An autodissemination strategy using entomopathogenic fungi and kairomonal attractants for managing thrips on grain legumes

BK Mfuti

25076558

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Promoter:	Prof MJ du Plessis
Co-promoter:	Dr NK Maniania
Assistant Promoter:	Dr S Subramanian

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DEDICATION

To my beloved wife Hermane Julienne **Longi** and my two sons Divin-Regis **Kupesa** and Joyce-Marlon **Mfuti** for their constant affection and motivation,

To my father **Kupesa,** my mother **Kolingila** and the entire **Kupesa** family for their encouragement and moral support,

I dedicate this dissertation.

DECLARATION AND APPROVAL

Declaration by the candidate

This research project is my original work and has not been presented for a degree in any other university.

[12]**Mfuti Batoba Kupesa** Registration No: 25076558

Signature: ..... Date: 6/11/2015

Approval by supervisors

This research project has been presented for examination with our approval as supervisors.

[1] **Prof. Magdalena Johanna du Plessis**

Signature: ..... Date: 13/11/2015

[2] **Dr. Nguya Kalemba Maniania**

Signature: ..... Date: 06/11/2015

[2] **Dr. Sevgan Subramanian**

Signature: ..... Date: 06/11/2015

[1]*Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom, 2520, South Africa;*

[2]*International Centre of Insect Physiology and Ecology, PO Box 30772-00100, Nairobi, Kenya.*

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ABSTRACT

Grain legumes are among the key economical crops widely grown in western and eastern Africa as important sources of food and animal fodder. However, the production of grain legumes in Kenya is seriously affected by a complex of insect pests particularly thrips. Yield losses of 20 to 100% have often been reported in some areas. The bean flower thrips (BFT), *Megalurothrips sjostedti* is considered to be the most important thrips pest of grain legumes. Chemical control is still the main management strategy, with detrimental consequences on the environment, users and consumers. Entomopathogenic fungi (EPF) are among the most promising alternatives to chemical pesticides. Inundative sprays are the most common application techniques for EPF. Although efficient and environmentally safe, the performance of entomopathogenic fungi is affected by several environmental parameters such as UV light, temperature, drought and rain. In order to improve the efficacy of EPF, an autodissemination system has been developed for the management of thrips in greenhouses. In this system, thrips are attracted to an autoinoculator where they are infected with an EPF before returning to the environment to disseminate the EPF to conspecifics. It therefore provides promising prospects, but for effective control, the conidial persistence and thrips attraction need to be optimized, while the EPF and the semiochemical should be compatible. The objective of this study was therefore to optimize the autodissemination system for thrips management on grain legumes in Kenya.

The semiochemical Lurem-TR, has been found to inhibit conidia of EPF when put together in an autoinoculation device. The effect of spatial separation of Lurem-TR on the persistence of conidia of EPF, *Metarhizium brunneum* and *Metarhizium anisopliae* was therefore evaluated to

develop an autodissemination strategy for the management of *M. sjostedti*. Influence of spatial separation of the semiochemical on thrips attraction and conidial acquisition by thrips from the autoinoculation device was also investigated in the field. This study showed that conidia persistence of both fungal species increased with distance of separation from Lurem-TR. Attraction of thrips to the device also varied significantly according to distance between the device and semiochemical. More thrips were attracted when Lurem-TR was placed in a container below the device and at 10 cm distance from the device. Conidial acquisition by thrips was not significantly different between spatial separation treatments of conidia and Lurem-TR.

Seven alternative thrips attractants, namely 4-anisaldehyde, ethyl benzoate, cis-jasmone, linalool, methyl anthranilate, trans-caryophyllene and phenylethanol were also screened for their compatibility with *M. anisopliae* ICIPE 69 in autodissemination devices and for their attraction to *M. sjostedti* in the field. Methyl anthranilate (MA) was found to be the attractant most compatible with *M. anisopliae* and its attractiveness to *M. sjostedti* was similar to that of Lurem-TR.

The performance of the attractant, methyl anthranilate, was compared to the commercial attractant Lurem-TR in autoinoculation devices treated with *M. anisopliae* under field conditions for two seasons. Densities of *M. sjostedti* in plots with the two semiochemical-baited autoinoculation devices were less than in the control plots during both experimental seasons. Plots with MA-baited and Lurem-TR-baited devices had similar densities of *M. sjostedti* during both seasons. However in the second season thrips densities in plots with the Lurem-TR-baited devices did not differ significantly from the control plots. Conidial viability of *M. anisopliae* was

significantly higher in semiochemical-free baited devices (control) than in semiochemical-baited devices in both seasons. Conidial germination decreased over time in all the treatments but remained above 45%, 12-15 days post-exposure. The average number of conidia acquired by a single *M. sjostedti* ranged between 2.0 and 10.0 x 10^3 conidia in both semiochemical-baited device treatments during both seasons. Significantly more conidia were acquired by single thrips in MA-baited devices compared to Lurem-TR baited devices during the podding stage of the crop during the second season. Significantly higher mortality of *M. sjostedti* was caused in field plots by Lurem-TR baited and MA-baited autoinoculation devices compared to mortality of *M. sjostedti* collected from the control plots in both seasons. Cowpea yield also differed significantly between the treatment plots. The highest yield was recorded in plots where MA-baited devices were placed. From this study, it could therefore be recommended that methyl anthranilate be used in autoinoculation devices for the management of *M. sjostedti* on grain legumes. The success achieved with MA in these trials resulted in the evaluation of this EPF for possible use in a spot spray strategy.

The efficacy of spot spray and cover spray applications of *M. anisopliae* in combination with the thrips attractant Lurem-TR was compared in field experiments for the management of *M. sjostedti* on a cowpea crop in two seasons. Plants in the treatment plots where a spot spray application of *M. anisopliae* was done five days after the placement of Lurem-TR recorded the lowest densities of *M. sjostedti*. Fungal viability and thrips conidial acquisition did not differ between the two application methods. Compared to the control treatment plots, both application strategies resulted in yield increases of 34.1 and 46.2% with spot and cover spray treatments,

respectively. The cost benefit analysis suggests that the spot spray application was more profitable due to the reduction in labour and the quantity of inoculum used.

Key words: Biological control, cowpea, entomopathogenic fungus, grain legumes, lure and infect, *Megalurothrips sjostedti*, semiochemicals, thrips

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Grain legumes are cultivated on an estimated 27 million ha in Sub-Saharan Africa (SSA) with an estimated yield of 19 million metric tons (MT). In South Asia, 40 million hectares are planted with an estimated yield of 43 MT. The export value of grain legumes expressed in terms of global exports is estimated at 0.4% in SSA and 2% in South Asia, respectively (Abate *et al*., 2012). These grain legumes (Fabales: Fabaceae) include common beans, *Phaseolus vulgaris* L., cowpea *Vigna unguiculata* (L.) Walp. and pigeonpea, *Cajanus cajan* (L.) Willsp. These crops play an important role in tropical cropping systems in SSA (Singh and Van Emden, 1978). They are major sources of plant proteins, vitamins and animal fodder (Tarawali *et al*., 1997; Asiwe, 2009). Cowpea is among the most consumed grain legumes in eastern Africa (Uganda, Kenya and Tanzania) (Abate *et al*., 2012).

Insect pests, especially thrips, are regarded as mainly responsible for the low yield of grain legumes (Rachie, 1985; Jackai and Daoust, 1986; Abate and Ampofo, 1996). Thrips have a very short life cycle and overlapping of generations is frequently observed (Mac Donald *et al*., 1998). High infestation levels may result in complete grain yield losses if no control measures are taken (Asiwe *et al*., 2005). The most common thrips species (Thysanoptera: Thripidae) on cowpea in East Africa include the bean flower thrips (BFT), *Megalurothrips sjostedti* Trybom, *Frankliniella occidentalis* Pergande, *Frankliniella schultzei* Trybom and *Hydatothrips aldolfifriderici* Karny (Singh and Allen, 1979).

Chemical control is the main strategy for the management of thrips on grain legumes. However, most of the chemicals insecticides are toxic to humans and hazardous to the environment (Oparaeke, 2006; Nderitu *et al*., 2007). The effectiveness of synthetic chemicals is constrained and debatable due to the development of resistance to pesticide among thrips (Jensen, 1998; 2004; Espinosa *et al*., 2002), emergence of secondary pests (Graham-bryce, 1977) and the presence of toxic residues in the crop produce (Mitchell and Lykken, 1963). Hence, there is an urgent need for research on environmental-friendly alternatives.

Entomopathogenic fungi (EPF) are among the alternatives being considered (Ekesi *et al*., 2002). A *Metarhizium anisopliae* (Metchnikoff) Sorokin based biopesticide has been developed by *icipe* and was commercialized for thrips control by Real IPM (www.realipm.com; Ekesi *et al*., 2009). EPF are generally applied using the conventional insecticide application approach, *e.g.* inundative sprays. However, this approach has a number of disadvantages including short persistence of the inoculum due to detrimental effects of solar radiation and high costs as a result of repeated applications and high volume of inoculums required (Inglis *et al*., 2000; Leland and Behle, 2004; Jaronski, 2010).

Thrips generally respond to colour, odour, and shape (Terry, 1997; Teulon *et al*., 1999; Mainali and Lim, 2011). Coloured sticky traps were developed for monitoring of thrips in ornamental orchards and greenhouses (Cho *et al*., 1995) and blue sticky traps have been found to be the most attractive to *M. sjostedti* (Muvea *et al*., 2014). Semiochemicals (aggregation, pheromones or allelochemicals) have also been shown to attract thrips. For example, a commercial product Lurem-TR, whith the active compound, methyl isonicotinate, increase thrips catches up to 30

fold (Davidson *et al*., 2007; Teulon *et al*., 2010). Subsequently, the combination of semiochemicals and coloured sticky traps has become an important IPM tool for the management of thrips (Muvea *et al*., 2014; Niassy *et al*., 2012; Mfuti *et al*., 2016). Since EPF can be transmitted horizontally (Dimbi *et al*., 2013), their integration with semiochemicals provides new opportunities for use in an autodissemination /"lure and infect" device. This approach could be improved further to sustain both the thrips attraction and conidial persistence ensuring compatibility between EPF and the semiochemical.

