on *Anticarsia gemmatalis* Hübner was enhanced when larvae were reared on a resistant soybean genotype. The results of this study therefore, agree with these previous studies and suggest that the use of resistant variety and *M. anisopliae* may be a compatible IPM strategy for the control of *M. sjostedti*.

Temperatures of 15 and 20^{0}C caused significant decrease in development of fungal infections but did not significantly affect percentage mortality caused by fungus on the

susceptible and resistant varieties at all concentrations of inoculum. Most authors agree that incubation period of most fungal diseases in insects is temperature dependent (Ferron, 1978; Carruthers & Sopers, 1987). Mortality was however, higher at 25 and 30^{0}C compared to the lower temperatures and this is evidenced in the significant temperature x *M. anisopliae* interaction.

Generally very few data are available on the infectivity of conidia of entomopathogenic fungi on plant surfaces and some authors opined that spores adhere strongly to leaves and are not sufficiently picked up by insects (Gillespie and Jimenez, 1990). In this study, however, high mortality in *M. sjostedti* was observed as a result of uptake of conidia from floral tissues. Secondary uptake of spores from vegetation has been reported to be an important factor in the control of grasshoppers with entomopathogenic fungi (Moore and Caudwell, 1997). Thomas *et al.* (1998) have shown that acquisition of spores of *M. flavoviride* from vegetation had a significant effect on mortality of the rice grasshopper and a greater single effect on mortality than direct contact with spores under field conditions. The combination of direct contact of conidia on insects during spray application and uptake of spores from vegetation are likely to be vital components of good control of *M. sjostedti* under field conditions.

Interestingly, the mortality observed in *M. sjostedti* reared on the tolerant variety compared to the other two varieties was much lower. This observation prompted further studies to investigate the possible existence of antifungal substances in the tolerant variety. Germination and colony forming units from treated floral tissues differed significantly among the three varieties indicating that leaf surface chemistry of the tolerant variety affected the viability of the fungus. The effects of airborne volatiles and the hexane crude extract of the tolerant variety on fungal viability clearly indicate the existence of antifungal substances in the tolerant variety. Lwande *et al.* (1989) recorded the presence of 11 volatile compounds mostly mono- and sesqui-terpenes in the headspace collection of the cowpea variety, VITA 1. Some

authors have reported that sesqui-terpenoids posses antifungal properties (Richardson *et al.*, 1989; Pare *et al.* 1993). Vast majority of legumes including cowpea also produce antimicrobial phenolic substances generally referred to as phytoalexins (Allen, 1983). These compounds are known to be involved in multi-faceted disruption of fungal metabolisms resulting in the suppression of growth (Smith & Ingham, 1980; Smith, 1982). Vega *et al.* (1997) has also shown that phenolic compounds can reduce germination of blastospores of *Paecilomyces fumosoroseus* (Wize) Brown & Smith. Crude extracts of black gram, *Vigna mungo* (L.) Hepper which is closely related to cowpea can inhibit spore germination and growth of some phytopathogenic fungi (Roy & Sharma, 1982). Pathak and Olela (1986) noted that the tolerant variety used in this experiment is also tolerant to most plant diseases. These results reinforces this observation and suggest that antimicrobial factors affording protection to this cowpea variety against plant pathogens can also directly or indirectly affect the spores of entomopathogenic fungi either on the plant surfaces or be transferred to the host insect as it feeds on the plant. Ramoska and Todd (1985) have observed similar mechanism of fungal suppression in chinch bug reared on sorghum. Significant variability in mortality caused by *M. anisopliae* in *Chilo partellus* (Swinhoe) reared on different varieties of sorghum have also been reported in the laboratory (Maniania *et al.*, 1998).

This study has revealed both positive and negative effects in a relationship involving entomopathogenic fungus, insect and host plant genotype. These results have shown that *M. sjostedti* are more susceptible to infection by *M. anisopliae* when reared on a moderately resistant variety (ICV 8), thus supporting the general hypothesis that the susceptibility of insects to entomopathogens increases as host plant suitability decreases (Hare, 1992). On the other hand, it also revealed that the fungus might not be compatible with all varieties of cowpea as observed with the tolerant variety (ICV 7). The precise mechanisms surrounding these two effects are not well known. Although it has been reported that the mechanism of

resistance in cowpea variety ICV 8 used in this study is mainly antibiotic (Ekesi *et al.* 1998), factors in this variety that may determine plant resistance and that are compatible with *M. anisopliae* are not known. It may therefore, be interesting to know how this factors influences the compatibility of host plant resistance with *M. anisopliae* as well as examining the potential for increasing these mortality factors when both control strategies are used together. Further investigations are also required to determine whether temperature/resistant variety/*M. anisopliae* interactions exert an important influence on infection of field populations of *M. sjostedti*. Additionally, factors in the tolerant variety that affect the susceptibility of *M. sjostedti* to *M. anisopliae* merit further attention. Finally, this study underlines the importance of studying tritrophic interactions in the development of fungal pathogens for insect pest management.

CHAPTER EIGHT

Field evaluation of the entomopathogenic fungus, *Metarhizium anisopliae* for the control of the legume flower thrips, *Megalurothrips sjostedti* on cowpea

8 Introduction

In previous chapters, it has been demonstrated that *M. anisopliae,* strain ICIPE 69, could cause significant mortality in adult *M. sjostedti*. All of the bioassays in the aforementioned studies were conducted in the laboratory with field grown leaf buds, flower buds and flowers. This present chapter examines the potential of this strain for the management of the legume flower thrips under field conditions. The Null hypothesis that entomopathogenic fungus cannot cause mortality/reduction in thrips population and damage under field conditions and hence cannot increase cowpea yield will be rejected if analysis of variance and/or mean separation show significance differences in mortality, thrips population and damage and cowpea grain yield between control plots and *M. anisopliae* treated plots.

8.1 Materials and methods

8.1.1 Study sites

The studies were carried out at the International Centre of Insect Physiology and Ecology's Mbita Point Field Station on the shores of Lake Victoria, Kenya. Mbita Point is on latitude 0 degrees 34 minutes S and longitude 34 degrees 10 minutes E and altitude 1170 m. The site has two rainy seasons: (March-July and September-December) with an annual rainfall of 1150 mm. The soil is a clay loam [pH 6.9; C, 0.9%; cation exchange capacity 30 meq $(100\ g)^{-1}$ soil; N, 0.19; P, 36 meq $(100\ g)^{-1}$ soil; K, 0.1 meq $(100\ g)^{-1}$ soil].

8.1.2 Field plots

Cowpea variety ICV 2, was planted in 4 x 4 m plots with intra-row spacing of 20 cm and inter-row spacing of 60 cm for two seasons: first season (September-December, 1996) and second season (March-July, 1997). ICV 2 is characterized by spreading, indeterminate-growth habit, tolerant to pod sucking bugs and legume pod borer; but highly susceptible to the legume flower thrips and matures in about 75 days (Pathak and Olela, 1986; J.C. Olela, personal comm.). The plants were top-dressed with diamonium phosphate at 45kg ha^{-1} after thinning to one plant per hill at 14 days after emergence (DAE). Plots were hand-weeded with hoe as necessary.

8.1.3 Fungus and production of inoculum

The strain of *M. anisopliae* used in this experiment was isolated from a soil sample from Kinshasa, Democratic Republic of the Congo in 1990 using the "*Galleria* bait method" (Zimmermann, 1986a). The virulence of the strain was maintained by regular passage through *M. sjostedti* (Schaerffenberg, 1964). Conidia were produced on a substrate of ground maize/vermiculite (Plate 6). The substrate was autoclaved for 1 h at 121^{0}C. This was then transferred into plastic buckets (33 x 25 x 13 cm) and inoculated with a 3 day-old culture of blastospores (50 ml). The buckets were covered with autoclavable polyethylene bags to prevent any contamination. The culture was incubated for 21 days in a controlled environment room (25 ± 2^{0}C, 60-70% RH) (Maniania, 1993a). The culture was then allowed to dry for 5 days at room temperature and conidia were harvested by sieving, and stored in the refrigerator (4-6^{0}C) before use in the field. In viability tests, between 84-90% of conidia germinated after 24 h in medium containing yeast extract (0.1%), chloramphenicol (0.1%), Tween 80 (0.01), benlate 0.001%) and water (1000 ml) (Milner *et al.*, 1991).

8.1.4 Treatments

Metarhizium anisopliae was tested as two different formulations: an ultra-low volume (ULV) oil/aqueous formulation and a high volume (HV) aqueous formulation. Six treatments were applied: (1) control ULV, (2) control HV, (3) ULV formulation at 1 x 10^{11} conidia ha^{-1}, (4) ULV formulation at 1 x 10^{13} conidia ha^{-1}, (5) HV formulation at 1 x 10^{11} conidia ha^{-1}, and (6) HV formulation at 1 x 10^{13} conidia ha^{-1}. During the second season, treatment 1 (control ULV) was replaced with a synthetic insecticidal treatment, lambda cyhalothrin (Karate® 17.5 EC) (Zeneca Ltd, Fernhurst, Haslemere, UK), applied at the recommended rate of 1 litre ha^{-1} of the commercial formulation which contains 17.5 g a.i. ha^{-1}. The treatments were arranged in a six by six quasi-complete Latin-square design. This design ensured that uneven pest invasion of the crop from one side did not bias the experiment as each treatment appeared next to every other treatment twice in both rows and columns (Bailey, 1984; Bailey *et al.*, 1989).

For spray application, conidia were suspended in water containing 0.05% Silwet L-77® (organo-silicone surfactant/emulsifier) (Loveland Industries, Greeley, USA) for the HV aqueous formulation. ULV oil/aqueous formulation contained 50: 50 corn oil (Elianto Kenya, Nakuru, Kenya) and water and 0.1% Silwet L-77. Nutrient agar (0.1%) (Unipath, Basingstoke, UK), glycerine (0.1%) (BDH Lab. Supplies, Poole, UK) and molasses (0.5%) (Muhoroni Sugar, Kisumu, Kenya) were added to the inoculum as protectants and bait to complete the formulation (Maniania, 1993b). Concentrations of inoculum were determined with a Malassez-cell-counting chamber.

In both seasons, three applications were performed. The first spray applications were administered at 44 and 42 DAE for the first and second seasons, respectively. Subsequent spray applications were at 5 day intervals. The ULV formulation was administered with a Micron-ulva spinning disk sprayer (Micron Sprayers, Bromyard, UK) with 6 new batteries at an output of 1 litre

ha^{-1}. The wind speed was a steady 2-3 m s^{-1}. The HV formulation and the chemical insecticide were applied with a separate CP15® knapsack sprayer (Cooper Pegler, Sussex, UK) at an output of 350 litres ha^{-1}. Control plots for ULV formulation consisted of 50: 50 corn oil and water containing 0.1% Silwet, 0.5% molasses, 0.1% nutrient agar and 0.1% glycerine applied with a Micon-Ulva sprayer. Control plots for HV formulation consisted of water spray containing 0.05% Silwet, 0.5% molasses, 0.1% nutrient agar and 0.1% glycerine, applied with a CP15 knapsack sprayer. Spray applications were performed in the evenings between 17 h and 18:30 h to lessen the adverse effect of ultraviolet radiation (Moore and Prior, 1993).

8.1.5 Evaluation

Plots were sampled for *M. sjostedti* by randomly picking 20 flowers from each plot into a vial containing 30% alcohol (Salifu and Singh, 1987). The samples were brought to the laboratory and flowers were dissected and washed to separate the insects from the plant parts, and later counted. The first flower samples were taken before the first treatment at 43 DAE (first season) and 41 DAE (second season). Subsequent samples were taken at five days interval after treatments.

To assess for mortality caused by *M. anisopliae*, 20 adult insects per plot were aspirated at random using a cotton-plugged aspirator from flowers which, were also randomly picked from each plot. The insects were placed individually in screened transparent plastic vials (50 x 100 mm) and fed with surface sterilized floral tissues and pollen from untreated cowpea plots. The insects were maintained in a controlled environment room (26 ± 2^0C, 60 ± 5% RH and a photoperiod of L: D 12: 12). Mortality was recorded daily for 14 days. Dead insects were transferred to petri dishes lined with damp filter paper. Mortality due to fungus was confirmed by microscopic examination of hyphae and spores on the surface of the dead body.

Table 17. Scale for rating flower thrips damage to cowpea[a]

Score	Symptoms
1	No browning drying of stipules, leaf buds or flower buds; no bud abscission
3	Initiation of drying of stipules, leaf buds and flower buds not flower bud abscission
5	Distinct browning drying of stipules, leaf buds and flower buds; start of flower bud abscission
7	Serious flower bud abscission and failure of peduncles to elongate
9	Very severe bud abscission. Most plants carry short barren peduncles

[a]After Jackai and Singh (1988)

Plate 6. Conidia of *Metarhizium anisopliae* produced on a substrate of maize and vermiculite

Ten plants per plot were randomly selected to assess for damage using the scale of 1-9 (Table 17) (Jackai and Singh, 1988). Sampling dates were the same as for population density assessment. Flower production per plant was monitored on 10 plants per plot picked at random. Pre-spray counts were taken at 43 and 41 DAE, and subsequently at 4 day intervals for the first and second season, respectively. Pod production per plant was assessed on 10 randomly selected plants per plot at 51, 58 and 65 DAE, in the first season and at 49, 56 and 63 DAE during the second season. Grain yield data was collected from the entire length of each plot at harvest. This was extrapolated to kg ha^{-1}.

8.1.6 Statistical analysis

The data were subjected to analysis of variance for a Latin-square design and means were separated by Student-Newman-Keuls (SNK) test (P=0.05) using the ANOVA or GLM Procedure of SAS (SAS Institute Inc., 1985). Analysis of thrips count and damage score were based on data transformed to log (x+1). Similarly, percentage mortality data were transformed by arcsine to standardize mean percentages (Gomez and Gomez, 1984) before analysis.

8.2 Results

8.2.1 Efficacy of fungus application

8.2.1.1 Thrips population.

Spraying of cowpea plants with *M. anisopliae* significantly reduced the population of *M. sjostedti* in both seasons (Figures 11 and 12) (rejection of Null hypothesis). In the first cropping season (Figure 11), the number of thrips before the first spray application varied between 150-218 thrips/20 flowers. Five days after treatment (equivalent to 48 DAE), significant difference (P<0.05) in thrips population was observed among treatments. Similar differences (P<0.05) among treatments were also observed at 58 DAE and 63 DAE.

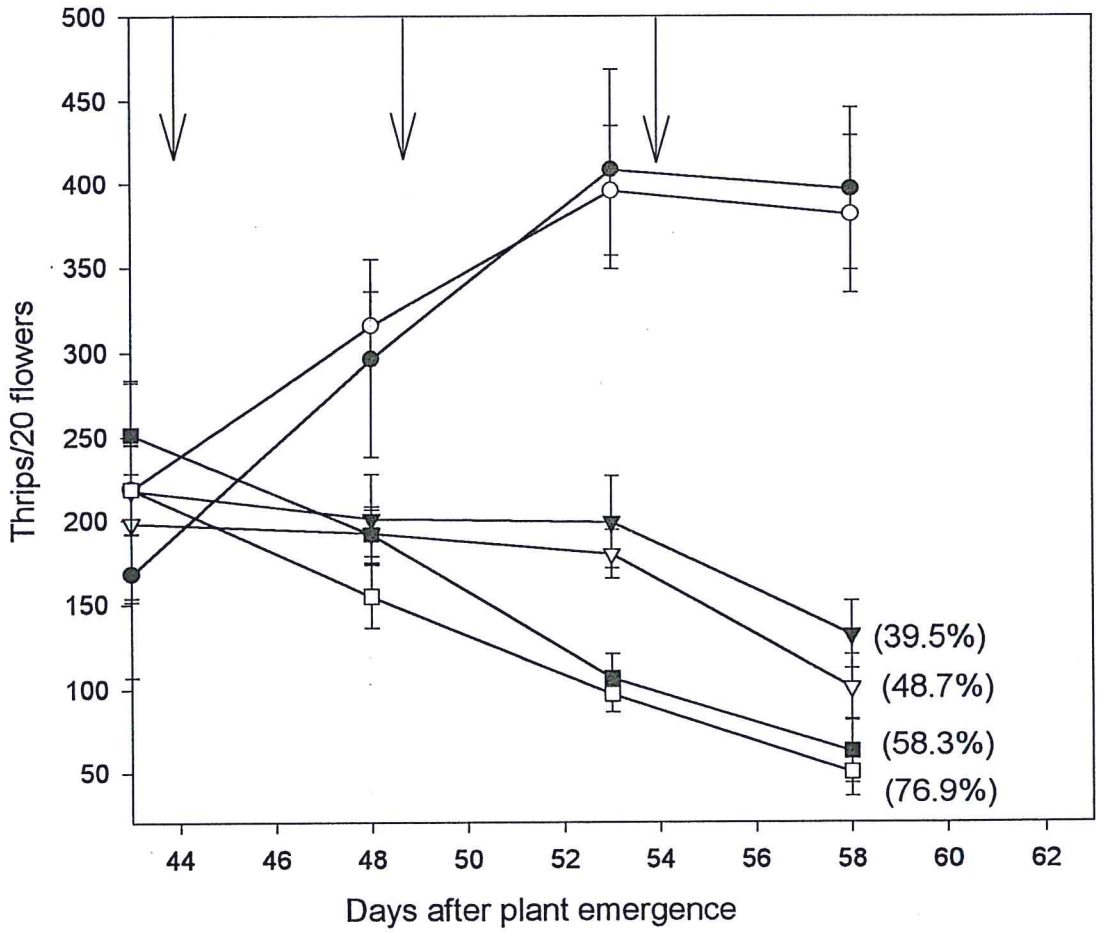


Figure 11. Fluctuation in the population of *Megalurothrips sjostedti* on cowpea plots treated with different formulations and concentrations of *Metarhizium anisopliae* at ICIPE Mbita Point Field Station during the first season. Arrows indicate date of spray. Values in parentheses are percentage reduction in thrips density over the season.

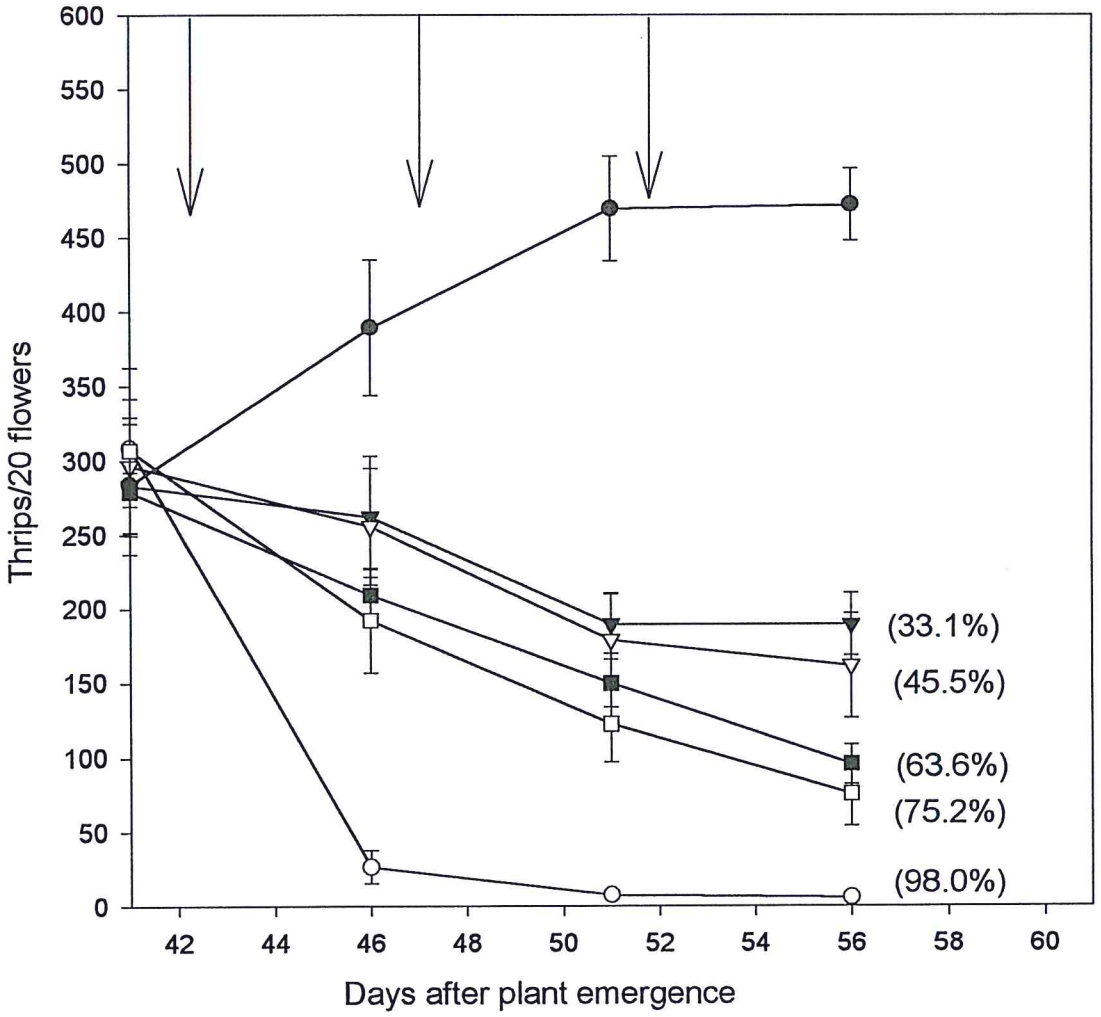


Figure 12. Fluctuations in the population of *Megalurothrips sjostedti* on cowpea plots treated with different formulations and concentrations of *Metarhizium anisopliae* and with Karate at ICIPE Mbita Point Field Station during the second season. Arrows indicate date of spray. Values in parentheses denote percentage reduction in thrips density over season.

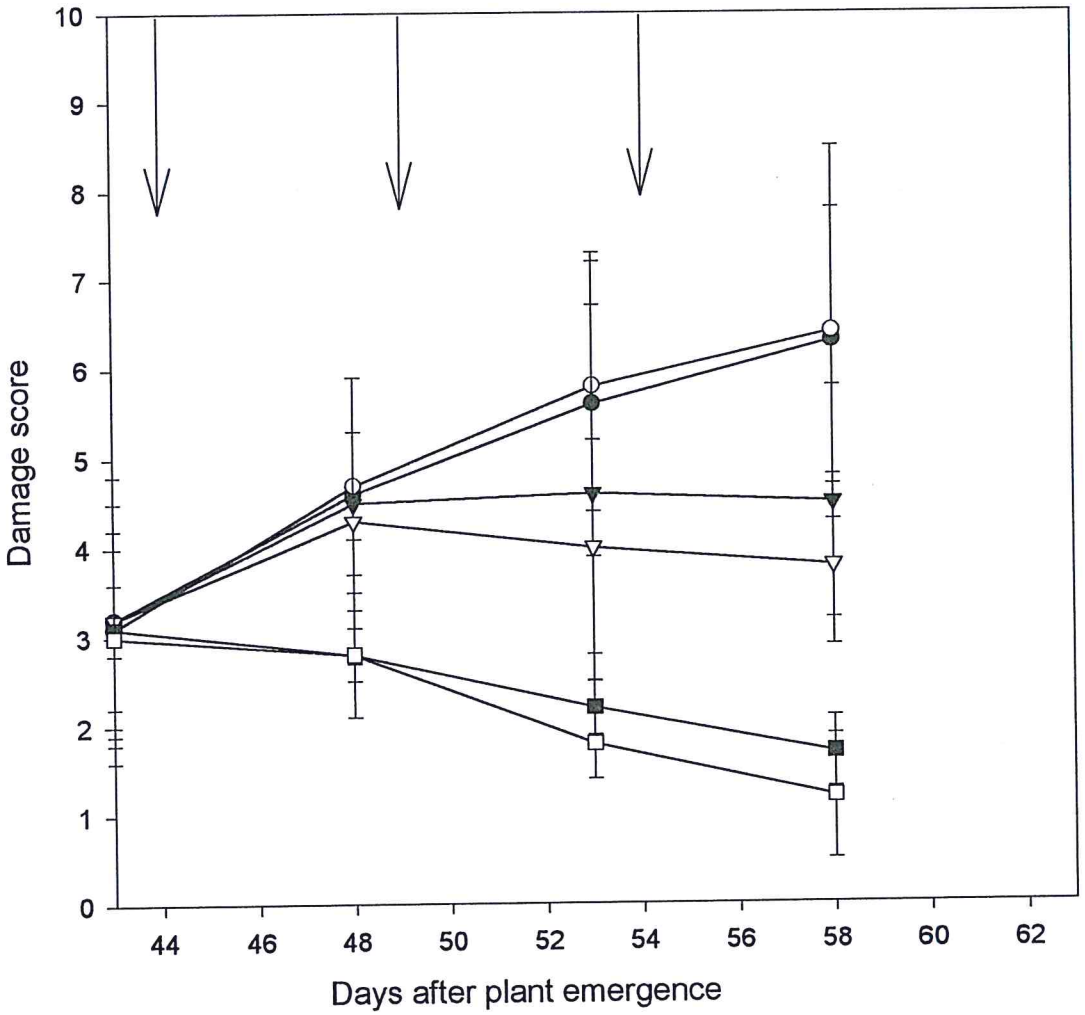


Figure 13. Trends in thrips (*Megalurothrips sjostedti*) damage scores in cowpea plots treated with different formulations and concentrations of *Metarhizium anisopliae* at ICIPE Mbita Point Field Station during the first season. Arrows indicate date of spray.

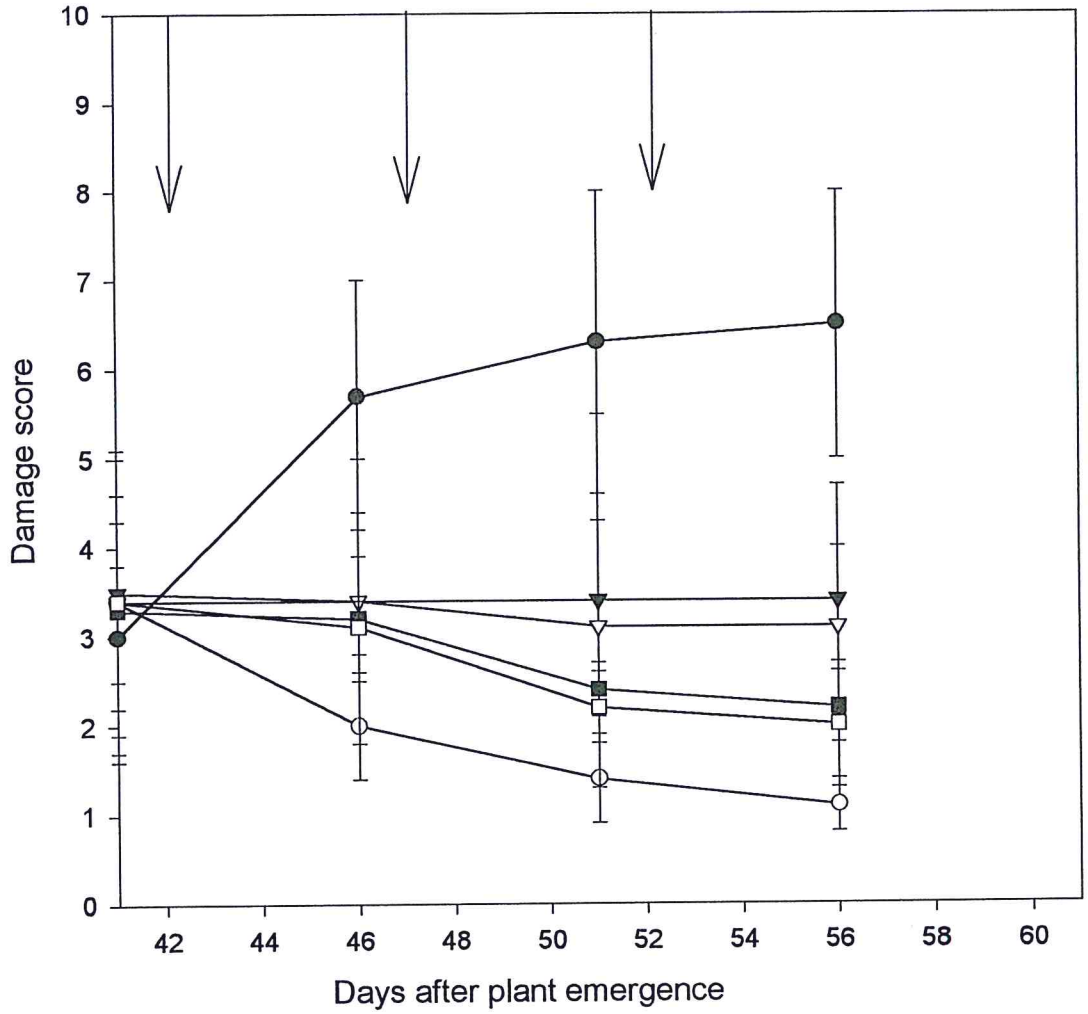


Figure 14. Trends in thrips (*Megalurothrips sjostedti*) damage scores in cowpea plots treated with different formulations and concentrations of *Metarhizium anisopliae* and with Karate during the second season at ICIPE Mbita Point Field Station. Arrows indicate days of spray.

The percentage reduction in the population of thrips over the season varied between 40% (ULV at 1 x 10^{11} conidia ha^{-1}) to 77% (HV 1 x 10^{13} conidia ha^{-1}) (Figure 11).

During the second cropping season (Figure 12), thrips counts taken before the first spray application varied from 278-301 thrips/20 flowers. Following spray application significant reduction ($P<0.05$) in thrips population was observed in both the fungus and Karate treated plots compared with the control plots during all days of sampling dates (rejection of Null hypothesis). The average percentage reduction over the season varied from 33% (ULV treatments at 1 x 10^{11} conidia ha^{-1}) to 75% (HV at 1 x 10^{13} conidia ha^{-1}). The synthetic insecticidal treatment provided 98.0% reduction in thrips population (Figure 12).

Mortality caused by application of *M. anisopliae* was observed in all treatments (Table 18) (Rejection of Null hypothesis). During the first season, mortality varied from 29%-61%, 40-71% and 49-73% after the first, second and third spray, respectively. In the second season, mortality due to mycosis ranged from 22-44% following the first fungal application, from 38-53% after the second spray and from 41-63% after the third spray (Table 18). In both seasons, some insects from control plots were observed to have died from *M. anisopliae* infection (Table 18).

8.2.1.2 Thrips damage

In the first season, significant reduction ($P<0.05$) in plant damage among treatments was observed at 58 DAE (Figure 13) (rejection of Null hypothesis). The HV formulation treatment at 1 x 10^{13} conidia ha^{-1} gave the lowest damage at all days of sampling (Figure 13). During the second season, the amount of damage in plots sprayed with the HV formulation at 1 x 10^{13} conidia ha^{-1} did not differ significantly ($P>0.05$) from the Karate treated plots (Figure 14).

8.2.1.3 Flower and pod production.

Application of *M. anisopliae* increased flower production in both seasons (Table 19). At 51 DAE flower production was significantly higher ($P<0.05$) in all fungal treated plots compared to the controls (Table 19). HV formulation at both concentrations produced significantly more flowers after spraying, compared to other treatments (Table 19). During the second cropping season, mean flower production per plant following treatments were also significantly higher in fungal and synthetic insecticidal plots compared to the controls (Table 19). By 49 DAE, the synthetic insecticidal treatment had the highest number of flowers per plant which did not differ significantly from HV treatment at 1 x 10^{13} conidia ha^{-1} (Table 19).

Mean pod production per plant during the first season were significantly higher ($P<0.05$) in fungal treated plots compared to the control plots at all sampling dates (Table 20). Treatment with the HV aqueous formulation at 1 x 10^{13} conidia ha^{-1} produced significantly more pods compared to other treatments (Table 20). In the second cropping season, application of both *M. anisopliae* or Karate significantly increased ($P<0.05$) pod production compared to the control (Table 20). By 63 DAE, there was no real difference in pod production per plant between the HV formulation at 1 x 10^{13} conidia ha^{-1} and the synthetic insecticidal treatment (Table 20).