The presence of the semiochemical Lurem-TR in an autodissemination device has been reported to have a negative effect on the viability of conidia of *Metarhizium anisopliae* (Metchnikoff) Sorokin (Niassy *et al*., 2012). This finding led to the current study to investigate the compatibility between *M. anisopliae* and Lurem-TR in the autodissemination.

1.2. Problem statement and justification

Biological control using predators and parasitoids is effective in screen houses but not in open fields. The use of EPF is therefore considered as a component for integrated thrips management under field conditions.

EPF are generally applied through an inundative approach, which requires high volumes of inoculum, resulting in high costs. In addition, the short persistence of the inoculum in the field as a result of breakdown by solar radiation necessitates frequent applications, which further increases the cost. The high cost of biopesticides in general has always been considered as one of the limiting factors for their adoption by the small-scale farmers (Samuel and Graham, 2003). A

strategy by which insects are infected by a pathogen after being attracted to a semiochemical-baited inoculation device containing it, and disseminating the pathogen to other insects in the population after its return to the environment, could address the shortcomings described above. Alternatively, EPF could be used in combination with a semiochemical in spot spray applications, thereby reducing the quantity of inoculum needed and the resultant cost thereof.

1.3. Objectives

1.3.1 General objective

To develop efficient, economical and sustainable strategies for the management of thrips on grain legumes using a "lure and infect'' approach

1.3.2 Specific objectives

The specific objectives were:

- To investigate the compatibility between *M. anisopliae* and Lurem-TR in an autodissemination device for thrips management on grain legumes, using distance and time of separation
- To identify other potential attractants that could be compatible with *M. anisopliae*
- To evaluate the performance of the selected attractant in an autodissemination device for the management of thrips on grain legumes
- To evaluate the efficacy and cost benefit of spot spray and cover spray applications of *M. anisopliae* through the use of the attractant Lurem-TR for the management of *M. sjostedi* on cowpea crops

1.3.3 Research Hypotheses

- Distance separation of Lurem-TR from *M. anisopliae* in an autodissemination device will enhance their compatibility and infectivity for thrips management on grain legumes.
- Thrips attractants (other than Lurem-TR) are compatible with *M. anisopliae.*
- Alternative attractants to Lurem-TR will perform as well as Lurem-TR in an autoinoculation device for the management of thrips on grain legumes.
- Spot spray and cover spray applications of *M. anisopliae* in combination with thrips attractants are effective and can be used for the management of *M. sjostedti* on cowpea.

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CHAPTER 2: LITERATURE REVIEW

2.1 Thrips taxonomy and identification

Thrips belonging to order Thysanoptera, are present worldwide and only 5000 of an estimated 8000 extant species have been described (Mound and Houston, 1987). The Thysanoptera are divided into two suborders: Terebrantia and Tubilifera (Lewis, 1997; Mound, 2009). Most pest thrips species found on grain legumes belong to the suborder: Terebrantia. Among them, bean flower thrips (BFT), *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae) is the most common thrips species found on cowpea (*Vigna unguiculata* L. Walp) in tropical Africa (Moritz *et al*., 2013).

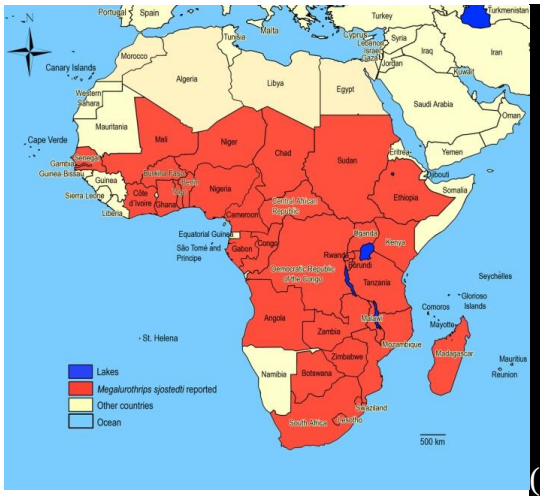
2.2 Geographical Distribution

Thrips are widespread throughout the world and are found in various habitats including forests, grasslands, and areas of low vegetation and deserts as well as on most cultivated crops. The different thrips species can be classified as phytophagous, carnivorous species, gall-makers or inquilines (Lewis, 1973). Species that feed on a wide range of plants and are crop pests are mostly in the family Thripidae (Moritz *et al*., 2004). Some flower thrips reproduce in flowers and feed on the cells of the flower tissue, on pollen grains and on small developing fruits (Mortiz *et al*., 2004). Many of the flower-dwelling species are partly predatory on small insects whilst other species primarily feed on leaves (Lewis, 1973; Mortiz *et al*., 2004).

The Bean flower thrips, *M. sjostedti* occurs throughout tropical Africa (Figure 2.1) (Singh and Van Emden, 1978; Moritz *et al*., 2013). Adults can prevail in the dry savannah throughout the

year (Bottenberg *et al*., 1997), indicating a much higher degree of adaptability to unfavourable conditions, which might be a consequence of their capability to feed and reproduce on more diverse types of plants (Tamò *et al*., 1993). Legumes (Fabales: Fabaceae) are the main host plants of *M. sjostedti* and include cowpea [*V. unguiculata*], pigeon pea [*Cajanus cajan* (L.) Willsp], common beans/French beans [*Phaseolus vulgaris* L.] (Tamo *et al*., 1993; Moritz *et al*., 2013). They also attack other plant species which are considered as minor hosts such as groundnut [*Arachis hypogaea*] (Tamo *et al*., 1993) and wild host plants (Tamo *et al*., 1997).

(a)



(b)

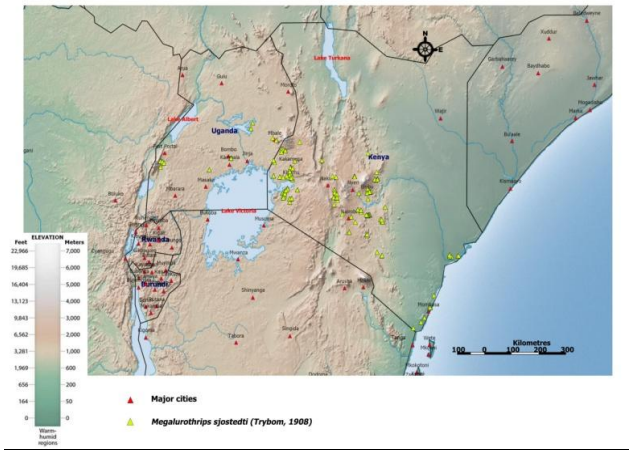


Figure 2.1: Geographic distribution of bean flower thrips, *Megalurothrips sjostedti* in Africa (a) and in east Africa (b) (Moritz *et al*., 2013).

2.3 Biology

Development rate of thrips is highly dependent upon environmental conditions and nutrient quality of their food sources (Mound, 1997). All described genera of thrips are haplodiploid organisms capable of parthenogenesis, with some favoring arrhenotoky (unfertilized eggs develop into males) and others, thelytoky (unfertilized eggs develop into females) (Lewis, 1997; Kumm and Moritz, 2008).

Thysanoptera species are hemimetabolous insects with an incomplete metamorphosis (Mound, 1997; 2005). Females of *M. sjostedti* undergo a pre-oviposition period which lasts from a day to a week during which their eggs mature, and before they mate. Although mated females of *M. sjostedti* laid eggs that produce both sexes, a very high percentage of their offspring is females (Lewis, 1997; Kumm and Moritz, 2008). The life cycle has six distinct stages. Eggs are very tiny (0.25 mm long and 0.1 mm wide). They are white when freshly laid and turn pale yellow toward maturation. Eggs are usually laid singly inside the plant tissue, and are therefore not visible (Lewis, 1997). They hatch within 3 to 20 days, depending on temperature.

The first and second instars are very small (0.5 to 1.2 mm). They are wingless and usually lighter in colour than the adults. The larval stage lasts for 8 to more than 20 days in total, followed by non-feeding prepupal and pupal stages. The pupal stages are usually completed in the soil at the base of the plant. After 3 to 6 days, the adult thrips emerge (Mound and Kibby, 1998). Although most adult thrips possess long fringed wings, wingless adults also occur (Lewis, 1973; 1997; Mound, 1997).

2.4 Economic importance of thrips

Yield losses caused by *M. sjostedti* have been estimated to be between 20 and 100% in various parts of Africa (Singh and Allen, 1980). In Kenya for instance, 94% yield loss has been reported on cowpea (Ampong-Nyarko *et al*., 1994).

Thrips damage mainly the floral parts (flowers, buds and pods) of plants. Infested flower buds become brown and eventually abort leaving behind dark red scars (Singh *et al*., 1997). Damaged flowers are characterized by distortion, malformation and discoloration of floral parts (Singh and Van Emden, 1978).

Figure 2. 2: Female of the bean flower thrips *Megalurothrips sjostedti*.

2.5 Control strategies for thrips

2.5.1 Chemical control

Chemical insecticide application is the most widely used thrips control method. Diverse insecticides such as chlorpyrifos-methyl, methiocarb, methamidophos, acrinathrin, endosulfan, deltamethrin and formetanate are often used (Singh and Rachie, 1985; Jackai and Adalla, 1997). However, development of pest resistance to insecticides has resulted in higher dosages and more frequent insecticide applications with more environmental hazards and negative effects on

human, environment and non-target insect species. Rotation of insecticides with different modes of action has been suggested to reduce pest resistance (Alghali, 1992) but not all the farmers can afford it.

2.5.2 Intercropping

Cultural control practices such as intercropping have been reported to reduce *M. sjostedti* infestations on crops (Kyamanywa and Ampofo, 1988; Kyamanywa and Tukahirwa, 1988; Kyamanywa *et al*., 1993; Ampong-Nyarko *et al*., 1994). For example, yield loss caused by *M. sjostedti* was reduced from 94% to 51% in cowpea/sorghum intercrop which also received chemical treatment (Ampong-Nyarko *et al*., 1994). Ekesi *et al*. (1999) also reported reductions in *M. sjostedi* numbers by 72 and 96% in cowpea monocrop and cowpea intercrop treated with *M. anisopliae*, respectively.

2.5.3 Thrips monitoring and trapping

Early detection of thrips infestation could be crucial for their successful control. Visual inspection by tapping plants on a tray or checking flowers at regular time intervals are often used (Pearsall and Myers, 2000). Thrips monitoring should be done at least once a week, and more often when an infestation is detected. Coloured sticky cards are currently the best monitoring tool for thrips populations (Plimmer *et al*., 1982; Cho *et al*., 1995; Koschier *et al*., 2000; Muvea *et al*., 2014). Blue and yellow are the colours mostly recommended (Blumthal *et al*., 2005; Muvea *et al*., 2014). It is recommended that sticky traps should be placed above the crop canopy so that the bottoms of the traps are just above the crop, at a rate of one or two traps per 1,000 square feet (Greer and Diver, 2000). Regular monitoring is crucial for effective control.

2.5.4 Semiochemicals

Thrips respond to olfactory cues (pheromones, semiochemiclas or allelochemicals) (De Kogel and Koschier, 2003; Kirk and Terry, 2003; Hamilton *et al*., 2005; Muvea *et al*., 2014). Subsequently, semiochemical-based products such as Lurem-TR and Thripline have been developed for use in thrips monitoring and management (Sampson and Kirk, 2013; Teulon *et al*., 2014; Broughton *et al.*, 2015). These semiochemicals can be integrated with other control strategies to improve thrips management in horticulture (Suckling *et al*., 2012; Sampson and Kirk, 2013). Lurem-TR is a commercial semiochemical whose active ingredient is methyl-isonicotinate. It was previously reported to be effective in monitoring *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Davidson *et al*., 2007) and several other pest thrips (Nielsen *et al*., 2010). More recently, it has been also reported to be effective against *M. sjostedti* populations (Muvea *et al*., 2014; Mfuti *et al*., 2016).

2.5.5 Biological control

2.5.5.1 Predators

Larvae of thrips are easy prey for a wide range of general arthropod predators but those more specific to thrips include members of the Aeolothripidae, the anthocorid genera *Orius* and *Montandoniola*, the Cecidomyiid genus *Thripsobremia* and the Sphecidae genus *Microstigmus* (Mills, 1991). Some of them are commercially available and are currently used as biological control agents in a variety of crops (Driesche *et al*., 1998; Van Lenteren and Loomans, 1998; Loomans, 2003). In Africa, Fritzsche and Tamo (2000) reported *Orius albidipennis* Reuter (Heteroptera: Anthocoridae) to be a natural enemy of *M. sjostedti* on cowpea and other host plants.

2.5.5.2 Parasitoids

Parasitoid species identified for *M. sjostedti* control include *Ceranisus menes* Walker (Hymenoptera: Eulophidae), (Diop, 1999), *C. femoratus* Gahan (Hymenoptera: Eulophidae) (Tamo *et al*., 1997; 2012), *Megaphragma priesneri* Kryger (Hymenoptera: Trichogrammatidae) and *M. mymaripenne* Timberlake (Hymenoptera: Trichogrammatidae) (Tamo *et al*., 1993; Loomans, 2003; Noyes, 2014).

2.5.5.3 Entomopathogenic fungi

Entomopathogenic fungi (EPF) are among the entomopathogens being considered for biological control of thrips (Butt and Brownbridge, 1997; Ekesi and Maniania, 2002). EPF are generally applied through inundative sprays, which require high quantities of inocula, thereby encreasing its cost (Jaronski, 2010). The persistence of conidia applied on foliage is influenced by several environmental parameters such as UV light, rain, temperature (Inglis *et al*., 2000; Jaronski, 2010), which necessitates an improvement in application technique. There are many published reports on successful control of thrips by EPF (Ekesi *et al*., 1998, 1999; Maniania *et al*., 2003). A number of fungus-based products are now registered or marketed for the control of thrips worldwide (Faria and Wraight, 2007; Lacey *et al*., 2015). In Kenya, an isolate of *M. anisoplaie* ICIPE 69 is commercialized for the control of thrips by RealIPM (www.realipm.com; Ekesi *et al*., 2009).

2.5.5.4 Current strategies for delivery of entomopathogenic fungi in the field

Entomopathogenic fungi are generally applied using inundative sprays similar to the conventional insecticide application approach (Jaronski, 2010). However, this technique has a number of shortcomings including the use of high volumes of inoculum, short persistence in the field due to breakdown by solar radiation which leads to repeated applications that are too expensive (Fargues *et al*., 1996; Inglis *et al*., 2000; Jaronski, 2010). Responses of insects to visual and olfactory cues are exploited for their management. For example, semiochemicals are used to lure large numbers of insects into a trap, inoculated with EPF as with termites (Alves *et al*., 2002). This strategy has led to the concept of autodissemination/autoinoculation. It consists of a semiochemical-baited inoculation device containing the pathogen. The insects are attracted to the device. On entering, they are infected with the pathogen and on return to the environment they disseminate the pathogen among the insects in the population (Vega *et al*., 2007). This strategy has been developed against a number of insects including fruit flies, *Ceratitis* spp. (Diptera: Tephritidae) (Dimbi *et al*., 2003), tsetse flies, *Glossina* spp. (Diptera: Glossinidae) (Maniania, 1998, 2002), pea leafminer, *Liriomyza huidobrensis* (Diptera: Agromyzidae) (Migiro *et al*., 2010) and recently against *F. occidentallis* (Thysanoptera: Thripidae) (Niassy *et al*., 2012). The cost of this technique is low in comparison to cover-spray applications. The integration of pheromones and kairomones in thrips management (Teulon *et al*., 2014; Broughton *et al*., 2015) therefore offers new perspectives for application of EPF for the control of thrips (Niassy et *al*., 2012; Mfuti *et al*., 2016).

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CHAPTER 3: SPATIAL SEPARATION OF SEMIOCHEMICAL LUREM-TR AND ENTOMOPATHOGENIC FUNGI TO ENHANCE THEIR COMPATIBILITY AND INFECTIVITY IN AN AUTOINOCULATION SYSTEM FOR THRIPS MANAGEMENT

Abstract

The effect of spatial separation of the semiochemical Lurem-TR, which has been found to inhibit conidia of entomopathogenic fungi when put together, on the persistence of conidia of *Metarhizium brunneum* and *M. anisopliae* was evaluated in the greenhouse and field in order to develop an autodissemination strategy for the management of bean flower thrips, *Megalurothrips sjostedti* on cowpea crop. Influence of spatial separation of the semiochemical on thrips attraction and conidial acquisition by thrips from the autoinoculation device was also investigated in the field. Persistence of conidia of *M. brunneum* and *M. anisopliae* increased with distance of separation of Lurem-TR. Direct exposure of fungus without separation from Lurem-TR recorded the lowest conidial germination as compared to the other treatments. Attraction of thrips to the device also varied significantly according to distance between device and semiochemical, with a higher number of thrips attracted when Lurem-TR was placed in a container below the device and at 10 cm distance. There was no significant difference in conidia acquisition between spatial separation treatments of conidia and Lurem-TR. Attraction of other insect pests to the device did not significantly vary between treatments. Positive correlations were found between conidial acquisition and thrips attraction. This study suggests that spatial separation of fungal conidia from Lurem-TR in an autoinoculation device could provide a low-cost strategy for effective management of thrips in grain legume cropping systems.

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3.1 Introduction

Grain legumes are among the key economical crops widely grown in eastern and western Africa as important sources of food and animal fodder (Abate *et al*., 2012; Tarawali *et al*., 1997). In Kenya, the annual bean production is estimated at 577,674 MT (USAID, 2013). However, the production of grain legumes is compromised by a complex of insect pests such as the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae), bean stem maggots, *Ophiomyia* spp. (Diptera: Agromyzidae), aphids (Hemiptera: Aphididae) and thrips (Thysanoptera: Thripidae) (Abate and Ampofo, 1996). Among the thrips, the bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae), is considered as the most important pest attacking the reproductive structures of grain legumes (Tamò *et al*., 1993). Damage by *M. sjostedi* includes early flower blemishes, abscission and necrosis with yield losses ranging between 20 to 100% (Singh and Allen, 1980).

Thrips are difficult to control owing to their cryptic flower dwelling behaviour and their minute size (Lewis, 1997). Chemical control is the most widely adopted management strategy by farmers who often resort to using obsolete or banned chemical pesticides with detrimental consequences to human, environmental and animal health (Nderitu *et al*., 2007). The introduction of stringent regulations by European importing countries such as the Maximum Residue Limit (MRL) has led to several crop rejections and economical losses. In addition, thrips have developed resistance to most of the chemical insecticides and hence the need to explore other control strategies including biological control (Brødsgaard, 1994; Espinosa *et al*., 2002; Jensen, 1998; 2004).