Table 18. Percentage mortality (mean ± SE) in adult *Megalurothrips sjostedti* caused by spray application of *Metarhizium anisopliae*

	Spray application		
Season/Treatment	First	Second	Third
First season			
Control (ULV)	0.0 ± 0.0d	3.3 ± 1.7c	17.7 ± 2.2b
Control (HV)	0.0 ± 0.0d	4.2 ± 1.6c	15.8 ± 5.8b
ULV 1 x 10^{11}	29.2 ± 6.6c	40.8 ± 5.1b	49.2 ± 7.9a
ULV 1 x 10^{13}	45.8 ± 4.5b	42.5 ± 5.4b	59.2 ± 8.1a
HV 1 x 10^{11}	53.3 ± 1.7ab	59.2 ± 4.5a	66.7 ± 8.6a
HV 1 x 10^{13}	60.8 ± 10.4a	70.8 ± 4.8a	72.5 ± 6.1a
Second season			
Karate	-	-	-
Control	0.0 ± 0.0c	0.0 ± 0.0b	12.5 ± 7.3d
ULV 1 x 10^{11}	21.7 ± 13.1b	38.3 ± 12.9a	40.8 ± 15.4c
ULV 1 x 10^{13}	25.0 ± 14.4b	41.7 ± 5.5a	47.5 ±10.1bc
HV 1 x 10^{11}	40.8 ± 4.1a	43.3 ± 17.4a	52.5 ± 11.6a
HV 1 x 10^{13}	44.2 ± 10.3a	53.3 ± 20.1a	63.3 ± 19.7a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls test (P=0.05). Means were angularly transformed before analysis but values represent detransformed means.

Table 19. Mean (± S.E) flower production plant^{-1} in cowpea plots treated with different formulations and concentrations of *Metarhizium anisopliae*

	Days after emergence		
Season/Treatment	43 (41)	47 (45)	51 (49)
First season			
Control (ULV)	6.0 ± 2.4ab	7.5 ± 4.4b	6.5 ± 1.5e
Control (HV)	6.7 ± 1.8ab	8.2 ± 2.7b	7.3 ± 2.5d
ULV 1 x 10^{11}	7.1 ± 2.1a	8.2 ± 5.1b	8.2 ± 0.7c
ULV 1 x 10^{13}	6.3 ± 2.3ab	7.9 ± 1.9b	8.6 ± 2.3b
HV 1 x 10^{11}	6.8 ± 2.0ab	10.2 ± 3.6a	12.3 ± 4.7a
HV 1 x 10^{13}	6.7 ± 1.8ab	10.2 ± 4.1a	12.4 ± 4.1a
Second season			
Karate	5.8 ± 1.8ab	10.4 ± 2.5a	13.1 ± 3.6a
Control	5.6 ± 1.6ab	6.7 ± 3.2f	6.1 ± 1.7d
ULV 1 x 10^{11}	6.0 ± 2.3a	7.2 ± 1.8e	10.2 ± 2.7c
ULV 1 x 10^{13}	5.8 ± 3.1ab	7.9 ± 2.0d	10.1 ± 3.1c
HV 1 x 10^{11}	5.2 ± 1.6b	8.2 ± 1.7c	11.9 ± 2.3b
HV 1 x 10^{13}	5.6 ± 1.7ab	9.7 ± 2.9b	12.5 ± 3.4a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls test (P=0.05). Days in parentheses are for second season.

Table 20. Mean (± S.E) pod production plant^{-1} in cowpea plots treated with different formulations and concentrations of *Metarhizium anisopliae*

	Days after emergence		
Season/Treatment	51 (49)	58 (56)	65 (63)
First season			
Control (ULV)	9.9 ± 1.8d	12.1 ± 3.1d	11.6 ± 1.7d
Control (HV)	10.3 ± 3.6d	12.3 ± 1.2d	12.0 ± 4.2d
ULV 1 x 10^{11}	12.5 ± 2.7c	16.7 ± 2.7c	18.2 ± 3.7c
ULV 1 x 10^{13}	12.9 ± 1.9c	17.6 ± 1.8c	18.5 ± 2.9c
HV 1 x 10^{11}	16.7 ± 4.6b	20.8 ± 4.7b	21.3 ± 5.6b
HV 1 x 10^{13}	19.0 ± 3.4a	23.1 ± 7.9a	23.2 ± 5.3a
Second season			
Karate	20.3 ± 5.6a	23.7 ± 3.7a	22.9 ± 5.7a
Control	8.1 ± 1.8e	10.8 ± 5.1e	10.7 ± 2.7e
ULV 1 x 10^{11}	10.0 ± 2.4d	13.6 ± 3.2d	16.3 ± 2.4d
ULV 1 x 10^{13}	12.4 ± 3.6c	16.6 ± 4.1c	17.4 ± 3.8c
HV 1 x 10^{11}	16.7 ± 4.3b	19.7 ± 2.6b	21.1 ± 6.1b
HV 1 x 10^{13}	17.8 ± 2.4b	20.7 ± 3.5b	22.1 ± 5.7a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls test (P=0.05). Days in parentheses are for second season.

Table 21. Effect of *Metarhizium anisopliae* and Karate application on mean (± SE) cowpea grain yield (kg ha^{-1})

Treatment	First season	Second season
Control (ULV)*	572.6 ± 85.3c	1826.3 ± 200.7a
Control (HV)	872.6 ± 52.4c	348.0 ± 46.2b
ULV 1 x 10^{11}	1028.3 ± 170.7bc	1007.5 ± 268.9ab
ULV 1 x 10^{13}	1021.1 ± 206.9bc	1013.4 ± 183.4ab
HV 1 x 10^{11}	1544.4 ± 198.8ab	1495.1 ± 209.3a
HV 1 x 10^{13}	1897.2 ± 74.9a	1730.4 ± 205.4a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls test (P=0.05). *Replaced with Karate in the second season.

8.2.1.4 Grain yield

In both seasons, grain yield increased significantly (P=0.05) in treated plots compared to the controls (Table 21) (rejection of Null hypothesis). HV aqueous formulation produced yields, which were significantly higher than the controls in the first season. In the second season, mean separation by SNK showed that yields from fungal treated plots and the Karate treated plots were not significantly different (Table 21).

8.3 Discussion

The results of the present study show the potential of *M. anisopliae* for the control of *M. sjostedti* on cowpea. Application of the fungus resulted in significant reduction of pest population and plant damage, and subsequently increased flower and pod production as compared to the controls in both cropping seasons. Entomopathogenic fungi are being developed worldwide for the control of insect pests of agricultural importance and some are available commercially (Zimmermann, 1986b; Ferron *et al.*, 1991; Hsu and Quarles, 1995). Studies carried out by other workers on other species of thrips have shown that hyphomycetes fungi can effectively control thrips on different host plants. For example, *Verticillium lecanii* (Zimmermann) Viegas gives good control of onion thrips, *T. tabaci* Lindeman and the western flower thrips (WFT), *F. occidentalis* in greenhouses and in the field (Gillespie, 1986; Van der Schaaf *et al.*, 1990; Beerling *et al.*, 1996). Weekly sprays of *V. lecanii* applied as a wettable powder caused 60% infection rates in WFT on cucumber. *M. anisopliae* applied as greenhouse soil drench reduced WFT population on chrysanthemum by about 72% while *B. bassiana* (Bal.) Vuill. applied to the plants reduced the population by about 47% (Brownbridge *et al.*, 1994; Brownbridge, 1995).

Following the success obtained during our experiment of the first season, a synthetic insecticidal treatment was introduced in the second season. This replaced the ULV control since the difference was not significant between the controls in the first season (Table 21). As expected,

application of Karate resulted in rapid decline of the pest population within 3-5 days. In contrast, application of *M. anisopliae* caused a gradual decline of *M. sjostedti* population over 5-10 days. Slow speed of kill has been identified as a disadvantage of bioinsecticides compared to conventional insecticides (Dent, 1991), although the debilitating effects of infection due to fungi has also been reported to reduce insect's capacity to harm crops several days before death (See Chapter six).

A high proportion of adult insects collected from the field died from mycosis in both seasons. However, few mycosed insects were also observed from control plots in both seasons. This could be attributed to drift or migration of infected insects. The ecological niche associated with *M. sjostedti* within leaf buds and flowers of cowpea probably provided sufficient microclimate humidity for fungal infection to take place (Hall and Papierok, 1982; Benz, 1987) and this may have contributed to the high level of mortality observed from the fungal treated plots. Meteorological data were not collected during the experimental period because of breakdown of the weather equipment. However, relative humidity of between 60-78% have been reported during the first and second seasons at the study site (Maniania *et al.*, 1993a), suggesting that the fungus benefited from both atmospheric RH and the microclimate humidity within the leaf buds and flowers. Additionally, the fact that these insects have the tendency to aggregate together means that dead and/or infected insects may have served as a source of inoculum to infect other insects, hence the high level of mycosed insects.

Metarhizium anisopliae applied as HV aqueous formulation performed better than ULV oil/aqueous formulation. This is in general agreement with observation of Brownbridge *et al.*, (1996) who attributed it to better penetration of flowers by the infective propagules of *B. bassiana* to sites of high WFT population, as a result of the large volumes applied to the plant. Spray droplets force under pressure from HV application into flowers probably enhance direct contact of the pathogen with the insect, hence the superior level of control. The two dosages of 1×10^{11} and 1×10^{13} conidia ha^{-1} used for both formulations in these experiments are within the range of dosages

widely applied against aerial pests in different agroecosystems (Rombach, 1986ab; Ibrahim and Low, 1993; Maniania 1993ab; Maniania *et al.*, 1994).

Grain yields in HV aqueous formulation at 1 x 10^{13} conidia ha^{-1} was significantly higher compared to the ULV formulation and the controls during the first season. In the second season, grain yields from all fungal treated plots were not significantly different from the Karate treated plots. The second cropping season recorded low yields compared to the first cropping season. This could be attributed to the high thrips population during the second cropping season. Populations of *M. sjostedti* are generally higher during the second cropping season than the first cropping season (Ampong-Nyarko *et al.*, 1994). The rapid build-up of the pest in the second season prompted earlier intervention during this season, although it would appear that some damage had been done before treatments were performed. Since mycopathogens are slower-acting mortality agents, this observation clearly calls for further investigations on timing and frequency of applications, if full use of the pathogen is to be made in the management of the legume flower thrips.

In conclusion, this study has revealed that *M. anisopliae* is an effective biological control agent for the legume flower thrips and this suggests its inclusion in the integrated pest management strategies for *M. sjostedti*.

CHAPTER NINE

Effect of intercropping cowpea with maize on the performance of the entomopathogenic fungus, *Metarhizium anisopliae* against the legume flower thrips, *Megalurothrip sjostedti*

9 Introduction

Intercropping is usually defined as the practice of growing two or more crops simultaneously on the same field (Blade *et al.* 1997). In the tropics, subsistence farmers who practice low-input agriculture are particularly dependent upon this form of crop production to minimize the risk of crop failure, improve nutrition and produce high yields of particular crops (Ofori and Stern 1987). For example, in western Kenya, 95% of the farmers grow cowpea as an intercrop (Saxena *et al.* 1989). There is generally very little information available on the exploitation of cultural practices to favour the development of fungal infection in insect population (Ferron, 1981; Ferron *et al.* 1991). Burliegh (1975) reported that the incidence of *Nomuraea rileyi* (Farlow) Samson in *Heliothis* species was higher in close canopy cotton than in open canopy variety. Early-planted soybeans in narrow row and at high seedling rate increased the incidence of *N. rileyi* in lepidopterous pests (Sprenkel, 1979). Habitat management practices that incorporate the use of fungal pathogens against insect pests should provide much information on the nature and extent of application likely to provide better control strategies (Ferron, 1981). A review of literature indicates that there is no information on the effect of intercropping on the performance of entomopathogenic fungi, although cases of enhanced activity of *Bacillus thuringiensis* (Berliner) under intercropping have been reported (Baliddawa, 1985). In previous chapters we have demonstrated the efficacy of *Metarhizium anisopliae* (Metsch.) Sorokin as a potential bioinsecticide against *M. sjostedti*. In the present study, further exploration on the possibility of integrating *M. anisopliae* and intercropping for the management of *M. sjostedti* on cowpea is investigated. Application of entomopathogens

within a cowpea intercrop also raises concerns for non-target organisms as their activity is believed to increase within this cropping system (Matteson, 1982; Ezueh and Taylor, 1984). In this regard, the populations of some predators in treated and control plots to assess the impact of the fungus on the density of the non-target organisms was also examined. In this study, the null hypothesis that *M. anisopliae* caused equal reduction in thrips population and damage, and no significant difference in yield parameters between the monocrop and intercrop would be rejected if analysis of variance shows significant differences among the treatments. Similarly, the null hypothesis that the populations of predators in *M. anisopliae* treated plots is not equal to the density in control plots would be rejected if analysis of variance and/or mean separation reveals a significant difference among the treatments.

9.1 Materials and Methods

9.1.1 Study sites

The experiment was carried out in the same site as described in the previous experiment.

9.1.2 Fungus and production of inoculum

The fungus used and the production protocol are the same with the previous experiment.

9.1.3 Field plots and treatments

Cowpea variety, ICV 2, was planted as a monocrop and intercrop with maize variety, Hybrid 512, during the first and second season of 1997. The main crop, ICV 2 cowpea, is characterized by spreading, indeterminate growth habit; tolerant to pod sucking bugs and legume pod borer, but highly susceptible to the legume flower thrips and matures in about 75 days (Pathak and Olela, 1986; J.C. Olela Pers. comm.). The treatments consisted of: (1) cowpea monocrop non-protected, (2) cowpea intercrop non-protected, (3) cowpea monocrop protected with *M. anisopliae,* (4)

cowpea intercrop protected with *M. anisopliae,* (5) cowpea monocrop protected with Karate® (Lambda-cyhalothrin), and (6) cowpea intercrop protected with Karate. The treatments were arranged in a six by six quasi-complete Latin square design. This design ensured that uneven pest invasion of the crop from one side did not bias the experiment as each treatment appeared next to every other treatment twice in both rows and columns (Bailey, 1984; Bailey *et al.* 1989). The plant spacing for cowpea monocrop was 30 cm intra-row x 60 cm inter-row to give a plant population density of 82500 plants ha^{-1} in both seasons. Plant arrangement for the intercropping was a single alternating rows of cowpea and maize. In the first season, plant spacing were 30 cm intra-row x 120 cm inter-row for both cowpea and maize. This gave a respective plant population of 41250 plants ha^{-1} for the cowpea and maize. During the second season, cowpea plant population in the intercrop was adjusted to give an intra-row spacing of 15 cm and inter-row spacing of 120 cm but maize spacing were similar to that of the first season. This gave a cowpea density of 82500 plants ha^{-1}.

For spray application, conidia of *M. anisopliae* were suspended in water containing 0.05% Silwet L-77®. Nutrient agar (0.1%), glycerin (0.1%) and molasses (0.5%) were added to the inoculum as protectant and bait to complete the formulation (Maniania, 1993b). The concentration of inoculum was determined with a Malassez-cell-counting chamber. The fungus was applied at the rate of 1×10^{12} conidia ha^{-1} and Karate was applied at the recommended rate of 17.5 g a.i. ha^{-1}. In both seasons, treatments were performed three times. The first spray applications were administered 42 days after plant emergence (DAE) and subsequently at 5 day intervals in both seasons. Both fungus and Karate were applied with a separate CP 15® knapsack sprayer at an output of 350 litres ha^{-1}. Control plots were sprayed with water containing 0.05% Silwet, 0.5% molasses, 0.1% nutrient agar and 0.1% glycerin. Spray applications were performed in the evenings between 17: 00 h and 18: 30 h to lessen the adverse effects of ultraviolet radiation (Moore and Prior, 1993).

9.1.4 Evaluation

Plots were sampled for *M. sjostedti* by randomly picking 20 flowers from each plot and placing them into a vial containing 30% alcohol. The samples were brought to the laboratory and flowers were dissected and washed to separate the insects from the plant parts, and later counted. The first flower samples were taken before the first treatment at 41 DAE and subsequently at 5 day intervals.

To assess for mortality caused by *M. anisopliae*, 20 adult insects per plot were aspirated at random using a cotton plugged aspirator from flower samples which were also randomly picked from each plot one day after each spray application. The insects were placed individually in screened transparent plastic vials (50 x 100 mm) and fed with surface sterilized floral tissues and pollen from untreated cowpea plots. The insects were maintained in a controlled environment room (26 ± 2^0C, $60 \pm 5\%$ RH and photoperiod of L: D 12: 12). Mortality was recorded daily for 14 days. Dead insects were transferred to Petri dishes lined with damp filter paper. Mortality due to fungus was confirmed by microscopic examination of hyphae and spores on the surface of the cadaver.