Entomopathogenic fungi (EPF) are among the most promising alternatives to synthetic chemical pesticides (Butt and Brownbridge, 1997; Ekesi *et al*., 2001; Niassy *et al*., 2012b). Fungal-based biopesticides for control of thrips are commercially available and include *Metarhizium anisopliae* (Metschnikoff) Sorokin ICIPE 69 marketed as Campaign® by the RealIPM, Kenya. The most common application technique of EPF is through inundative sprays (Hajek and St-Leger, 1994). However, EPF conidia applied on foliage have short persistence owing to environmental factors such as UV light, temperature and rain (Daoust and Pereira, 1986; Hong *et al*., 1999; Inglis *et al*., 2000; Jaronski, 2010). For instance, Ekesi *et al*. (2001) reported persistence of *M. anisopliae* conidia for 3-4 days on cowpea leaves. Such short persistence in the field requires frequent applications of EPF, resulting in higher inoculum requirement and high costs. Another application technique referred to as autodissemination or autoinoculation consisting of attracting insects to an autoinoculator where they are infected with a pathogen before returning to the environment to disseminate the pathogen to conspecifics is also being

considered (Vega *et al*., 2007). This approach has already been tested against *Frankliniella occidentalis* Pergande on French bean (Niassy *et al*., 2012a) and is based on combined use of visual cues (blue color), the semiochemical attractant Lurem-TR and the entomopathogenic fungus *M. anisopliae*. However, Lurem-TR was found to negatively affect conidial germination and infectivity of *M. anisopliae* in field cages (Niassy *et al*., 2012a). Introduction of Lurem-TR in a dessicator containing a culture of *M. anisopliae* resulted in complete inhibition of its germination after 48 hrs, confirming field results (S. Niassy, pers. observation). In order to improve the performance of autodissemination device for thrips management, we explored the effect of distance separation of Lurem-TR from fungal conidia on the persistence of *M. brunneum* in greenhouse and *M. anisopliae* under field conditions. We also evaluated the influence on thrips attraction and conidial acquisition in various distance separation treatments under field conditions.

3.2 Materials and methods

Study site

The study was conducted in the greenhouse at Plant Research International, Wageningen, The Netherlands (51.986: 5.663, 13 m above sea level) (T = 20 °C, 16:8 L:D photoperiod), and in the field of Kamiti, Kiambu County, Kenya (1.191S: 36.883E, 1640 m above sea level) and at *icipe*, Nairobi (1.221S, 36.896E; 1616 m above sea level). In the greenhouse, the experiments intended to assess the effect of Lurem-TR on the persistence of *M. brunneum* while experiments in the field assessed the effect of Lurem-TR on the persistence of *M. anisopliae* strain ICIPE 69, attraction of thrips and other insects, and conidial acquisition by thrips. Experiments were carried

out during the dry season of May-August 2013. Average temperatures and relative humidity of 20.8 ˚C and 74.2%, respectively, were recorded in the experimental field.

Entomopathogenic fungi

Conidia of *M. brunneum* were obtained from the commercial product BIO1020 (strain Met52) (Bayer CropScience, The Netherlands). They were cultured on Sabouraud dextrose agar medium (SDA) at 25-27 ˚C, pH = 5.6±0.2 (Cooke *et al*., 2002). Conidia were harvested from the plate and suspended in 0.01% Triton X-100 and conidial concentration determined using a haemocytometer (Fuchs-Rosenthal 0.2 mm). A spore suspension of approximately 10^9 conidia ml^{-1} was prepared and stored for 2 days at 5 ^oC until use in the experiment. *Metarhizium anisopliae* isolate ICIPE 69 is commercially available and marketed as Campaign® by the RealIPM, Kenya. Conidia of *M. anisopliae* were mass-produced on long rice substrate in Milner bags (60 cm long by 35 cm wide). Rice was autoclaved for 1 h at 121 ^oC and inoculated with a 3-day-old culture of blastospores (Jenkins and Goettel, 1997). The rice containing fungal spores was then allowed to dry for five days at room temperature. Conidia were harvested by sifting the substrate through a 295-µm mesh sieve and stored for 2 days at 5 ^oC until use. Conidial viability was determined before any experiment by spread-plating 0.1ml of the suspension (3 x 10^6 conidia ml^{-1}) on Sabouraud Dextrose Agar (SDA) plates. Sterile microscope cover slips were placed on each plate. Plates were then incubated at 24-28 ^oC, 12:12 L:D photoperiod and examined after 16-20 hours. Percentage germination was determined by counting the number of germ tubes formed among 100 random conidia for each plate at 400 x under a light microscope (Goettel and Inglis, 1997). Conidial germination of approximately 90% was considered acceptable.

Semiochemical

Lurem-TR, a commercial semiochemical whose active ingredient is methyl-isonicotinate, previously reported to be effective in monitoring thrips populations was used in this study (Davidson *et al*., 2007). It was obtained from Pherobank (Wageningen, The Netherlands).

3.2.1 Effect of spatial separation of Lurem-TR on the persistence of conidia of *Metarhizium brunneum* in the greenhouse

Four 9 cm Petri dishes without cover were placed at 0, 5, 10 and 20 cm, corresponding to treatments P0, P5, P10 and P20, respectively, on a rack with platforms connected with a stick in such a way that all platforms/Petri-dishes were vertically under each other (Figure 3.1). Lurem-TR was placed above the top Petri dish (P0). Petri dishes contained water agar (1.5% w/w) on which eight cover slips of 10 mm diameter (0.79 cm^2) previously atomized with a spore suspension of *M. brunneum* were placed. Atomization was done by spraying 4 ml conidial suspension (approximately equivalent to 600 l/ha) of *M. brunneum* on eight glass cover slips placed on Petri dishes without water agar at a pressure of 7.5 bar using a Potter Precision Laboratory Spray Tower (Burkard Manufacturing Co Ltd., Rickmansworth, United Kingdom). Petri dishes were allowed to dry for 20-30 min, after which cover slips were transferred to the Petri-dishes containing water agar and then placed in the rack. The treated Petri dishes were exposed to Lurem-TR for 24 h. As a control, a Petri dish was atomized with conidial suspension as described above and allowed to dry, and conidial germination determined immediately. All treatments were replicated two times and repeated four times.

To determine the maximum effect of Lurem-TR on inhibition of conidial germination, in addition to the four treatments described above, Petri dishes were prepared as detailed above and placed in closed boxes (diameter 10 cm, height 10 cm) with or without Lurem-TR. After 24 h the spore germination was determined. The persistence of conidia was determined after a period of 24 h for all the treatments including the control. Conidial viability was determined according to an adapted method of Faria et *al.* (2010). Each cover slip with conidia was removed from the Petri dish, placed in a 10-ml Greiner tube containing 1 ml of 0.01% Triton X-100 water solution and vortexed for 20s to dislodge conidia. From each Greiner tube, three samples of each 10 µl were pipetted separately on one glass slide covered with a thin layer of SDA and incubated in a closed container on humidified filter paper in the dark for 24 h at 25 ^{o}C. Percentage germination was determined by pipetting one droplet of lactophenol on each sample after 24 h, covering it with a cover slip and counting the number of germinating and non-germinating conidia (minimum count was 200 spores per droplet).

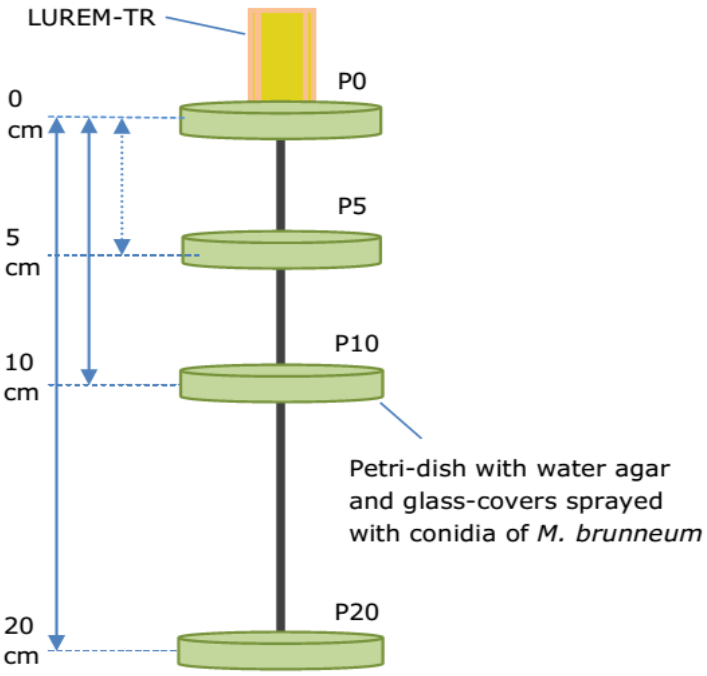

Figure 3.1: Experimental design for the evaluation of the effect of distance separation of Lurem-TR on *Metarhizium brunneum* conidial persistence in the greenhouse.

Field experiment with autoinoculation device

The effect of spatial separation of Lurem-TR on the persistence of *M. anisopliae,* attraction of *M. sjostedi* and other insects, and conidial acquisition by thrips was evaluated in field experiments. Cowpea, *Vigna unguiculata* L. Walp variety *Ken-Kunde1,* was planted in 10 m^2 plots with inter- and intra- row spacing of 10 and 45 cm, respectively. No fertilizers, organic matter or insecticides were applied during the experimental period.

The autoinoculation device used in the present study and procedure for the inoculation of device is as described by Niassy *et al.* (2012a). Briefly, a Lynfield trap (11 cm diameter x 10 cm height) was perforated with six entry ⁄ exit holes (2 x 3 cm) near the top and bottom of the bottle at alternate positions. Velvet (8 x 8.5 cm) and blue netting (3.5 x 11 cm) were wrapped around a smaller inner cylindrical bottle (5.2 cm diameter x 6 cm high) that was then hung inside the trap. The semiochemical dispenser used to lure thrips was placed in different positions (see Figure 3.2). Approximately, 2–3 g of dry conidia was spread evenly on the velvet cloth of the autoinoculation device. Blue netting was then wrapped around the velvet cloth containing spores and tightened with two office pins. The device was then hung at crop canopy level (35 cm).

The following treatments were used in the field with the autoinoculation device: T_1 – Direct exposure of fungal conidia to Lurem-TR; T_2 – Conidia separated from Lurem-TR placed inside a small container fixed just below the device, hereafter also referred to 0 cm; T_3 - Conidia separated from Lurem-TR at 10 cm above the device; T_4 - Conidia separated from Lurem-TR at 20 cm above the device and T_5 - Control (device without Lurem-TR) (Fig. 3.2). Treatments were laid out in a complete randomized block design with three blocks as replicates. The blocks and

treatments were separated by a distance of at least 15 m to avoid interferences between treatments and within blocks. Each of the five treatments was deployed in a single plot so there were five plots and these were repeated three times. For conidial viability, five treatments replicated four times were used, giving a total of 20 experimental units. The experiment on thrips conidial acquisition and attraction, five treatments were replicated three times (15 experimental plots in total).