Plant damage was assessed using the scale of Jackai and Singh (1988) on ten plants tagged at random in each plot. The same plants tagged for plant damage assessment were used to monitor for flower and pod production per plant. Sampling dates for plant damage and flower production were the same as for thrips population density assessment. Pod production was assessed at 53, 60 and 67 DAE. At harvest, because of the variation in plant density within the monocrop and intercrop in the first season, grain yield data were recorded both on a per plant and a per plot basis from the entire length of each row (Terao *et al.* 1997). Yield per plot was extrapolated to kg ha^{-1}.

The population density of predatory bugs (*Orius* spp.) was estimated using the same flower samples for thrips density. Staphylinid beetles (*Paederus* spp.), earwigs, spiders (Araneidae, Theridiidae and Thomisidae) and ants (*Dorylus* spp. and *Camponotus* spp.) were monitored using pitfall traps constructed from cylindrical metal cans (10 cm dia. x 13 cm deep). Traps were placed

diagonally across each plot at 1 m intervals (Kyamanywa *et al.* 1993) with each plot having 4 traps. The rims of the trap were made level with the ground so as not to obstruct movement of insect and the area around them was kept bare to standardize catching conditions (Baars, 1979). Traps were half-filled with aqueous solution of 2% formalin to preserve the insects, stop predation and increase retaining efficiency (Kyamanywa *et al.* 1993). Plastic rain guards on a single wire strand were placed over each trap to minimize flooding by rain and predation of the cup contents by birds. The traps were placed at 36 dae. Pitfalls were emptied pre-spray at 41 dae and subsequently at 5 day intervals.

The populations of cocinellid beetles (*Scymnus* spp., *Cheilomenes* spp.) were estimated using the direct count method (Kyamanywa *et al.* 1993). This was done on a 4 m row in each plot. Starting at one end of the plot, beetles were counted on each plant until all plants in the row were inspected. Spiders found on the plants were also counted and the data were pooled with that of the pitfall traps. Sampling dates were the same as for pitfall traps.

9.1.5 Statistical Analyses

The data were subjected to analysis of variance for a Latin square design and means were separated by Student-Newman-Keuls (SNK) test (P=0.05) using the ANOVA or GLM procedure of SAS (SAS Institute Inc., 1985). Analysis of thrips counts and non-target organisms were based on data transformed to log (x+1). Post spray samples of non-target organisms were pooled over the various days after emergence and mean values of 3 sampling dates are presented. Percentage mortality data were transformed by arcsine to standardize mean percentages before analysis.

9.2 Results

During the first and second seasons, thrips population samples taken before spray application varied from 147-217 thrips/20 flowers and 96-171 thrips/20 flowers, respectively. Following spray

applications, there were significant reduction in thrips populations in all fungal and Karate treated plots in both seasons compared to the controls (Figs. 15 and 16). Thrips populations were generally lower in the intercrop compared to the monocrop in both trials. In the first season, the mean percentage reduction in thrips population over season in fungal treated plots was 66% for monocrop and 83% for intercropped cowpea (rejection of Null hypothesis) while thrips reduction in Karate treated plots was 98% in the monocrop and 91% within the intercrop (F=47.5, DF=5,20, P=0.0001). During the second season, percentage thrips reduction in cowpea monocrop and cowpea intercrop treated with *M. anisopliae* was 72% and 96%, respectively (rejection of Null hypothesis), while Karate treated plots incurred 95% reduction in the monocrop and 99% reduction in the intercrop (F=23.8, DF=5,20, P=0.0013).

Metarhizium anisopliae infection was observed in adult *M. sjostedti* in all fungal treated plots. In the first season, fungal infection ranged from 42% to 52% in cowpea monocrop, and from 41% to 68% in cowpea intercrop after the first and third spray, respectively (Table 22). During the second season, infection after the first and third spray ranged from 48% to 56% in cowpea monocrop and 48% to 76% in cowpea intercrop, respectively (Table 22).

Application of *M. anisopliae* and Karate significantly reduced damage by *M. sjostedti* with the least damage occurring within the intercrop in all the trials (rejection of Null hypothesis) (Figs.17 and 18).

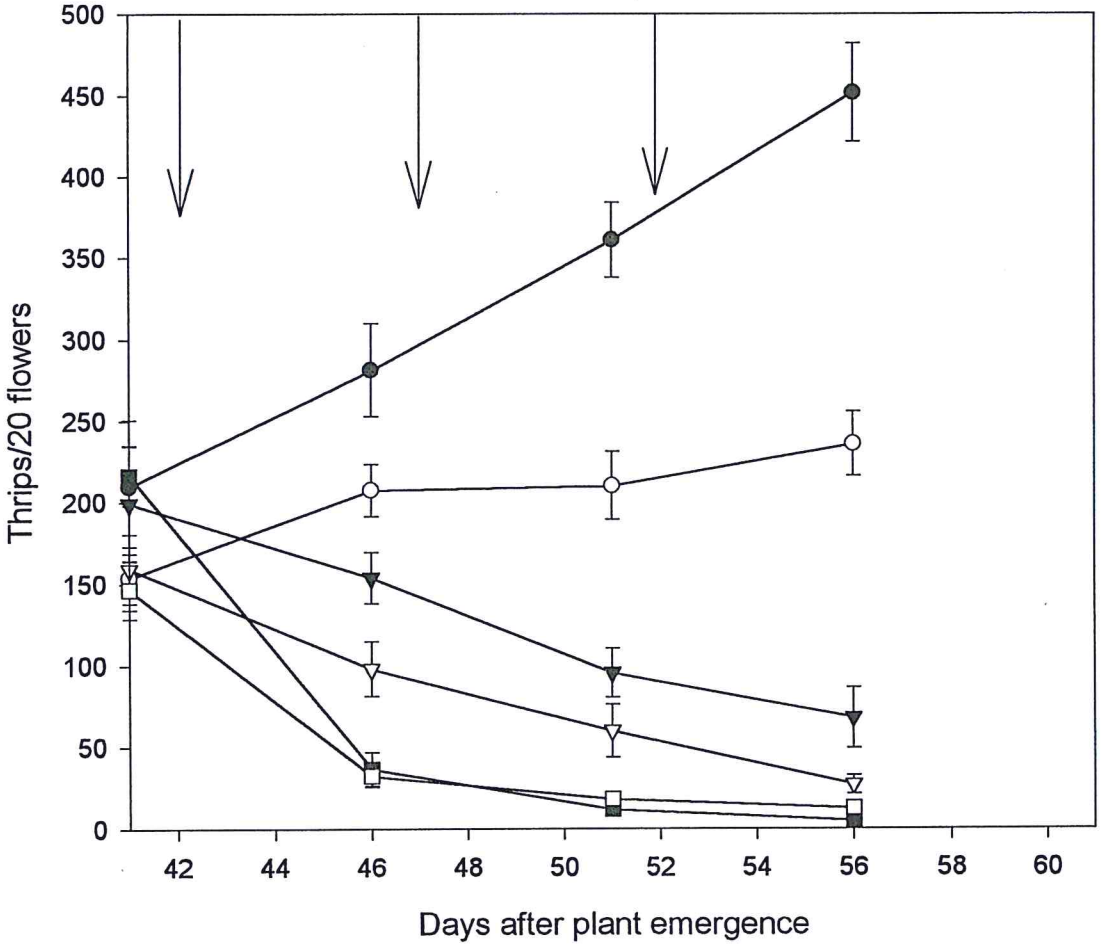
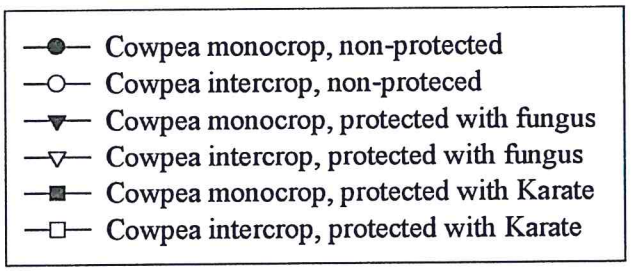


Figure 15. Trends in the density of *Megalurothrips sjostedti* in cowpea treated with *Metarhizium anisopliae* and Karate during the first season. Arrows indicate days of spray.



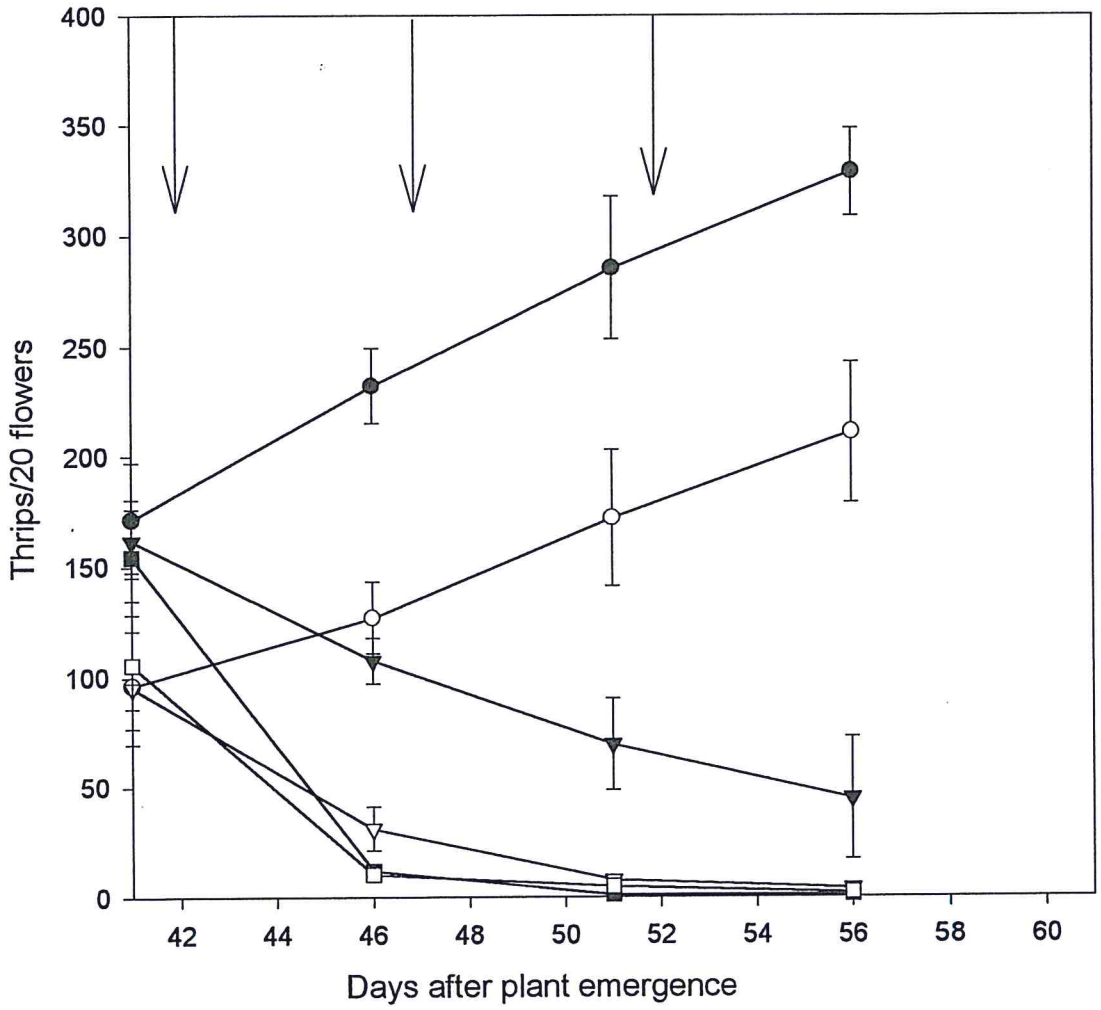


Figure 16. Trends in the density of *Megalurothrips sjostedti* in cowpea treated with *Metarhizium anisopliae* and Karate during the second season. Arrows indicate days of spray.

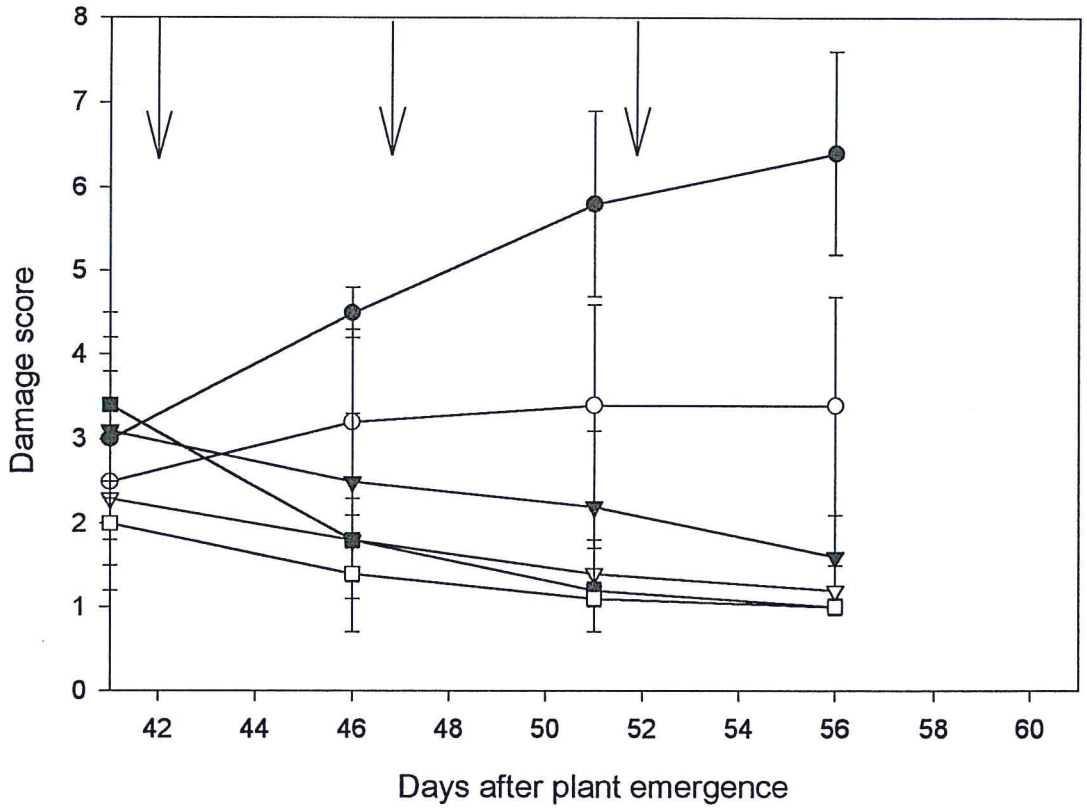


Figure 17. Trends in *Megalurothrips sjostedti* damage scores in cowpea treated with *Metarhizium anisopliae* and Karate during the first season. Arrows indicate days of spray.

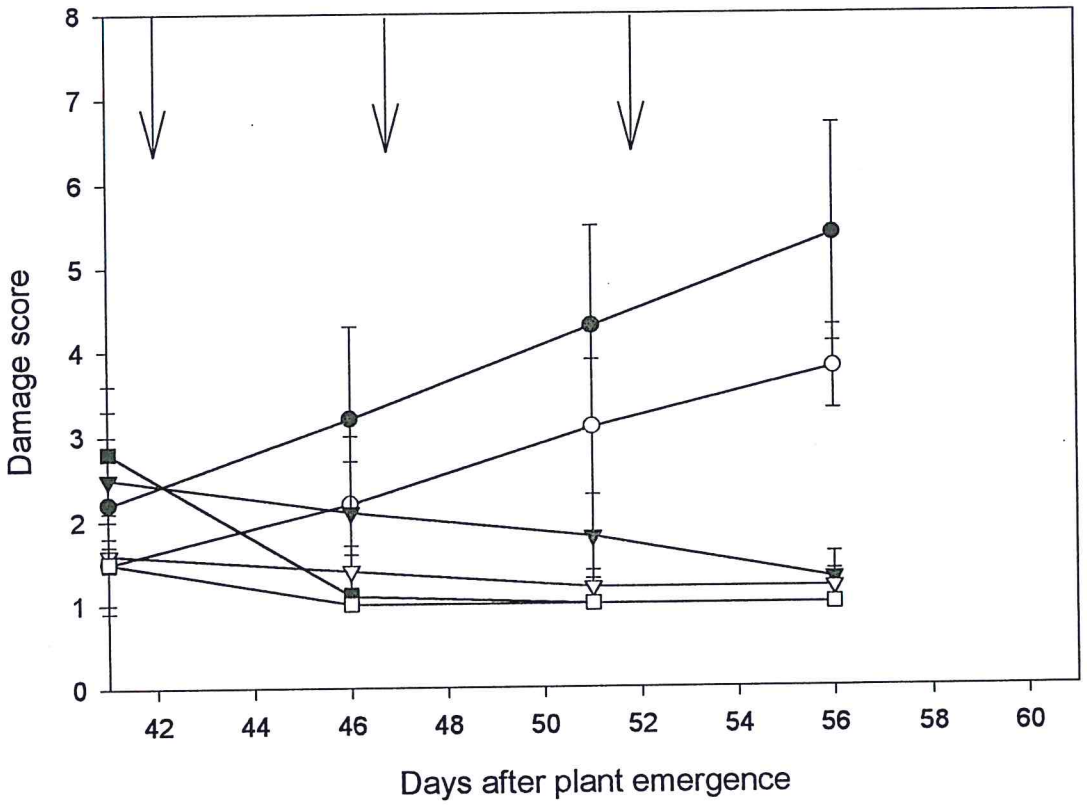


Figure 18. Trends in *Megalurothrips sjostedti* damage scores in cowpea treated with *Metarhizium anisopliae* and Karate during the second season. Arrows indicate days of spray.