Experiments were conducted during peak flowering stage of the crop which corresponds to the period of peak infestation of the crop by thrips necessitating control measures. The crop was planted 14 June 2013, and experiments run from July to August 2013. The flowering stage occurred from 24 July 2013 to 7 August 2013 while the podding stage started from 7August 2013 up to harvest.

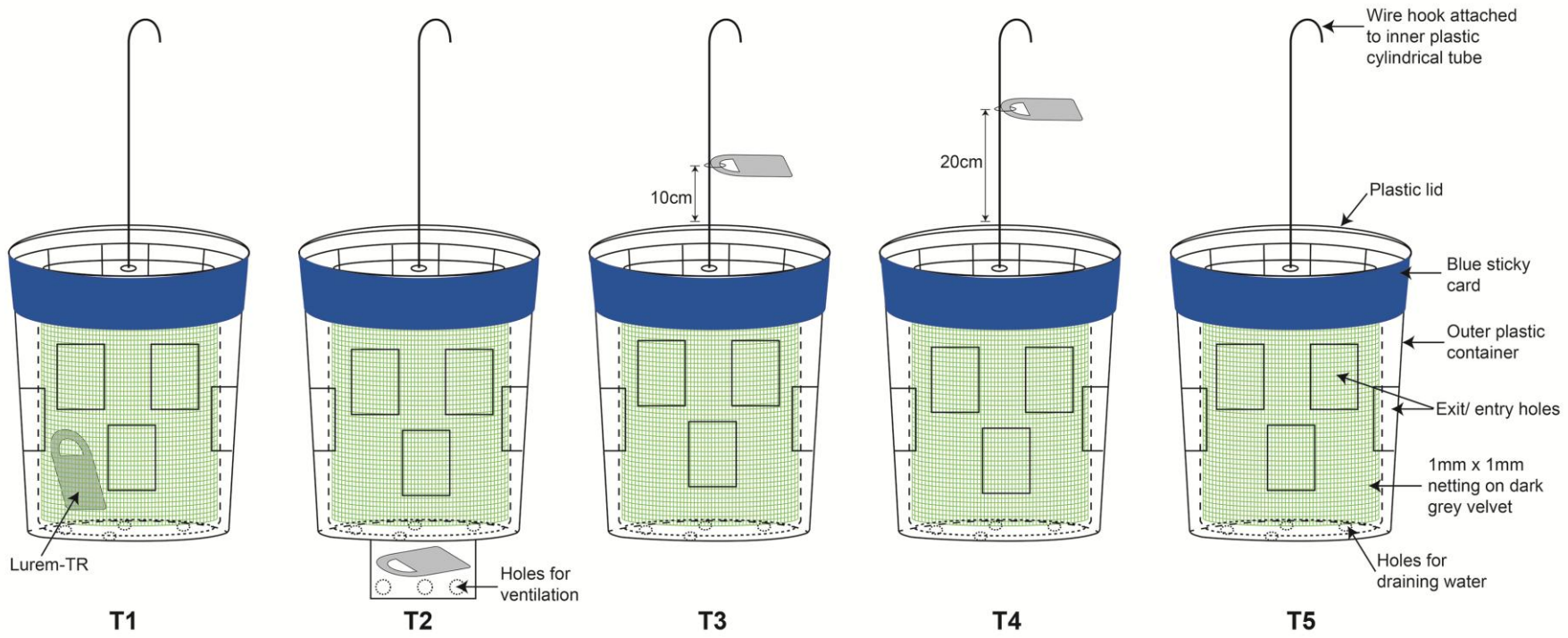

Figure 3.2: Description of spatial separation of Lurem-TR on *Metarhizium anisopliae* conidial persistence in an autoinoculation device in the field. Treatments: T_1 – Direct exposure of conidia to Lurem-TR; T_2 – conidia separated from Lurem-TR placed inside a small container fixed just below the device; T_3 - conidia separated from Lurem-TR at10 cm above the device; T_4 - conidia separated from Lurem-TR at 20 cm above the device and T_5 - control, device without Lurem-TR.

3.2.2 Effect of spatial separation of Lurem-TR on *Metarhizium anisopliae* conidia persistence in the field

The persistence of conidia of *M. anisopliae* was evaluated for a period of two weeks after the onset of the experiment. At three–day intervals, samples of conidia were collected from the autoinoculation devices from the five treatments using a moist cotton bud. The end of the cotton bud was cut, suspended in 10-ml 0.05% (wt ⁄vol) Triton X-100 and vortexed for 1 min to dislodge conidia. A sample of 100 µl was spread-plated on SDA and incubated for 16 h at 25 ± 2 ^{o}C and L12:D12 photoperiod. Germination of conidia was determined as described above.

3.2.3 Attraction of *Megalurothrips sjostedti* and other pests

A blue sticky card (5 cm × 10 cm) was fixed to the side of the autoinoculaion device with or without Lurem-TR to determine the number of insects, including *M. sjostedti* visiting the device. The sticky cards were replaced every three days. Kerosene was used to dissolve the glue on the sticky cards and insects were removed with a fine brush. Thrips specimens were then cleared, mounted on slides and identified as described in the Lucid Key Pest thrips of the world and Pest thrips of east Africa (Moritz *et al*., 2004; Moritz *et al*., 2013). The number of thrips and other insect pests such as leaf miners and bean stem maggot were recorded.

3.2.4 Conidial acquisition by *Megalurothrips sjostedti*

To assess the number of conidia acquired by a single thrips visiting the autoinoculation device, 5 to 10 cowpea plants from a distance of 2 m around the autoinoculation device were randomly sampled using a whole plant tapping technique (Pearsall and Myers, 2000). The latter consists of

tapping plants on a white barber tray (25 x 45 cm) where the tray is held underneath the selected plant, while the plant is tapped gently by hand (5 taps). In each treatment, five cowpea plants were sampled around the autoinoculation device (1-2 m radius) and 20 insects were collected in separate glass containers (10-ml) using an aspirator. Containers were labelled and stored in the fridge for immobilization. Insects were transferred individually into 2-ml cryogenic tubes containing 1 ml of sterile 0.05% Triton X-100. The tube was vortexed for 2–3 min to dislodge conidia from the insect and the concentration of conidia was determined using a Neubauer haemocytometer.

3.2.5 Statistical analysis

In the greenhouse experiment, differences in germination rate of conidia of *M. brunneum* between treatments were assessed by linear logistic regression analysis of the observed counts of germinated spores over the total number of spores examined for the replicate. The data Y were treated as pseudo-binomial data, taking the variance to be proportional to binomial variance, *i.e.* var (Y) = $\sigma^2 np\ (1-p)$. Here p ($0<p<1$) denotes the expected germination rate Y/n of germinated spores Y. Here n stands for the number of spores examined from a replicate, σ^2 denotes the dispersion parameter. A linear logistic model with main effects of batch and treatment has been used to describe the relationship between the expected germination rate p and effects of batch and treatment. The model reads:

$$\ln\left(\frac{p}{1-p}\right) = constant + batch + \ treatment$$

Estimates for the dispersion parameter σ^2, main effects and F-tests for the main effects were obtained from fitting the model using the generalized linear model procedure in GenStat (VSN-International, 2013). The dispersion parameter σ^2 was estimated from Pearson's chi-square statistic. Apart from F-tests for main effects, differences between batches and treatments were assessed by t-tests on all pairwise differences of fitted means on the logistic scale. Data shown are back-transformed data from the analysis and present the predicted germination rates.

For field experiments, ANOVA (repeated measures) was used to analyse *M. anisopliae* conidial viability, conidial acquisition, *M. sjostedi* counts and other insect counts. *Megalurothrips sjostedi* and other insect counts were log-transformed prior to repeated measures analysis of variance to normalize the data and stabilize variance between treatments. Means were separated using Tukey's HSD test at $\alpha = 0.05$. A linear regression model was used to study the relationship between distance of Lurem-TR and device separation and *M. sjostedti* attraction. Pearson correlation was used to analyze the association between distance of separation and conidial counts. The repeated measure ANOVA was implemented in R 3.0.1(R Development Core Team, 2014).

3.3 Results

3.3.1 Effect of spatial separation of Lurem-TR on conidial viability in the greenhouse

In the greenhouse, the distance from which Lurem-TR was placed away from conidia had a significant effect on the viability of conidia of *M. brunneum* both over treatments ($F = 19.4$; $df = 6,52$; $P<0.001$) and over times of observation ($F = 41.6$; $df = 3,52$; $P<0.001$). The lowest conidial germination (0.6%) was observed when conidia were in presence of Lurem-TR inside the closed

box (Lmax), followed by Lurem-TR in immediate proximity (0 cm) of the conidia (13.8%) in the open air inside the greenhouse. However, there was no significant difference in conidial viability when Lurem-TR was placed at distance of 5, 10 or 20 cm in the open air in the greenhouse, conidial germination being 29.0, 37.4 and 32.8%, respectively. The control treatment in the open air (33.1% germination) was also only significantly different from the 0 cm distance treatment and not from the other distance treatments. In the control treatment (Lmin) where conidia in a closed box were not exposed to Lurem-TR, conidial viability was the highest (49.8% conidial germination) and significantly different from all other treatments (Figure 3.3).

3.3.2 Effect of spatial separation of Lurem-TR on conidial viability in the field

In the field, the separation distance of Lurem-TR and *M. anisopliae* had a significant effect on overall viability of conidia, $F = 24.0$; $df = 4,12$; $P<0.0001$ (Table 3.1A). The lowest conidial germination (39%) was obtained when conidia were in direct contact with Lurem-TR, placed within the autoinoculation device. However, there was no significant difference in conidial viability when Lurem-TR was not in direct contact with *M. anisopliae*, at 0 cm, 10 and 20 cm away from the autoinoculation device, conidial germination being 46, 47 and 45%, respectively. In the control treatment, conidial viability was 52% and significantly different from the other treatments (Figure 3.4). Conidial viability decreased significantly over time ($F = 40.0$; $df = 3,45$; $P<0.0001$) (Table 3.1A). Direct exposure resulted in the lowest conidial viability at all observation times and after 15 days, the viability was only 25%, whereas the control recorded the highest viability at all observation times with 41% viability after 15 days (Table 3.2). The other treatments were intermediate to the direct exposure and control treatments. The differences observed between treatments were consistent over time; therefore, no significant interactions

were observed between treatment and exposure time ($F = 0.33$; $df = 12,45$; $P = 0.98$) (Table 3.1A).

Table 3.1: Repeated measures ANOVA table for the response variable: *Metarhizium anisopliae* conidial viability (A) and acquisition (B) in autoinoculation devices as affected by spatial separation of Lurem-TR position and *Metarizhium anisopliae*.

Conidial viability (A)

Source of variation	df	Sum of squares	Mean square	F-value	P-value
Between plot					
Block	3	306	102	6.90	0.006
Treatment	4	1417	354	23.95	<0.0001
Residuals	12	178	15		
Within plot					
Time	3	5715	1905	39.99	<0.0001
Time x Treatment	12	186	16	0.33	0.981
Residuals	45	2144	48		

Conidial acquisition (B)

Source of variation	df	Sum of squares	Mean square	F-value	P-value
Between plot					
Block	2	10.19	5.09	11.02	0.005
Treatment	4	4.20	1.05	2.27	0.150
Residuals	8	3.70	0.46		
Within plot					
Time	4	6.49	1.62	15.73	<0.0001
Time x Treatment	16	1.06	0.07	0.64	0.828
Residuals	40	4.13	0.10		

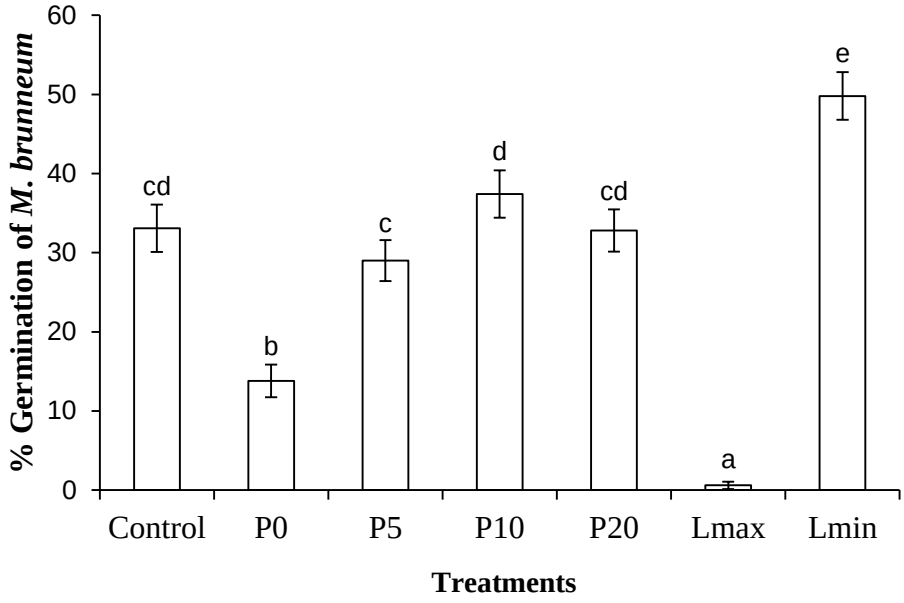

Figure 3.3: Effect of spatial separation of Lurem-TR from *Metarhizium brunneum* (Met52) on conidial germination. Treatments: P0, P5, P10 and P20 are respectively Petri-dishes with conidia directly exposed, 5 cm above, 10 cm above and 20 cm above Lurem-TR. Lmin and Lmax represent the minimum and the maximum effect of Lurem-TR on inhibition of spore germination when placed in closed boxes with or without Lurem-TR. Control: Petri dish atomized with conidial suspension and germination determined immediately.

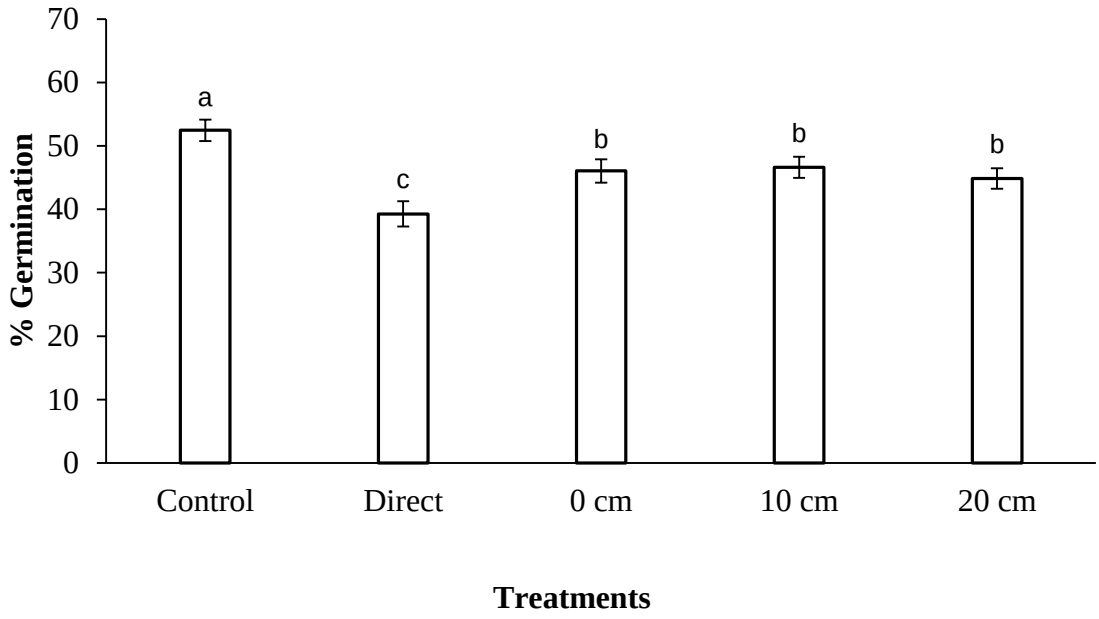

Figure 3.4: Effect of spatial separation of Lurem-TR on conidial viability of *Metarhizium anisopliae* in autoinoculation devices. Bars denote means ± one standard error at P = 0.05 (Tukey HSD). Means (±SE) of three replicates of five autoinoculation devices.

Treatments: Control, device without Lurem-TR; Direct, direct exposure of conidia to Lurem-TR; 0 cm, conidia separated from Lurem-TR placed inside a small container fixed below the device; 10 cm, conidia separated from Lurem-TR at 10 cm above the device and 20 cm, conidia separated from Lurem-TR at 20 cm above the device.

Table 3.2: Effect of spatial separation of Lurem-TR on the persistence of conidia of *Metarhizium anisopliae* (% germination) in autoinoculation devices over time.

Distance of separation	Mean conidial germination ± SE				Mean[a]
	Days after treatment				
	3	6	9	15	
Control	60.6±2.5	58.2±2.8	50.2±1.7	40.8±4.2	52.5±2.5a
Direct	51.2±3.3	46.1±3.5	35.2±2.9	24.5±4.0	39.3±3.3c
0 cm	61.4±3.0	57.5±3.3	50.9±2.3	33.8±4.1	50.9±2.2b
10 cm	61.0±2.0	58.5±3.3	50.0±3.2	39.1±3.6	52.2±2.5b
20 cm	53.4±2.2	48.5±3.0	52.4±2.8	35.9±3.6	47.6±2.5b
Mean[b]	57.5±1.3a	53.8±1.2b	47.7±1.8b	34.8±2.2c	

[a]Means (±SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.

[b]Means (±SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

3.3.3 Effect of spatial separation of Lurem-TR on attraction of *Megalurothrips sjostedti*

The position of Lurem-TR had a significant effect on thrips attraction ($F = 15.1$; $df = 4,8$; $P<0.001$) (Table 3.3A). Thrips were signifivantly more attracted to the device when Lurem-TR was placed at 0 cm and 10 cm distance compared to all other treatments (Figure 3.5). The control treatment recorded the lowest number of thrips (80.2 ± 11.3) and was significantly different from direct exposure (99.2 ± 16.5) and 20 cm separation treatments (97.8 ±11) (Table 3.4). The mean number of *M. sjostedi* attracted to the device increased over time: 100.0 ± 16.5 at day 3 and 167.8±25.1 at day 15 ($F = 6.3$; $df = 4,40$; $P<0.0001$) (Table 3.3A) and this did not vary significantly between treatments ($F = 0.73$; $df = 16,40$; $P = 0.75$) (Table 3.3A).

Table 3.3: Repeated measures ANOVA table for the response variable: *Megalurothrips sjostedti* attraction (A) and other insect attraction (B) (log-transformed counts) in autoinoculation devices as affected by spatial separation of Lurem-TR position and *Metarhizium anisopliae*.