There was a significant increase in flower production following treatment of cowpea with *M. anisopliae* and Karate (Table 23). For example, in both trials, the number of flowers per plant at 51 DAE were significantly higher ($P<0.05$) in treated plots compared to the control plots. In both seasons, cowpea intercrop treated with *M. anisopliae* produced significantly more flowers than cowpea monocrop receiving the same spray application (Table 23) (rejection of Null hypothesis). However, there was no real difference between cowpea intercrop treated with *M. anisopliae* and those treated with Karate in both cropping systems (Table 23).

Treatment of cowpea with *M. anisopliae* and Karate significantly increased ($P<0.05$) pod production compared to the controls in both seasons. (Table 24). Pod production was generally higher in the intercrop treated with *M. anisopliae*, and plots treated with Karate in both cropping systems in all the trials (Table 24).

Grain yield per plant were significantly higher ($P<0.05$)in fungal and Karate treated plots compared to the controls in both seasons (Table 25). Cowpea intercrop treated with *M. anisopliae* produced significantly more grains per plant compared with cowpea monocrop receiving the same fungal application (rejection of Null hypothesis). Because of the variation in plant density between the cropping pattern, grain yields in kg ha^{-1} during the first season were compared separately within monocrop and within intercrop. Grain yields were significantly higher in fungal and Karate treated plots within both cropping systems compared to the controls: monocrop (F=19.5; DF=2,2; P=0.0001), intercrop (F=17.9; DF=2,2; P=0.0001). In the second season, grain yields in kg ha^{-1} were compared across all cropping patterns since cowpea plant densities were similar. Grain yields were significantly higher ($P<0.05$) in fungal and Karate treated plots compared to the controls (Table25).

Table 22. Percentage mortality caused by *Metarhizium anisopliae* in adult *Megalurothrips sjostedti* following spray application

	Mean ± S.E.	
Season/Spray	Cowpea monocrop	Cowpea intercrop
First season		
Spray I	41.7 ± 5.6a	40.8 ± 7.3a
Spray II	45.8 ± 6.3b	53.3 ± 8.1a
Spray III	51.7 ± 5.1b	67.5 ± 5.6a
Second season		
Spray I	48.3 ± 11.3a	47.5 ± 8.6a
Spray II	50.8 ± 9.4b	60.8 ± 10.3a
Spray III	55.8 ± 8.7b	75.8 ± 12.4a

Means within a row followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test. Means were angularly transformed before analysis but values represent detransformed means.

Table 23. Flower production (mean plant^{-1} ± S.E.) in cowpea following application of *Metarhizium anisopliae* and Karate

Season/ Treatment	Days after emergence 41	46	51	56
First season				
Cowpea monocrop NP	5.2 ± 1.7b	6.3 ± 2.1d	6.1 ± 1.3d	4.7 ±1.2d
Cowpea intercrop NP	6.6 ± 1.3a	7.2 ± 1.8c	8.3 ± 1.5c	5.1 ± 1.6c
Cowpea monocrop PF	5.7 ± 0.8ab	8.2 ± 1.5b	10.5 ± 2.3b	6.8 ± 2.1b
Cowpea intercrop PF	6.4 ± 1.4a	9.7 ± 1.7a	12.5 ± 2.5a	8.8 ± 1.5a
Cowpea monocrop PK	5.9 ± 1.6ab	10.2 ± 2.3a	12.6 ± 1.7a	8.2 ± 2.3a
Cowpea intercrop PK	6.7 ± 2.1a	9.6 ± 1.1a	12.9 ± 1.9a	7.7 ± 1.7ab
Second season				
Cowpea monocrop NP	6.1 ± 2.1b	6.3 ± 1.1d	5.7 ± 0.8d	5.3 ± 1.3d
Cowpea intercrop NP	7.2 ± 1.7a	8.1 ± 1.7c	8.2 ± 1.7c	6.3 ± 1.5c
Cowpea monocrop PF	6.8 ± 1.5ab	10.1 ± 2.1b	12.0 ± 2.4b	8.2 ± 1.8b
Cowpea intercrop PF	6.2 ± 1.2b	12.7 ± 3.4a	14.2 ± 2.6a	10.2 ± 2.3a
Cowpea monocrop PK	6.2 ± 0.8b	11.8 ± 2.7a	14.5 ± 3.1a	9.8 ± 1.8a
Cowpea intercrop PK	7.5 ± 1.5a	11.3 ± 2.3a	13.2 ± 2.8ab	9.3 ± 1.7a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test. NP = Non-protected, PF = Protected with fungus, PK = Protected with Karate.

Table 24. Pod production (mean plant^{-1}± S.E.) in cowpea following application of *Metarhizium anisopliae* and Karate

Season/ Treatments	Days after emergence 53	60	67
First season			
Cowpea monocrop NP	8.6 ± 1.7d	10.2 ± 2.1d	10.8 ± 2.2d
Cowpea intercrop NP	11.7 ± 2.1c	13.0 ± 1.8c	13.5 ± 2.7c
Cowpea monocrop PF	13.1 ± 1.8b	17.0 ± 2.7b	18.8 ± 3.8b
Cowpea intercrop PF	18.7 ± 3.3a	21.4 ± 4.1a	21.4 ± 2.5a
Cowpea monocrop PK	19.0 ± 2.8a	21.9 ± 3.2a	21.4 ± 2.0a
Cowpea intercrop PK	18.3 ± 3.1a	20.8 ± 3.7a	22.0 ± 1.7a
Second season			
Cowpea monocrop NP	9.4 ± 1.8d	12.0 ± 1.8d	12.2 ± 1.6d
Cowpea intercrop NP	13.4 ± 1.5c	14.6 ± 2.3c	14.9 ± 1.3c
Cowpea monocrop PF	15.8 ± 1.3b	16.7 ± 2.1b	19.0 ± 1.8b
Cowpea monocrop PF	19.7 ± 2.1a	22.8 ± 3.1a	23.4 ± 2.4a
Cowpea monocrop PK	21.6 ± 1.8a	23.8 ± 2.5a	25.8 ± 1.6a
Cowpea intercrop PK	19.8 ± 2.4a	22.7 ± 1.7a	24.4 ± 2.7a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test. NP = Non-protected, PF = Protected with fungus, PK = Protected with Karate.

Table 25. Effect of *Metarhizium anisopliae* and Karate application on cowpea grain yield

Treatment	First season		Second season	
	Yield (g plant^{-1})	Yield[a] (kg ha^{-1})	Yield (g plant^{-1})	Yield (kg ha^{-1})
Cowpea monocrop NP	3.7 ± 0.7d	306.2 ± 34.6	3.8 ± 0.5d	312.5 ± 23.9d
Cowpea intercrop NP	6.9 ± 0.8c	283.8 ± 27.5	6.5 ± 0.7c	542 .7± 32.1c
Cowpea monocrop PF	9.6 ± 1.2b	795.8 ± 41.8	9.7 ± 0.8b	801.5 ± 51.1b
Cowpea intercrop PF	14.0 ± 1.6a	577.4 ± 38.7	12.3 ± 2.4a	1017.3 ± 67.5a
Cowpea monocrop PK	12.8 ± 1.5a	887.5 ± 35.3	14.7 ± 2.1a	1213.1 ± 56.4a
Cowpea intercrop PK	13.2 ± 2.1a	542.6 ± 42.3	13.8 ± 1.9a	1141.8 ± 75.2a

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test. NP = Non-protected, PF = Protected with fungus, PK = Protected with Karate. [a]Means not separated across cropping pattern because of variation in plant density.

Table 26. Trends in the number of non-target organisms (mean ± S.E.) in cowpea plots treated with *Metarhizium anisopliae* and Karate

Non-target organisms/ Treatment	First season		Second season	
	Pre-spray	Post-spray	Pre-spray	Post-spray
Coccinellid beetles				
Cowpea monocrop NP	2.3 ± 0.5b	3.1 ± 0.8a	1.8 ± 0.5c	2.3 ± 0.6b
Cowpea intercrop NP	2.0 ± 0.3b	2.2 ± 0.7b	2.5 ± 0.3ab	2.8 ± 0.5a
Cowpea monocrop PF	2.3 ± 0.6b	3.1 ± 1.2a	2.3 ± 0.7ab	3.0 ± 0.5a
Cowpea intercrop PF	2.2 ± 0.1b	2.6 ± 0.3b	3.0 ± 1.2a	2.5 ± 0.3ab
Cowpea monocrop PK	3.8 ± 1.3a	0.0 ± 0.0c	1.8 ± 0.2c	0.0 ± 0.0c
Cowpea intercrop PK	2.5 ± 0.3b	0.0 ± 0.0c	2.7 ± 0.1a	0.0 ± 0.0c
Ants				
Cowpea monocrop NP	10.2 ± 3.7ab	10.4 ± 3.1a	9.3 ± 2.1a	10.7 ± 1.5a
Cowpea intercrop NP	8.2 ± 2.1b	10.3 ± 4.3a	9.8 ± 1.3a	10.1 ± 1.8a
Cowpea monocrop PF	10.5 ± 1.7ab	10.1 ± 2.3a	8.7 ± 1.1b	9.8 ± 1.1a
Cowpea intercrop PF	7.5 ± 2.4b	9.4 ± 0.5ab	10.2 ± 2.1a	10.7 ± 1.5a
Cowpea monocrop PK	12.3 ± 1.5a	1.2 ± 0.2c	9.8 ± 1.2a	1.5 ± 0.3b
Cowpea intercrop PK	9.8 ± 0.8b	1.3 ± 0.1c	8.2 ± 0.7b	1.2 ± 0.1b

Table 26 continued. Trends in the number of non-target organisms (mean ± S.E.) in cowpea plots treated with *Metarhizium anisopliae* and Karate

Non-target organisms/ Treatment	First season		Second season	
	Pre-spray	Post-spray	Pre-spray	Post-spray
Predatory bugs				
Cowpea monocrop NP	2.2 ± 0.4a	3.1 ± 1.1a	2.0 ± 0.3a	1.5 ± 0.1a
Cowpea intercrop NP	1.7 ± 0.3b	2.3 ± 0.8b	1.7 ± 0.1a	1.9 ± 0.1a
Cowpea monocrop PF	2.3 ± 0.8a	3.1 ± 0.4a	2.0 ± 0.3a	2.0 ± 0.4a
Cowpea intercrop PF	1.5 ± 0.3b	2.6 ± 1.2ab	2.0 ± 0.2a	1.8 ± 0.2a
Cowpea monocrop PK	2.0 ± 0.4a	0.0 ± 0.0c	2.0 ± 0.1a	0.0 ± 0.0b
Cowpea intercrop PK	1.3 ± 0.1b	0.0 ± 0.0c	1.8 ± 0.1a	0.0 ± 0.0b
Spiders				
Cowpea monocrop NP	4.8 ± 0.6b	2.4 ± 0.4b	6.8 ± 1.1b	5.8 ± 1.3b
Cowpea intercrop NP	6.3 ± 1.4a	6.4 ± 1.7a	9.3 ± 1.3a	8.6 ± 1.4a
Cowpea monocrop PF	5.0 ± 1.3b	3.3 ± 1.2b	6.1 ± 0.6b	5.8 ± 1.1b
Cowpea intercrop PF	6.1 ± 1.3a	6.5 ± 1.5a	8.9 ± 1.4a	8.6 ± 0.6a
Cowpea monocrop PK	5.1 ± 0.8b	1.4 ± 0.1c	5.8 ± 0.7c	1.3 ± 0.7c
Cowpea intercrop PK	6.0 ± 1.4a	1.2 ± 0.1c	9.1 ± 2.1a	1.1 ± 0.1c

Table 26 continued. Trends in the number of non-target organisms (mean ± S.E.) in cowpea plots treated with *Metarhizium anisopliae* and Karate

Non-target organisms/ Treatment	First season		Second season	
	Pre-spray	Post-spray	Pre-spray	Post-spray
Staphylinid beetles				
Cowpea monocrop NP	3.5 ± 1.2b	3.1 ± 0.4b	2.8 ± 0.7a	2.5 ± 0.1a
Cowpea intercrop NP	4.8 ± 1.3a	4.6 ± 0.8a	3.1 ± 0.5a	3.1 ± 0.7a
Cowpea monocrop PF	3.2 ± 0.8b	3.3 ± 1.3b	2.5 ± 0.6a	2.4 ± 0.3a
Cowpea intercrop PF	4.5 ± 0.6a	4.2 ± 0.5a	2.7 ± 0.4a	2.5 ± 0.1a
Cowpea monocrop PK	4.1 ± 1.3a	1.8 ± 0.1c	2.6 ± 0.3a	0.0 ± 0.0b
Cowpea intercrop PK	4.3 ± 0.8a	1.1 ± 0.1c	3.2 ± 0.5a	0.0 ± 0.0b
Earwigs				
Cowpea monocrop NP	5.1 ± 0.6a	3.2 ± 1.1a	3.4 ± 0.4a	2.7 ± 0.4a
Cowpea intercrop NP	3.0 ± 1.2b	2.8 ± 0.5a	1.9 ± 0.1ab	2.8 ± 0.3a
Cowpea monocrop PF	3.0 ± 0.2b	2.7 ± 0.7a	2.9 ± 0.5a	3.0 ± 0.1a
Cowpea intercrop PF	2.7 ± 1.1b	3.1 ± 0.5a	3.2 ± 1.1a	2.9 ± 0.6a
Cowpea monocrop PK	3.2 ± 0.5b	0.7 ± 0.1b	2.7 ± 0.5a	0.0 ± 0.0b
Cowpea intercrop PK	4.8 ± 1.1a	0.0 ± 0.0b	2.7 ± 0.1a	0.0 ± 0.0b

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (*P*=0.05) test. NP=Non-protected, PF=Protected with fungus, PK=Protected with Karate

Application of *M. anisopliae* had no adverse effect on the populations of non-target organisms compared with theKarate treated plots. Post treatment samples taken at various days after emergence showed that there was no significant difference between fungal treated plots and the control plots in both seasons (Table 26) (rejection of Null hypothesis).

9.3 Discussion

Although examples abound on the practical performance of mycopathogens against various species of thrips on glasshouse crops, relatively few field trials have been undertaken to evaluate entomopathogenic fungi specifically for thrips control on field crops (Brownbridge *et al.* 1994; Brownbridge, 1995; Butt and Brownbridge, 1997). The results of the present study have shown that *M. anisopliae* can provide good control of *M. sjostedti* which is comparable to that achieved with Karate on field crop. The potential of *M. anisopliae* for the control of *M. sjostedti* has been demonstrated in previous chapter. Conidia of the fungal pathogen when applied as an ultra-low volume oil/aqueous or high volume aqueous formulation caused a significant reduction of *M. sjostedti* density and increased yield. In the present study, application of *M. anisopliae* to intercropped and monocropped cowpea significantly suppressed thrips densities and damage and also increased cowpea yield in both cropping systems, thus confirming previous studies on the potentials of this pathogen in the management of *M. sjostedti*. Reduction in thrips densities in both cropping patterns were gradual in fungal treated plots compared to the rapid decline observed in the Karate treated plots. Compared with chemical insecticides, mycoinsecticides have relatively slow speed of kill but this does not necessarily result in reduced crop protection from insects. Moore *et al.* (1992) has shown that adult *S. gregaria* infected with *M. flavoviride* reduced feeding within 24 h. It has also been demonstrated in Chapter six of this project that adult *M. sjostedti* infected with *M. anisopliae* reduced feeding within 24 h following infection and ceased feeding up to 2 days before death.