***Megalurothrips sjostedti* attraction** (A)

Source of variation	df	Sum of squares	Mean square	F-value	P-value
Between plot					
Block	2	3.31	1.66	70.94	<0.0001
Treatment	4	1.41	0.35	15.11	0.001
Residuals	8	0.19	0.02		
Within plot					
Time	4	0.57	0.14	6.32	0.000
Time x Treatment	16	0.26	0.02	0.73	0.746
Residuals	40	0.90	0.02		

Attraction of other insects (B)

Source of variation	df	Sum of squares	Mean square	F-value	P-value
Between plot					
Block	2	0.35	0.18	3.13	0.099
Treatment	4	0.20	0.05	0.90	0.507
Residuals	8	0.45	0.06		
Within plot					
Time	4	0.96	0.24	17.25	<0.0001
Time x Treatment	16	0.12	0.01	0.54	0.909
Residuals	40	0.56	0.01		

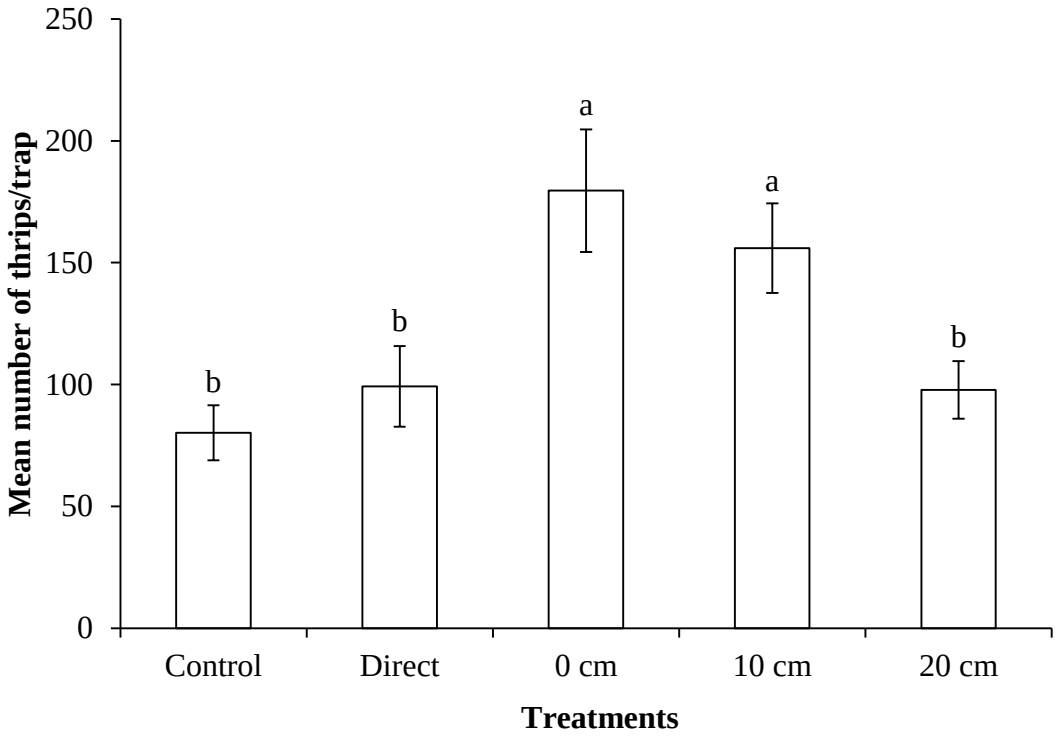

Figure 3.5: Effect of spatial separation of Lurem-TR and *Metarhizium anisopliae* on overall attraction of *Megalurothrips sjostedti*. Bars denote means ± one standard error at P = 0.05 (Tukey HSD). Means (±SE) of three replicates of five autoinoculation devices. Treatments: Control: device without Lurem-TR, Direct: direct exposure of conidia to Lurem-TR; 0 cm: conidia separated from Lurem-TR placed inside a small container fixed below the device; 10 cm: conidia separated from Lurem-TR at 10 cm above the device and 20 cm: conidia separated from Lurem-TR at 20 cm above the device and T_5 - Control, device without Lurem-TR.

Table 3.4: Effect of spatial separation of Lurem-TR and *Metarhizium anisopliae* on *Megalurothrips sjostedti* attraction (mean number of thrips per trap) on autoinoculation devices over time.

Distance of separation	Mean number of thrips/trap± SE					Mean[a]
	Days after treatment					
	3	6	9	12	15	
Control	64.7±25.44	97.0±37.4	71.3±24.8	79.7±36.5	88.3±12.9	80.2±11.3e
Direct	75.3±43.8	104±46.8	89.7±66.5	92.3±49.5	134.7±54.3	99.2±16.5c
0 cm	114.3±34.8	172.3±53.9	168±37.1	141.3±34.1	302±26.5	179.6±25.2a
10 cm	141.7±44.9	163.7±56.9	136.7±22.9	118.3±10.6	219.7±30.5	156.0±18.3b
20 cm	101.7±42.2	123.0±36.7	79.7±24.8	90.3±36.5	94.3±23.9	97.8±11.8d
Mean[b]	100±16.5e	132±19.6b	109±17.9c	104±14.1d	167.8±25.1a	

[a]Means (±SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.

[b]Means (±SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

3.3.4 Effect of spatial separation of Lurem-TR on conidial acquisition by *Megalurothrips sjostedti*

The interaction between treatment and time was not significant ($F = 0.64$; $df = 16,40$; $P = 0.83$) (Table 3.1B).

Overall, there was no significant difference in conidial acquisition by *M. sjostedti* between the different treatments ($F = 2.27$; $df = 4,8$; $P = 0.15$) (Table 3.1B). However, conidial acquisition increased significantly with time ($F = 15.7$; $df = 4,40$; $P<0.0001$) (Table 3.1B), ranging from 0.14×10^5 on day 3 to 0.96×10^5 on day 15 (Table 3.5).

Table3.5: Effect of spatial separation of Lurem-TR position and *Metarhizium anisopliae* on conidial acquisition (mean number of spores per individual thrips) on autoinoculation devices over time.

Distance of separation	Mean number of conidia/thrips ± SE					
	Days after treatment					Mean[a] x10^5
	3	6	9	12	15	
Control	0.1±0.1	0.2±0.1	0.2±0.1	0.7±0.2	0.8±0.3	0.4±0.1a
Direct	0.1±0.1	0.3±0.2	0.7±0.4	0.7±0.6	0.9±0.6	0.5±0.2a
0 cm	0.4±0.3	0.6±0.4	1.1±0.6	1.1±0.3	1.7±0.6	1.0±0.2a
10 cm	0.0±0.0	0.3±0.3	0.3±0.2	0.4±0.5	0.5±0.3	0.3±0.1a
20 cm	0.1±0.1	0.2±0.2	0.6±0.3	0.9±0.2	0.9±0.5	0.6±0.2a
Mean[b] × 10^5	0.2±0.1c	0.3±0.1bc	0.6±0.2bc	0.7±0.2ab	1.0±0.2a	

[a]Means (±SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.

[b]Means (±SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

3.3.5 Effect of spatial separation of Lurem-TR on the attraction of other insects

In addition to the attraction of *M. sjostedti*, other insects such as leafminers, whiteflies and bean stem maggot were also attracted to the device baited with Lurem-TR (Table 3.6). The attraction did not vary significantly among the treatments (F = 0.9; df = 4,8; P = 0.5) (Table 3.3B). The mean number of other insects attracted to the device increased over time (F = 17.3; df = 4,40; P

<0.0001) (Table 3.3B) with day 15 recording the highest number of other insects attracted to the device (62.8±5.8) (Table 3.5). The interaction between treatments and time was not significant (F = 0.54; df = 16,40; P = 0.9) (Table 3.3B).

A Pearson correlation test indicated a significant positive correlation between conidial acquisition and *M. sjostedti* attraction (r = 0.77; P = 0.0001). There was also a significant correlation between *M. sjostedti* attraction and attraction of other insects (r = 0.9; P = 0.0001). However, a negative correlation was found between *M. anisopliae* conidial persistence and *M. sjostedti* attraction (r = -0.7; P = 0.0001) and also between persistence and *M. anisopliae* conidial acquisition (r = -0.8; P<0.0001).

Table 3.6: Effect of spatial separation of Lurem-TR position and *Metarhizium anisopliae* on the attraction of other insects (mean number per trap) on autoinoculation devices over time.

Distance of separation	Mean number of insects/trap ± SE					Mean[a]
	Days after treatment					
	3	6	9	12	15	
Control	31.7±10.1	41.7±9.0	40.7±9.5	48.7±13.7	58.3±21.3	44.2±5.6b
Direct	23.0±5.0	34.3±4.5	33.7±8.3	45.0±10.8	56.7±1.2	38.5±3.9b
0 cm	30.7±3.4	47.3±10.3	48.3±9.1	72.3±6.4	66.3±8.4	52.9±5.0a
10 cm	42.0±14.2	34.0±3.1	45.3±7.0	65.3±24.4	78.3±19.3	52.9±7.4a
20 cm	28.7±4.2	35.3±6.8	35.7±9.5	55.7±3.8	54.3±9.7	41.9±3.9b
[b]Mean	31.0±3.6e	38.5±3.1d	40.7±3.4c	57.4±5.9b	62.8±5.8a	

[a]Means (±SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.

[b]Means (±SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

3.4 Discussion

The concept of autoinoculation has been tested against various insect pests and disease vectors (Maniania, 2002; Niassy *et al*., 2012a; Vega *et al*., 2007). One of the advantages of the autoinoculation device is long persistence of the inoculum which is protected against environmental factors. For instance, Maniania (2002) reported viability of over 60% of conidia of *M. anisopliae* in a contamination device after 31 days post-exposure under field conditions. However in the present study, only 41% of conidia of *M. anisopliae* remained viable after 15 days post-treatment. This could be explained by the difference in the autoinoculation devices and fungal isolates used in both studies. Entomopathogenic fungus applied in autoinoculative devices has the potential to suppress insect pest as reported earlier (Maniania, 2002; Dimbi *et al*., 2003). For instance, Dimbi *et al*. (2003) reported mortality of between 70–93% of fruit flies *Ceratitis rosa* (Karsch) and *C. fasciventris* (Bezzi) (Diptera: Tephritidae) after being attracted to a *M. anisopliae* treated autoinoculator baited with brewer's yeast in field cage experiments. In another study, 100% mortality of leaf miner *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) was observed after visiting *M. anisopliae*-treated autoinoculation devices (Migiro *et al*., 2010). No antifungal effect was observed in both studies although no semiochemical was involved in the second study. The addition of the semiochemical in present study was intended to increase the attraction of thrips and subsequently the infection by fungus. However, direct exposure of conidia of both *M. brunneum* and *M. anisopliae* to Lurem-TR resulted in reduced conidial viability as compared to control treatments, which confirms the antifungal effect of Lurem-TR as reported earlier (Niassy *et al*., 2012a). Conidia viability increased when the inoculum was separated from Lurem-TR, implying that the negative effects of Lurem-TR on conidial viability can be minimized through distance of separation.