Cowpea intercrop had a low number of thrips compared to the monocrop and this further increased the effectiveness of both the fungus and the synthetic insecticide. Various workers have shown that mixed cropping cowpea with maize and/or sorghum reduces the population of *M. sjostedti* (Kyamanywa and Tukarhirwa, 1988; Kyamanywa *et al.* 1993; Ampong-Nyarko *et al.* 1994). The population of the pest was higher during the first season than in the second season. Correspondingly, yields were lower during the first season than during the second. This is in agreement with previous studies (Ampong-Nyarko *et al.* 1994; See previous chapter).

In both seasons, flower and pod production were significantly higher in cowpea intercrop treated with *M. anisopliae* compared to cowpea monocrop receiving the same spray application. This effect could not be detected in the overall grain yield (kg ha^{-1}) in the first season since plant populations within the intercrop were lower than the monocrop population. Plant populations in the intercrop were adjusted in the second season to equal that of the monocrop, and the results showed that the cowpea intercrop treated with fungus out yielded the cowpea monocrop receiving the same fungal treatment. This shows that there is some yield advantage in the incorporation of a fungal entomopathogen into an intercropping system. In both seasons, there were no significant differences in yields between cowpea intercrop treated with *M. anisopliae* and cowpea intercrop treated with Karate. Yield trends were however, not consistent within the monocrop. The effective dosage equivalent to 1×10^{12} conidia ha^{-1} used in our studies is within the range of dosages widely applied against insect pests in different agroecosystem (Rombach 1986, Maniania, 1993ab).

Several factors are probably responsible for the good level of control in the intercrop receiving fungal application. Intercropping is known to be associated with high relative humidity and increased light interception within the crop canopy (Kyamanywa and Ampofo, 1988; Terao *et al.* 1997). A high relative humidity is an essential factor for fungal infection in insects (Hall and Papierok, 1982; Benz, 1987). Possibly, the high relative humidity within the intercrop favoured the fungal infection process and this is evident from the large number of insects dying from mycosis

within this cropping system. The efficacy of entomopathogens in the field is also affected by high light intensities and radiation in the ultraviolet region is very harmful to conidia of entomopathogenic fungi (Moore and Prior, 1993; Smits *et al.* 1996). Increased light interception within the intercrop suggests that loss of activity through UV degradation is reduced, hence good level of control achieved with the pathogen.

Risk assessment of fungal pathogens on non-target organisms constitutes one of the most important criteria in the development of mycoinsecticides as biological control agents (Moore and Prior, 1993). The results of this study showed no significant adverse effect of the fungus on predator populations when compared with the controls. Rath *et al.* (1995) observed that application of *M. anisopliae* for the control of subterranean scarab, *Adoryphorus couloni* (Burmeister) in pasture had no adverse effect on non-target invertebrates. *Metarhizium flavoviride* Gams and Rozsypal have been reported to be safe to non-target Hymenoptera, Coleoptera and Homoptera (Ball *et al.* 1994; Lutte Biologique contre les Locustes et Sauteriaux (LUBILOSA), unpublished data). Entomopathogenic fungi are frequently more specific to target organisms under field conditions and especially during epizootic (Goettel and Johnson, 1992), and this specificity evidently reduces the level of hazard to non-target organisms. Therefore, the results of this study confirm the conclusion of Goettel *et al.* (1990) that fungal pathogens pose minimal risk to non-target invertebrates. The non-target organisms recorded in these studies are all known to be generalists predators feeding on thrips and other insects (Lewis, 1973; Matteson, 1982; Kyamanywa *et al.* 1993; Tamo *et al.* 1997). These predators together with the fungus may complement each other in the overall management of *M. sjostedti* because susceptibility to predation will also increase due to infection by the fungus (Thomas *et al.*, 1998).

In conclusion, the efficacy of *M. anisopliae* against *M. sjostedti*, especially within the intercrop coupled with its safety to non-target organisms suggests that the use of intercropping and fungal pathogen may be a compatible IPM strategy. This compatibility is a distinct advantage as it

would not only reduce the over dependence on broad spectrum synthetic insecticides but would also reduce the disruption to the natural enemy complex while promoting biodiversity and sustaining environmental quality. However, since this study used only a single cropping pattern of alternating cowpea rows with maize, further studies on the benefits of these two control strategies under various intercrop situations such as strip cropping are required before final recommendations on the integration of the two management practices can be made.

CHAPTER TEN

Timing of application and persistence of *Metarhizium anisopliae* for the control of *Megalurothrips sjostedti* on cowpea

10 Introduction

Damage by *M. sjostedti* begins at preflowering stage on leaf buds and bracts/stipules of the cowpea plant. As the flower buds and flowers begin to form, the pest migrate to these structures which become progressively damaged (Ezueh, 1981). Because of this nature of damage, timing of application of chemical insecticides based on crop growth stages has been recommended for *M. sjostedti* control on cowpea (Alghali, 1992; 1995). It is however, opined that timing of spray application of entomopathogens are likely to differ from those of chemical insecticides because pathogens are slower acting mortality agents.

In the field, insects can encounter infective propagules on leaf surfaces. Studies of the persistence of the fungus on non-host substrate are therefore important to its utilization because, the pathogen must remain viable to be effective on the target organism. This study was therefore, aimed at investigating the stage of cowpea plant at which *M. anisopliae* should be applied to minimize damage and yield loss, and also assess the persistence of the fungus on the surface of cowpea flowers and leaf buds which constitute the inhabiting sites of *M. sjostedti*. In this study, the Null hypothesis that application of entomopathogenic fungus given at different treatment regimes does not affect thrips population, damage and cowpea yield will be rejected if analysis of variance and/or mean separation show significant differences in thrips density, damage and cowpea yield among the various treatment regimes.

10.1 Materials and methods

10.1.1 Study sites

The study site is similar to that described in the previous chapter.

10.1.2 Fungus and production of inoculum

The fungus used and production protocols are same with the previous experiment. In viability test were conducted on Saboraud dextrose agar in 90 cm Petri dishes before field application, conidial germination varied between 85-90% after 24 h.

10.1.3 Timing of application

10.1.3.1 Field plots and treatments

Cowpea variety, ICV 2, was planted in a 4 x 5 m plots with intra-row spacing of 20 cm and inter-row spacing of 60 cm for two season: first season (September-December, 1997) and second season (March-July, 1998). Two applications of chemical insecticide (one at flower bud stage and another at flowering) are normally required for *M. sjostedti* control (Afun *et al.*, 1991; Alghali, 1992) and this was compared with various other spray regimes for *M. anisopliae*. Seven treatments were applied:

(1) 2 applications of *M. anisopliae* timed at 37 and 44 days after plant emergence (DAE) corresponding to 75-100% leaf bud formation and 40-60% flower bud formation.

(2) 2 applications of *M. anisopliae* timed at 44 and 51 DAE corresponding to 40-60% flower bud formation and 30-50% flowering.

(3) 2 applications of Karate® (Lambda-cyhalothrin) timed as in (2) above.

(4) 2 applications of *M. anisopliae* applied at 51 and 58 DAE corresponding to 30-50% flowering and 70-90% flowering.

(5) 3 applications of *M. anisopliae* timed at 37, 44, and 51 DAE

(6) 3 applications of *M. anisopliae* timed at 44, 51, and 58 DAE

(7) Control (water spray).

The treatments were arranged in a randomized complete block design with three replications. For spray application, conidia of *M. anisopliae* were suspended in water containing 0.05% Silwet L-77®. Nutrient agar (0.1%), glycerin (0.1%) and molasses (0.5%) were added to the inoculum as protectant and bait to complete the formulation (Maniania, 1993a). The concentration of inoculum was determined with a Malassez-cell-counting chamber. The fungus was applied at the rate of 1×10^{12} conidia ha^{-1} and Karate was applied at the recommended rate of 17.5 g a.i. ha^{-1}. The fungus and Karate were applied with a separate CP 15® knapsack sprayer at an output of 350 litres ha^{-1}. Controls were sprayed with water containing 0.05% Silwet, 0.5% molasses, 0.1% nutrient agar and 0.1% glycerin. Sprayings were done in the evenings between 17:00 h and 18:30 h to lessen the adverse effects of ultraviolet radiation (Moore and Prior, 1993).

10.1.3.2 Evaluation

Plots were sampled for *M. sjostedti* by randomly picking 20 leaf buds or flowers, depending on the stage of growth of the plant, from each plot into a vial containing 30% alcohol. Plots were sampled on 36, 43, 50, 57 and 64 DAE. Samples were taken to the laboratory and flowers were dissected and washed to separate the insects from the plant parts, and later counted.

To assess for mortality caused by *M. anisopliae*, 20 adult insects per plot were aspirated at random using a cotton plugged aspirator from flower samples which were also randomly picked from each plot. The samples were taken at 53 and 60 DAE. The insects were placed individually in screened transparent plastic vials (50 x 100 mm) and fed with surface sterilized floral tissues and pollen from untreated cowpea plots. The insects were maintained in a controlled environment room (26 ± 2^0C, 60 ± 5% RH and photoperiod of L:D 12:12). Mortality was recorded daily for 7 days.

Dead insects were transferred to Petri dishes lined with moistened filter paper. Mortality due to fungus was confirmed by microscopic examination of hyphae and spores on the surface of the cadaver.

Plant damage was assessed using the scale of Jackai and Singh (1988) on 10 plants tagged at random in each plot. Sampling dates for plant damage were the same as for thrips population density assessment. At harvest, yield data were recorded from the entire length of each row. Yield per plot was extrapolated to kg ha^{-1}.

10.1.4 Persistence

Seeds of cowpea variety, ICV 2, were sown in 0.85 litre plastic pots filled with soil and plants were left to grow in the open. At 50% flowering, they were sprayed with a fungal concentration of 1.0 x 10^7 conidia l^{-1} equivalent to 2.3 x 10^{12} conidia ha^{-1} and assuming an application volume of 350 litres ha^{-1} of finished spray used in the field trial. Silwet, molasses, nutrient agar and glycerin were added to the suspension. Control treatment consisted of water containing similar ingredients as the fungal suspension. Pots were placed outdoors to receive maximum incident sunlight. No rainfall was recorded during the trial period. Since thrips are encountered both on the leaf buds and flowers of cowpea, 5 leaf buds and 5 flowers were removed randomly per treatment as soon as the residue had dried (designated "day 0" after treatment) and subsequently at 1, 2, 3, 4, 5, 6, and 7 days after treatment. Collected leaf buds and flowers were placed in a sealed humidified containers and transported to the laboratory. For the bioassay, leaf buds and flowers were placed in rearing cages (30 x 95 mm) and 20 three-day old adult thrips were aspirated from a laboratory colony and transferred into the cages. Four replicates were maintained per treatment for each collection date. Thrips were allowed to feed on the leaf buds and flowers for 48 hrs after which they were fed on surface sterilized floral tissues for the remainder of the incubation period. Mortality was recorded daily for 7 days after exposure to treated plants.

10.1.6 Statistical analyses

The data were subjected to analysis for a randomized complete block design and means were separated by Student-Newman-Keuls using the ANOVA procedure of SAS (SAS Institute, 1985). Analysis of thrips count were based on data transformed to log (x + 1). Percentage mortality data were transformed by arcsine to standardize mean percentages before analysis.

10.2 Results

10.2.1 Timing of application

Application of *M. anisopliae* significantly reduced ($P<0.05$) *M. sjostedti* density in both seasons in all the treatment regimes but to varying degrees (Tables 27 and 28) (rejection of Null hypothesis). Application of the fungus timed at 37 and 44 DAE caused a gradual decline in thrips density during the first season (Table 28). This same regime was, however able to reduce thrips population for just one week (between 43-50 DAE) during the second season and thrips population increased drastically thereafter (Table 28). Compared to *M. anisopliae* treatments, application of Karate timed at 44 and 51 DAE caused a drastic reduction in thrips population in both seasons (Tables 27 and 28). *Metarhizium anisopliae* timed at 44 and 51 DAE and 37, 44 and 51 DAE showed no significant difference ($P>0.05$) in thrips population at 64 DAE in both seasons. In the treatment timed at 51 and 58 DAE, there was a general increase in population in both seasons until 50 DAE. This trend however fell immediately after the first treatment at 51 DAE (Tables 27 and 28). In both seasons, application timed at 44, 51 and 58 DAE gave the best control of thrips among the *M. anisopliae* treated plots (Tables 27 and 28).

Table 27. Effect of *Metarhizium anisopliae* and Karate application on *Megalurothrips sjostedti* population (Mean ± S.E. 20 flower-[1]) at various treatment regimes during the first season

	Days after plant emergence				
Treatment	36	43	50	57	64
Control	147.7 ± 15.7a	190.7 ± 17.5a	268.7 ± 31.3a	295.0 ± 21.3a	305.3 ± 19.3a
M. anisopliae at 37, 44	155.7 ± 11.8a	146.3 ± 11.4c	128.3 ± 14.1c	124.0 ± 14.1c	126.3 ± 10.1c
M. anisopliae at 44, 51	137.7 ± 19.3b	149.7 ± 13.5c	102.3 ± 9.6d	75.3 ± 9.6d	71.7 ± 9.3d
Karate at 44, 51	136.3 ± 17.3b	194.0 ± 18.6a	8.6 ± 1.3e	2.7 ± 0.7f	2.0 ± 0.8f
M. anisopliae at 51, 58	148.0 ± 10.7a	176.3 ± 20.1b	256.3 ± 21.4a	244.3 ± 18.6b	192.7 ± 13.1b
M. anisopliae at 37, 44, 51	159.0 ± 24.5a	157.7 ± 15.6c	142.0 ± 12.1b	115.0 ± 11.4c	83.0 ± 11.0d
M. anisopliae at 44,51,58	135.0 ± 18.1b	149.3 ± 23.3c	114.7 ± 9.7d	56.0 ± 8.6e	27.7 ± 3.7e

Means within a column bearing the same letter do not differ significantly by Student-Newman-Keuls (P=0.05).

Table 28. Effect of *Metarhizium anisopliae* and Karate application on *Megalurothrips sjostedti* population (Mean ± S.E. 20 flower-[1]) at various treatment regimes during the second season

	Days after plant emergence				
Treatment	36	43	50	57	64
Control	102.3 ± 11.3b	128.3± 24.5b	212.0 ± 21.4a	295.0 ± 19.4a	305.3 ± 28.3a
M. anisopliae at 37,44	109.0 ± 18.4b	94.3 ± 11.3c	82.3 ± 10.3d	134.0 ± 17.5b	161.7 ± 14.3b
M. anisopliae at 44,51	98.7 ± 9.4bc	136.0 ± 27.4a	105.3 ± 10.7b	65.3 ± 8.6d	59.0 ± 10.1d
Karate *at* 44,51	115.0 ± 21.3a	137.7 ± 18.7a	8.0 ± 1.1e	2.7 ± 0.5f	2.0 ± 0.3f
M. anisopliae at 51,58	101.0 ± 12.4b	134.7 ± 19.3a	167.0 ± 13.7b	118.7 ± 12.1c	87.7 ± 8.7c
M. anisopliae at 37,44,51	107.3 ± 10.7b	100.7 ± 12.1c	94.0 ± 9.7d	56.7 ± 10.3d	48.3 ± 9.4d
M. anisopliae at 44,51,58	98.3 ± 11.3bc	127.7 ± 14.3b	82.0 ± 10.1c	46.7 ± 10.7e	27.7 ± 7.4e

Means within a column bearing the same letter do not differ significantly by Student-Newman-Keuls (P=0.05).

Table 29. Incidence of *Metarhizium anisopliae* in adult *Megalurothrips sjostedti* following treatment: %mortality ± S.E.

Time of application	First season		Second season	
(DAE)	53 DAE	60 DAE	53 DAE	60 DAE
Control	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
37,44	51.4 ± 11.4b	40.3 ± 7.4b	47.3 ±7.5b	41.1 ± 8.7c
44,51	50.7 ± 9.6b	41.1 ± 8.6b	56.4 ± 11.1a	50.1 ± 10.3b
44,51*	–	–	–	–
51,58	47.1 ± 6.5b	56.4 ± 10.2b	45.6 ± 7.8b	58.3 ± 12.1b
37,44,51	63.1 ± 12.3a	66.1 ± 11.3a	60.4 ± 10.5a	68.3 ± 14.1a
44,51,58	53.1 ± 10.3b	67.3 ± 12.4a	61.7 ± 12.1a	66.5 ± 10.3a

Means within a column followed by the same letter do not differ significantly by Student-Newman-Keuls (P=0.05) test. *Karate treatment

Table 30. *Megalurothrips sjostedti* damage score in cowpea plots treated with *Metarhizium anisopliae* and Karate at various regimes during the first season

	Mean damage score ± S.E.				
	Days after plant emergence				
	36	43	50	57	64
Control	2.8 ± 1.1a	3.4 ± 1.2a	4.5 ± 1.3a	5.3 ± 1.1a	5.9 ± 1.3a
M. anisopliae at 37,44	2.5 ± 0.8a	2.5 ± 0.6b	2.2 ± 0.5c	2.6 ± 0.9c	2.8 ± 0.5b
M. anisopliae at 44,51	2.3 ± 0.9a	2.9 ± 0.9a	2.8 ± 0.3c	2.1 ± 0.3d	2.1 ± 0.6c
Karate at 44,51	2.6 ± 0.7a	3.0 ± 1.0a	1.3 ± 0.1d	1.0 ± 0.0e	1.1 ± 0.1d
M. anisopliae at 51,58	2.7 ± 1.1a	3.2 ± 1.1a	3.5 ± 0.8b	3.4 ± 0.9b	3.3 ± 1.1b
M. anisopliae at 37,44,51	3.1 ± 1.2a	2.8 ± 0.8a	2.8 ± 0.7c	2.6 ± 0.8c	2.4 ± 0.5c
M. anisopliae at 44,51,58	2.5 ± 0.5a	2.7 ± 0.8a	2.3 ± 0.4c	1.7 ± 0.1d	1.3 ± 0.3d

Means within a column bearing the same letter do not differ significantly by Student-Newman-Keuls (P=0.05).

Table 31 *Megalurothrips sjostedti* damage score in cowpea plots treated with *Metarhizium anisopliae* and Karate at various regimes during the second season

	Mean damage score ± S.E.				
	Days after plant emergence				
	36	43	50	57	64
Control	1.6 ± 0.3a	2.8 ± 0.8a	3.5 ± 0.7a	4.5 ± 1.0a	5.5 ± 1.2a
M. anisopliae at 37,44	1.7 ± 0.5a	1.8 ± 0.2bc	1.5 ± 0.3c	3.0 ± 0.8b	5.0 ± 0.9a
M. anisopliae at 44,51	1.5 ± 0.7a	2.9 ± 0.9a	2.3 ± 0.6b	2.0 ± 0.2c	1.8 ± 0.4c
Karate at 44,51	1.6 ± 0.5a	2.8 ± 0.8a	1.2 ± 0.2c	1.0 ± 0.0d	1.0 ± 0.0d
M. anisopliae at 51,58	1.4 ± 0.1a	2.6 ± 0.8a	3.5 ± 0.9a	3.1 ± 0.5b	2.9 ± 0.8b
M. anisopliae at 37,44,51	1.7 ± 0.3a	2.0 ± 0.7b	1.8 ± 0.4b	1.7 ± 0.2c	1.6 ± 0.3c
M. anisopliae at 44,51,58	1.5 ± 0.6a	2.8 ± 0.8a	2.0 ± 0.5b	1.5 ± 0.1c	1.1 ± 0.1d

Means within a column bearing the same letter do not differ significantly by Student-Newman-Keuls (P=0.05).

Table 32. Grain yield (kg ha^{-1}) in cowpea plots treated with *Metarhizium anisopliae* at various application regimes

Treatment	Mean yield ± S.E.	
	First season	Second season
Control	338.1 ± 31.0e	453.3 ± 27.6e
M. anisopliae at 37,44	387.5 ± 27.1e	494.7 ± 31.3e
M. anisopliae at 44,51	778.0 ± 37.5c	894.5 ± 48.3c
Karate at 44,51	1102.3 ± 98.7b	1231.6 ± 85.8b
M. anisopliae at 51,58	571.4 ± 47.5d	635.1 ± 36.1d
M. anisopliae at 37,44,51	806.3 ± 34.3c	902.8 ± 51.6c
M. anisopliae at 44,51,58	1441.3 ± 98.7a	1581.3 ± 103.2a

Means within a column bearing the same letter do not differ significantly by Student-Newman-Keuls (P=0.05) test.

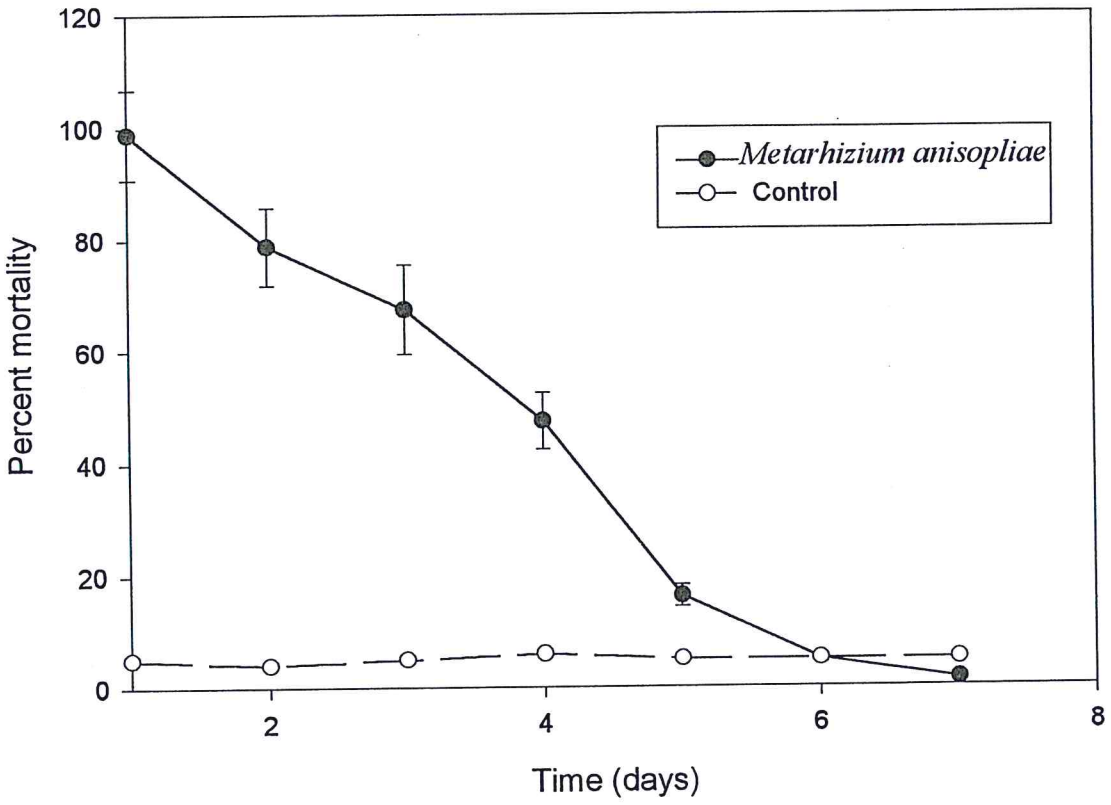


Figure 19. Persistence of *Metarhizium anisopliae* on cowpea foliage

Data in Table 29 shows the incidence of *M. anisopliae* in adult *M. sjostedti.* The incidence of mortality due to mycosis ranged from 47% to 63 % and 41 to 68% during the first and second seasons, respectively, in samples taken at 53 DAE. At 60 DAE, the incidence of mycosis ranged from 40% to 67% in the first season and 41 to 68% in the second season (Table 29).

Damage score also varied according to the treatment regimes in both seasons (Tables 30 and 31) (Rejection of Null hypothesis). There was a general reduction in damage with increased frequency of spray application. Application of the fungus timed at 37 and 44 dae recorded the highest amount of damage compared to other treated plots in both trials (Tables 30 and 31). There was a drastic reduction in damage in Karate treated plots compared with the *M. anisopliae* treatments, which caused gradual reduction in damage.

There was a significant difference in grain yield at various application regimes in both seasons (Table 32) (rejection of Null hypothesis). Grain yield was highest in *M. anisopliae* application timed at 44, 51 and 58 dae. Sprays applications of the fungus timed at 44 and 51 dae produced yield, which did not differ significantly from application timed at 37, 44 and 51 dae in both trials. Karate timed at 44 and 51 dae produced significantly higher yields compared with *M. anisopliae* receiving the same application regime. There was no significant difference in yield between application timed at 37 and 44 dae and the control in both seasons (Table 32).

10.2.2 Persistence

The study on the persistence of the fungus in terms of mortality in adult thrips showed that leaf/flower samples collected immediately after fungal application caused 100% mortality in *M. sjostedti* (Fig 19). Mortality was still high after 2 days but decreased thereafter. At the end of 5 days, persistence was less than 20% (Fig 19).

10.3 Discussion

Proper timing of application of insecticides is an important component of integrated pest management (Dent, 1991). Treatment application when made at the wrong time can lead to poor performance, resulting in the need for additional application at further cost to the grower. The different spray regimes tested in this study affected thrips densities and yield, and highlights the importance of timing of application of *M. anisopliae* for *M. sjostedti* control. Application of *M. anisopliae* at leaf bud stage and at flower bud stages alone does not protect yield because thrips population rebound at flowering. One application of *M. anisopliae* timed at flower bud (44 DAE) and another at flowering (51 DAE) also permitted some significant amount of damage to flowers but this same regime was enough to keep thrips populations in check for Karate application in both seasons. Three applications of the fungus corresponding to one at flower bud stage and two at flowering (44, 51 and 58 DAE, respectively) gave the highest grain yield. This suggests that spray application of *M. anisopliae* at leaf bud stage does not critically influence yield and application of *M. anisopliae* may not be necessary at this stage. The flower buds and flowers are also known to be particularly vulnerable to *M. sjostedti* attack (Ezueh, 1981; Akingbohungbe, 1982). Samples of thrips population density taken at 64 DAE in the treatment receiving three application of the fungus was significantly higher than in the Karate treatment, which received two applications. The final grain yield however indicated significantly higher yield in the fungus treatment compared with the Karate treatment. This observation agree with the studies of Afun *et al.* (1991) who showed that total insect killed by chemical insecticides on cowpea does not necessarily influence the optimum grain yields. It might however be interesting to determine the economic threshold level of *M. sjostedti* to assess the density at which control with *M. anisopliae* should be initiated to maintain the population below those causing economic injury level. A high number of adults insects aspirated from cowpea flowers and reared in the laboratory died from infection due to *M. anisopliae*. The

microclimate humidity within leaf/flower buds and flowers might have favoured the infection process (Benz, 1987), hence high number of insects dying from mycosis.

Studies of persistence showed that *M. anisopliae* remained active in the field for about 3-4 days. Most studies on infectivity of entomopathogenic fungi have revealed that conidia applied on foliage have short persistence (Gardner *et al.*, 1977; Ignoffo *et al.*, 1979; Daoust and Pereira, 1986). The radiation in the ultraviolet region is considered as the major factor affecting the survival of conidia on plant foliage (Smits *et al.*, 1996). Daoust and Pereira (1986) showed that conidia of *M. anisopliae* and *B. bassiana* persisted for only 1-2 days on cowpea leaves. The rate of inactivation observed in our study is slightly lower than those reported by these authors. Devi (1994) however, showed that conidia of *N. rileyi* persisted for 3-4 days on castor crop. Daoust and Pereira (1986) used fully formed cowpea leaves, which are attacked by cowpea leaf beetles, and the leaves were directly exposed to sunlight. In this study, flowers and leaf buds, which constitute the inhabiting sites of *M. sjostedti*, were used. Although flowers of the variety of cowpea used in this study were also exposed to sunlight, they open in the morning and evening and close in the daytime during periods of high light intensities. Since spray application was administered in the evening, the behaviour of the flowers ensures that spores, which are deposited inside the open flowers, are afforded some protection from direct sunlight. Cowpea leaf buds are also shaded from high light intensities by large leaves at the upper canopy. Under these circumstances, the plant parts evidently provides some protection to the conidia from inactivation by ultraviolet radiation, hence prolonged level of persistence. The position of conidial deposition and plant architecture have been reported to influence the rate of inactivation of infective propagules (Fargues *et al.*, 1988; Inglis *et al.*, 1993; James *et al.*, 1995).

In conclusion, this study has shown that *M. anisopliae* is a promising biological control agent for the legume flower thrips, if correct timing of spray application can be adopted. Two application of the *M. anisopliae* does not protect yield as does chemical insecticides. Instead, one

application of the fungus at flower bud stage and two applications at flowering are required to keep *M. sjostedti* population in check through these stages which are very sensitive to thrips damage. Because mass production of mycopathogen is easy (Prior, 1988) and cheaper substrate e.g. maize can be used for production of *M. anisopliae* (Maniania, 1993a), and since labour-cost in the developing countries are also cheap, production cost for mycoinsecticides can be lowered considerably below those of the conventional insecticides used for *M. sjostedti* management. For example, in this study, 1 kg of a substrate of maize/vermiculite could easily produce 1.6 kg of viable spores of *M. anisopliae* at the cost of around US$25.00. This is well within the range of production cost obtained by other workers (Langewald, per. comm.; Milner, per. comm.). Treatment of 1 ha of cowpea requires 1 kg of spores (1×10^{13} conidia ha^{-1}) at a production cost of approximately $16.00 (Ksh960.00). Treatment of 1 ha of cowpea with Karate requires 1litre of the chemical (17.5 g a.i. ha^{-1}) (Kenya On-label Approval) at the commercial cost of US$37.5 (Ksh2250.00) (Jumbo Agrovet, per. comm.). It is therefore likely that a commercial product of *M. anisopliae* should be cost competitive with conventional insecticide such as Karate which are used for *M. sjostedti* control. The low cost of production and high efficacy of the fungus will definitely contribute to an increase in the cost-benefit ratio of the control programme. This will not only help reduce the importation and use of rather expensive and toxic pesticides, but ensures that health hazard to the applicator as well as adverse effects on non-target beneficial organisms is reduced thus sustaining the quality of the environment. Further investigations are however, required on increasing the persistence of the pathogen in the field with ultraviolet protectants, as this might reduce the frequency of application and further minimize the cost of control to the farmer.

CHAPTER ELEVEN

General conclusions and recommendations

The results of the work presented here are the first of their kind to show the pathogenicity and field evaluation of entomopathogenic fungi for the control of legume flower thrips, *M. sjostedti*. Crop protection with biological insecticides are continually improving and increasing while problems associated with conventional insecticides is on the increase. Increasing regulatory constraints and cost of registration coupled with ecological risks associated with chemical insecticides makes the utilization of bioinsecticides more appealing today than in the recent past. By developing and commercializing biopesticides, the risk of misuse of synthetic pesticides among farmers who generally have inadequate knowledge of pesticide safety and who have insufficient means to purchase protective equipment is reduced. This work has shown that fungal pathogen can cause high mortality of *M. sjostedti* both in laboratory bioassays and in the field comparable to that achieved with a synthetic insecticide. There is therefore, a great potential for the use of mycopathogen for legume flower thrips control both as a stand alone control strategy and as a component of IPM. As observed in this study, the use of *M. anisopliae* could be compatible with the use of resistant varieties. Although it was observed that tolerant variety adversely affects the fungus, *M. sjostedti* reared on the resistant variety incurred significantly higher mortality compared with the susceptible variety. Further investigations on efficacy of this interaction under field condition require attention. The results of this study also revealed that *M. anisopliae* under intercropping condition enhance its efficacy against *M. sjostedti*. Since the pathogen has no adverse effect on non-target beneficial organisms, this is a distinct advantage in the developing countries where most peasant farmers grow their cowpea as an intercrop with various cereal crops. Three applications of the fungus commencing at flower bud stage should suffice to keep *M. sjostedti* population in check. Since the fungus persisted for a short time in the field, further studies on the

use of ultraviolet protectants to prolong persistence are needed. Additional studies on genetic manipulation could also increase the efficacy of the fungus under field conditions.

Application of modern agricultural technology, including that of crop protection, is necessary if the subsistence farmer is to increase his production output. However, acceptability of innovative technology by the small-scale farmer is an important factor in the development of that technology. Some farmers already appreciate the role of certain natural enemies, particularly predators in the reduction of pest population. The role of pathogens may as yet not have a clear impression of a pest-reducing factor in the mind of a farmer, probably because pathogens are slower acting mortality agents. Farmers must be made to understand that though pathogens act more or less slowly compared to chemical insecticides, the mere presence of the insect on the crop may not mean that the insect is causing an economic damage. As demonstrated in this study, the debilitating effect of infection in insect can reduce the amount of damage being done to the crop. Training of end-users is, therefore, necessary in the adoption of this technology. Further research to incorporate mycopathogen into *M. sjostedti* management programmes must also emphasize the involvement of farmers, extension services, national and non-governmental organizations and plant protection services in order to understand the socioeconomic constraints associated with the use of mycoinsecticides. Additionally, because research on entomopathogenic fungi has brought them closer to marketability, market evaluation and development are also required to assess markets and set cost criteria for use of mycoinsecticides at the local, national and regional levels.

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APPENDIX 1. LT_{90} values of different strains screened against adult *Megalurothrips sjostedti* at the concentration of 1 x 10^8 conidia ml^{-1} at 7 days post inoculation

Fungal species	Strain	LT_{90} (Fiducial limits)
Beauveria bassiana	ICIPE 48	14.6 (12.9-17.3)
	ICIPE 53	5.3 (5.0-5.5)
	ICIPE 59	9.5 (9.0-10.3)
	ICIPE 74	11.4 (14.8-15.3)
	ICIPE 78	14.0 (12.5-16.3)
	ICIPE 82	9.7 (9.2-10.4)
	ICIPE 83	10.4 (9.7-11.3)
	TP-GHA	6.1 (5.7-6.6)
Metarhizium anisopliae	ICIPE 18	11.8 (10.8-13.1)
	ICIPE 20	12.6 (13.8-15.2)
	ICIPE 30	6.3 (5.9-6.7)
	ICIPE 60	11.8 (10.8-13.1)
	ICIPE 62	7.0 (6.7-7.7)
	ICIPE 62	7.3 (7.0-7.5)
	ICIPE 66	6.7 (6.3-7.2)

APPENDIX 1 continued. LT_{90} values of different strains screened against adult *Megalurothrips sjostedti* at 7 days post inoculation at the concentration of 1×10^8 conidia ml^{-1} at 7 days post inoculation

Fungal species	Strain	LT_{90} (Fiducial limits)
Metarhizium anisopliae	ICIPE 67	8.2 (7.7-8.8)
	ICIPE 69	5.4 (4.9-5.9)
	ICIPE 74	4.9 (4.1-5.1)
	ICIPE 75	7.6 (7.1-8.3)
Verticillium lecanii	ICIPE 85	12.4 (13.4-18.3)
	ICIPE 86	17.6 (17.3-19.1)
Paecilomyces fumosoroseus	MY 613	(18.0 (17.9-18.3)

APPENDIX 2. LC_{90} values for the most active fungal strains against adult *Megalurothrips sjostedti* 7 days post inoculation

Fungal species	Strain	LC_{90} conidia ml^{-1} (Fiducial limit)
Beauveria bassiana	ICIPE 53	$2.5 \times 10^7 (1.8\text{-}3.9)10^7$
	TP-GHA	$4.6 \times 10^7 (4.0\text{-}6.8)10^7$
Metarhizium anisopliae	ICIPE 30	$5.3 \times 10^7 (3.2\text{-}6.7)10^7$
	ICIPE 66	$3.7 \times 10^7 (2.9\text{-}3.9)10^7$
	ICIPE 69	$4.5 \times 10^6 (3.3\text{-}5.7)10^6$
	ICIPE 74	$7.8 \times 10^6 (6.2\text{-}8.3)10^6$

APPENDIX 3. LT_{90} values of the most pathogenic strains screened against adult *Megalurothrips sjostedti* at different temperatures at concentration of 1 x 10^7 conidia ml^{-1}

Fungal species	Strains	15^{0}C	20^{0}C	25^{0}C	30^{0}C
B. bassiana	ICIPE 53	20.8 ± 3.4a	10.7 ± 1.7b	5.3 ± 0.8a	7.1 ± 0.9c
	TP-GHA	18.3 ± 3.1b	12.3 ± 1.8a	5.9 ± 0.7a	6.1 ± 0.8b
M. anisopliae	ICIPE 30	15.1 ± 2.1c	10.4 ± 2.1b	6.1 ± 1.1a	6.3 ± 0.7b
	ICIPE 66	10.7 ± 1.8d	9.8 ± 2.3b	6.4 ± 0.5a	12.5 ± 1.5a
	ICIPE 69	11.2 ± 2.1d	7.0 ± 0.5c	5.3 ± 0.3a	6.5 ± 0.5b
	ICIPE 74	19.6 ± 4.3b	10.3 ± 1.8b	5.2 ± 0.5a	7.2 ± 0.6c

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

APPENDIX 4. Slope of regression equation of the most pathogenic strains screened against adult *Megalurothrips sjostedti* at different temperatures at concentration of 1 x 10^7 conidia ml^{-1}

Fungal species	Strains	15°C	20°C	25°C	30°C
B. bassiana	ICIPE 53	0.53 ± 0.07*	0.43 ± 0.01*	1.53 ± 0.53**	0.32 ± 0.01ns
	TP-GHA	0.31 ± 0.03ns	0.71 ± 0.21*	1.72 ± 0.61**	1.67 ± 0.04**
M. anisopliae	ICIPE 30	0.41 ± 0.12*	0.53 ± 0.32s	1.63 ± 0.81**	2.11 ± 0.51**
	ICIPE 66	0.83 ± 0.08*	0.89 ± 0.07*	0.73 ± 0.03*	0.33 ± 0.06ns
	ICIPE 69	0.62 ± 0.37*	0.71 ± 0.24*	1.81 ± 0.07**	1.43 ± 0.31**
	ICIPE 74	0.41 ± 0.03*	0.52 ± 0.41*	1.01 ± 0.63*	1.18 ± 0.81*

t test on slope of regression; * significantly different from zero (P=0.05), ** significantly different from zero (P=0.01), ns = not significantly different from zero.

APPENDIX 5. LT_{90} values (in days) of the susceptibility of adult *Megalurothrips sjostedti* to *Metarhizium anisopliae* on three varieties of cowpea at different temperatures at concentration of 1×10^7 conidia ml^{-1}

Cowpea variety	Temperature 15^0C	20^0C	25^0C	30^0C
Insect Inoculation				
Resistant	14.3 ± 1.7c	12.6 ± 1.9c	5.8 ± 0.8c	5.7 ± 0.7c
Tolerant	27.6 ± 3.8a	28.4 ± 3.8a	25.7 ± 4.1a	25.6 ± 3.5a
Susceptible	18.1 ± 2.1b	15.2 ± 2.6b	8.1 ± 2.1b	7.6 ± 0.6b
Floral tissue inoculation				
Resistant	14.7 ± 2.1c	13.7 ± 1.6c	6.0 ± 1.6c	5.9 ± 0.7c
Tolerant	28.1 ± 4.3a	28.5 ± 3.5a	25.8 ± 3.7a	25.8 ± 3.7a
Susceptible	19.4 ± 2.4b	15.8 ± 1.9b	8.5 ± 1.3b	7.4 ± 0.5b

Means within a column followed by the same letter are not significantly different by Student-Newman-Keuls (P=0.05) test.

APPENDIX 6. Slope of regression equation on the susceptibility of adult *Megalurothrips sjostedti* to *Metarhizium anisopliae* on three varieties of cowpea at different temperatures at concentration of 1 x 10^7 conidia ml^{-1}

Cowpea variety	Temperature 15^{0}C	20^{0}C	25^{0}C	30^{0}C
Insect Inoculation				
Resistant	0.52 ± 0.02*	0.67 ± 0.25*	1.32 ± 0.61**	1.87 ± 0.04**
Tolerant	0.21 ± 0.03ns	0.32 ± 0.03ns	0.13 ± 0.07ns	0.12 ± 0.09ns
Susceptible	0.41 ± 0.79*	0.51 ± 0.54*	1.89 ± 0.09**	1.73 ± 0.03**
Floral tissue inoculation				
Resistant	0.53 ± 0.38*	0.73 ± 0.67*	1.10 ± 0.54**	1.01 ± 0.51**
Tolerant	0.31 ± 0.08ns	0.13 ± 0.03ns	0.27 ± 0.07ns	0.24 ± 0.03ns
Susceptible	0.48 ± 0.03*	0.67 ± 0.41*	1.21 ± 0.18**	0.86 ± 0.03*

t test on slope of regression; * significantly different from zero (P=0.05), ** significantly different from zero (P=0.01), ns = not significantly different from zero.

APPENDIX 7. LC_{90} values of the susceptibility of *Megalurothrips sjostedti* to *Metarhizium anisopliae* on different varieties of cowpea at different temperatures

Cowpea variety	Temperature 15°C	20°C	25°C	30°C
Insect inoculation				
Resistant	$7.3 \times 10^5 \pm 1.1 \times 10^5$c	$6.3 \times 10^5 \pm 0.4 \times 10^5$c	$4.3 \times 10^5 \pm 7.3 \times 10^4$c	$9.1 \times 10^4 \pm 0.4 \times 10^4$c
Tolerant	$5.6 \times 10^{12} \pm 6.1 \times 10^{11}$a	$6.1 \times 10^{11} \pm 0.1 \times 10^{11}$a	$4.1 \times 10^{11} \pm 0.3 \times 10^{10}$a	$3.5 \times 10^{10} \pm 0.3 \times 10^9$a
Susceptible	$2.4 \times 10^9 \pm 9.1 \times 10^8$b	$7.4 \times 10^7 \pm 0.2 \times 10^7$b	$1.3 \times 10^7 \pm 9.4 \times 10^6$b	$3.3 \times 10^6 \pm 1.0 \times 10^5$b
Floral tissue inoculation				
Resistant	$7.7 \times 10^5 \pm 8.3 \times 10^4$c	$8.4 \times 10^5 \pm 7.1 \times 10^4$c	$6.3 \times 10^5 \pm 8.9 \times 10^4$c	$2.3 \times 10^5 \pm 8.1 \times 10^4$c
Tolerant	$4.3 \times 10^{12} \pm 0.7 \times 10^{11}$a	$8.1 \times 10^{11} \pm 7.3 \times 10^9$a	$3.6 \times 10^{10} \pm 6.8 \times 10^9$a	$4.1 \times 10^{10} \pm 3.3 \times 10^9$a
Susceptible	$3.1 \times 10^9 \pm 7.1 \times 10^8$b	$2.3 \times 10^8 \pm 6.0 \times 10^7$b	$9.7 \times 10^6 \pm 0.8 \times 10^6$b	$5.1 \times 10^6 \pm 0.9 \times 10^6$b

For each bioassay, LC_{50} values within column followed by the same letter are not significantly different by SNK (P=0.05).

APPENDIX 8. Slope of regression equation of LC_{90} values on the susceptibility of *Megalurothrips sjostedti* to *Metarhizium anisopliae* on three varieties of cowpea at different temperatures

Cowpea variety	Temperature			
	15^0C	20^0C	25^0C	30^0C
Insect Inoculation				
Resistant	0.58 ± 0.04*	0.72 ± 0.23*	1.43 ± 0.57**	0.89 ± 0.06**
Tolerant	0.24 ± 0.02ns	0.22 ± 0.01ns	0.34 ± 0.07ns	0.13 ± 0.03ns
Susceptible	0.43 ± 0.09*	0.45 ± 0.41*	1.89 ± 0.05**	0.93 ± 0.06**
Floral tissue inoculation				
Resistant	0.45 ± 0.08*	0.77 ± 0.36*	1.14 ± 0.44**	1.44 ± 0.32**
Tolerant	0.21 ± 0.04ns	0.15 ± 0.01ns	0.23 ± 0.01ns	0.25 ± 0.01ns
Susceptible	0.44 ± 0.01*	0.67 ± 0.31*	1.28 ± 0.11**	0.96 ± 0.04**

t test on slope of regression; * significantly different from zero (P=0.05), ** significantly different from zero (P=0.01), ns = not significantly different from zero.

APPENDIX 9. Layout of six by six Quasi complete Latin square design

C	E	A	B	D	F
F	D	B	A	E	C
A	F	E	D	C	B
E	B	F	C	A	D
B	C	D	E	F	A
D	A	C	F	B	E

APPENDIX 10. Checklist of some registered entomopathogenic fungi

Pathogen	Trade name	Target pests	Country	References
Aschersonia aleyrodis	Aseronija®	Whiteflies Scales	U.S.S.R	Ferron *et al.*, 1991
Beauveria bassiana	Boverin®	*Leptinotarsa decemlineata*	U.S.S.R	Ferron, 1978
	Boverol®	*L. decemlineata*	U.S.S.R	Ferron *et al.*, 1991
	Ostrinil®	*Ostrinia nubilialis*	France	Riba, 1984
	Betel®	*Mahanarva posticata*	Brazil	Ferron *et al.*, 1991
	Naturalis®	Bollworm	U.S.A	Wright, 1993
	Basinil®	*Cydia pomonella*	China	Goettel *et al.*, 1990
		Neophotettix spp.	China	Goettel *et al.*, 1990
		Dendrolimus punctatus	China	Goettel *et al.*, 1990
		D. tabulaeformis	China	Goettel *et al.*, 1990
		Ostrinia furnacalis	China	Goettel *et al.*, 1990
	Mycotrol®	Grasshoppers	U.S.A.	Ferron *et al.*, 1991
Hirsutella thompsonii	Mycar®	*Phyllocoptruta oleivora*	U.S.A	Ferron, 1978

APPENDIX 10 continued. Checklist of some registered entomopathogenic fungi

Pathogen	Trade name	Target pests	Country	References
Metarhizium	Biocontrol®	Spittle bugs	Brazil	Goettel *et al.*, 1990
anisopliae	Biomax®	Spittle bugs	Brazil	Goettel *et al.*, 1990
	Combio®	Spittle bugs	Brazil	Goettel *et al.*, 1990
	Metabiol®	Spittle bugs	Brazil	Goettel *et al.*, 1990
	Metapol®	Spittle bugs	Brazil	Goettel *et al.*, 1990
	Metaquino®	Spittle bugs	Brazil	Goettel *et al.*, 1990
M. flavoviride	Green muscles®	Locust and Grasshoppers	Benin	Lomer *et al.*, 1997
Verticillium	Vertalac®	Aphids	U.K.	Zimmermann, 1986
lecanii	Mycotol®	Whiteflies	U.K.	Ferron *et al.*, 1991
	Thriptal®	*Thrips inconsequence*	U.K.	Goettel *et al.*, 1990

BIOGRAPHY

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9. PUBLICATIONS

1. Ekesi, S. and Onyeka, J.O.A. 1995. Potential biocontrol agents of *Acanthacris ruficornis* Fabricius (Othoptera: Acrididae) in Jos, Nigeria. pp. 56-58. In: *Insects and the Nigerian Environment. Proceedings of the 25th Annual Conference of Entomological Society of Nigeria, 5-8 December, 1993.* Obafemi Awolowo University, Ile-Ife, Nigeria (Uvah, I.I. and Badejo, M.A. Eds.). Osun, Nigeria.

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7. **Maniania, N.K., Ampong-Nyarko, K. Ekesi, S. and Sithanantham, S. 1998**. Microbial control of thrips. *1995-1997 ICIPE Annual Scientific Report*, pp.10-11. ICIPE Science Press, Nairobi.

8. **Ekesi, S. 1998.** Biology and seasonality of the Striped-bean weevil, *Alcidodes leucogrammus* Erichson in Samaru, Nigeria. *Journal of African Zoology* (in press).

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10. **Ekesi, S., Maniania, N.K., Ampong-Nyarko, K. and Onu, I. 1998.** Potential of the entomopathogenic fungus, *Metarhizium anisopliae* for the control of legume flower thrips, *Megalurothrips sjostedti* (Trybom) on cowpea in Kenya. *Crop Protection* 17: 661-668.

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10. MEMBERSHIP OF PROFESSIONAL BODY:

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