More thrips were attracted to the autoinoculation device when Lurem-TR was placed at 0 cm and 10 cm, which may be attributed to a better diffusion of the semiochemical due to ventilation (Teulon *et al*., 1993; Yang *et al*., 1995; Nielsen, 2013). Nielsen (2013) reported that several extrinsic factors such as airflow and type of dispensers affect methyl isonicotinate released rate. The higher thrips catches at 0 cm separation could be attributed to the proximity of Lurem-TR to the blue color as compared to 10 cm and 20 cm used as a lure (Teulon *et al*., 1999). This finding could also explain differences in *M. sjostedti* catches between direct exposure and separation treatments.

The present study has shown that *M. sjostedti* responds to methyl-isonicotinate, which is the active ingredient of Lurem-TR, and confirms a previous report by Muvea *et al*. (2014). The positive correlation observed with thrips attraction could be explained by frequent visits or longer stay of *M. sjostedti* in the device. Methyl-isonicotinate has been reported to stimulate walking and take-off behavior in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) female adults (Van Tol *et al*., 2012). This may explain the finding of Niassy *et al*. (2012a) who observed that conidial acquisition by *F. occidentalis* was greater in Lurem-TR baited device than a device without Lurem-TR. Maniania (2002) also observed that the time spent by individual tsetse flies (*Glossina* spp.) in the contamination device largely depended on the insect behaviour and varied between 5 to189 seconds, and the subsequent number of conidia collected varied between 1.6 and 40.5 $\times 10^5$ conidia per fly.

The effect of conidial acquisition on thrips mortality was not investigated in the present study. However, Niassy *et al*. (2012a) found that the overall *F. occidentalis* mean mortality and mean

number of conidia acquired per single thrips was significantly higher in field cages with a semiochemical-baited device 7 days after post-inoculation. Migiro *et al*. (2010) also reported a positive correlation between conidial acquisition and mortality on leaf miner *L. huidobrensis*.

Male aggregation and sexual behavior have been widely documented in thrips (Kirk, 1985) and such behavior are semiochemically mediated (Hamilton *et al*., 2005). Male thrips aggregate in numbers to demonstrate courtships (fighting, mounting) to females before mating (Kirk, 1985; Riefler and Koschier, 2009). Such behavioural elements can permit male-to-male or male-female transmission during leks. Similar sexual behaviour have been also reported in some fruit fly species (Hedström and Monge-Nájera, 1998; Prokopy and Hendrichs, 1979).

The negative correlation between conidial persistence and *M. sjostedti* attraction and between *M. anisopliae* conidial persistence and conidial acquisition observed in the present study suggest that the proximity of the attractant with color for attraction needs to be appropriately defined for the success of the lure and infect strategy.

3.5 Conclusion

Spatial separation of Lurem-TR with fungal conidia could reduce the negative effect of the semiochemical and subsequently enhance fungal persistence in an autoinoculation device. The distance of 0-10 cm away from the conidial source was found to be optimal for thrips attraction under field conditions. In addition to *M. sjostedti,* insect pests such as leafminers, bean stem maggot, and whiteflies also considered as important pests of cowpea in Kenya, can be attracted

to the autoinoculation device, which renders this strategy very viable for the management of thrips and other insect pests of grain legumes.

3.6 References

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CHAPTER 4: SCREENING OF ATTRACTANTS FOR COMPATIBILITY WITH *METARHIZIUM ANISOPLIAE* USE IN THRIPS MANAGEMENT

Abstract

Seven thrips attractants were screened for compatibility with *Metarhizium anisopliae* and a subset of these for attraction to bean flower thrips, *Megalurothrips sjostedti.* Conidial germination and germ tube length of *M. anisopliae* were used as indicators of its compatibility for use with thrips attractants. Conidial germination and germ tube length differed significantly following exposure to volatiles of different attractants. The highest conidial germination (76.5±3.5%) and longest germ tube length (130.3±13.4 µm) were recorded in the control, followed by methyl anthranilate (63.8±3.8%; 103.8±8.4µm), *cis*-jasmone (61.8±5.9%; 93.8±14.4 µm) and *trans*-caryophyllene (57.7±6.5%; 96.3±15.5 µm) which were found compatible with *M. anisopliae*. A Pearson correlation test indicated a significant positive correlation between conidial germination and germ tube length ($r = 0.8$; $P<0.0001$). The attraction of *M. sjostedti* also differed significantly between the attractants. Under field conditions, methyl anthranilate was equally attractive to *M. sjostedi* as Lurem-TR and could be recommended as a thrips attractant that can be combined with *M. anisopliae* in autodissemination devices for control of *M. sjostedti*.

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4.1 Introduction

In many flower dwelling thrips species, host finding is linked to visual, odour and morphological (shape) cues (Teulon *et al.*, 1993; Terry, 1997; Rieske and Raffa, 2003; Mainali and Lim, 2011). Subsequently, semiochemical-based products such as Lurem-TR and Thripline have been developed for use in thrips monitoring and management strategies (Broughton and Harrison, 2012; Sampson and Kirk, 2013; Teulon *et al.*, 2014; Broughton *et al.*, 2015). These semiochemicals can be integrated with other control strategies to improve thrips management in horticulture (Suckling *et al.*, 2008; Suckling *et al.*, 2012; Sampson and Kirk, 2013).

Entomopathogenic fungi (EPF) are among the alternatives to chemical pesticides being considered for the management of thrips in horticulture (Ekesi and Maniania, 2007). EPF are generally applied through inundative sprays, which requires a high volume of inoculums, thereby enhancing its cost (Jaronski, 2010). The persistence of conidia applied on foliage is also affected by several environmental parameters such as UV light, rain and temperature (Hong *et al*., 1999; Inglis *et al*., 2000; Jaronski, 2010). The use of a "lure and kill" strategy using autodissemination devices or spot spray applications could reduce the amount of inoculum, the cost of control and sustain fungal persistence in the field (Dimbi *et al.*, 2003; Nana *et al*., 2014; Mfuti *et al.*, 2016). However, the success of this technology depends on the use of attractants and their compatibility with the entomopathogens. For example, the tick attraction-aggregation-attachment pheromone (AAAP) could attract adult ticks from a distance of 6 m (Nchu *et al*., 2009) but could not be used in combination with conidia of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) because fungal conidia are inhibited by the pheromone (Nana *et al*., 2012). Inhibitory effects of conidia of *M. anisopliae* by the semiochemical Lurem-TR in

autoinoculation devices in a screenhouse and field experiments have also been reported by Niassy *et al*. (2012a) and Mfuti *et al*. (2016).

Considering the growing interest in integrating attractants with EPF in thrips management (Niassy *et al*., 2012a; Mfuti *et al*., 2016), there is a need to identify compounds that are both attractive to thrips and compatible with EPF. The objective of this study was therefore to identify thrips attractants that are compatible with *M. anisopliae* as indicated by conidial germination and germ tube length since the latter plays a crucial role in fungal infection (Ortiz-Ribbing and Williams, 2006).

4.2 Materials and methods

Study sites

Laboratory experiments were conducted at the Arthropod Pathology Unit of *icipe*, Duduville Kasarani (1° 13' 15.6" S; 36° 53' 45.6" E; 1,616 m above sea level). Field experiments were carried out at *icipe*'s Mbita Thomas Odhiambo Campus (ITOC) (0˚26' 06.19" S, 34˚12' 53.13" E; 1,137 m above sea level) in western Kenya, from April to June 2014.

Thrips attractants

Seven compounds with reported attraction to thrips belonging to the *Frankliniella* and *Thrips* genera (Table 4.1) were evaluated for their compatibility with *M. anisopliae* isolate ICIPE 69. These compounds were selected on the basis of structural analogies to a known attractant such as methyl isonicotinate (Lurem-TR) (Teulon *et al*., 2007; Teulon *et al*., 2010), but also based on previous studies by Koschier *et al*. (2000). The commercial attractant, Lurem-TR which was

earlier reported to have an antifungal effect (Niassy *et al*., 2012a) was included in the study as a reference. The latter is a commercial product of which the quantity and release rate are standardized and could therefore not be diluted. In preliminary bioassays with the seven candidate attractants, no significant effect of different concentrations (0.1%, 10% and 100%) was observed on conidial germination. Hence, subsequently only the recommended concentration of 10% of the pure product was used in the screening bioassays. The pure concentration of all attractants was diluted in paraffin oil.

Table 4.1: General information regarding the thrips attractant compounds that were evaluated.

Label Name	Chemical formula	CAS number	Chemical group	Company	Purity	Dilution range for thrips attraction and species attracted
4-anisaldehyde	$C_8H_8O_2$	19486-71-6	Aldehyde	Sigma-Aldrich, Germany	98%	0.1-10% (applied in 1microliter paraffin oil) (Koschier *et al*., 2000) *Frankliniella occidentalis*
Ethyl benzoate	$C_9H_{10}O_2$	93-89-0	Ester of benzoic acid and ethanol	Sigma-Aldrich, Germany	99%	*Thrips obscuratus*; *Thrips tabaci* (Koschier *et al., 2000)*
Cis-jasmone	$C_{11}H_{16}O$	488-10-8	Jasmonate	Sigma-Aldrich, Germany	≥ 99%	10mg/200 microliters hexane *T. obscuratus*, *T. tabaci* (El-Sayed *et al*., 2009)
Linalool	$C_{10}H_{18}O$	78-70-6	monoterpene	Sigma-Aldrich, Germany	97%	1-10% (in 1 microliter paraffin oil) *F. occidentalis*, *T.tabaci* (Koschier *et al*., 2000)
Methyl anthranilate	$C_8H_9NO_2$	134-20-3	Ester of anthranilic acid	Sigma-Aldrich, Germany	98%	*T. coloratus*, *T. hawaiiensis* (Murai *et al*., 2000; Imai *et al*., 2001)
trans caryophyllene	$C_{15}H_{24}$	87-44-5	Sesquiterpene	Sigma-Aldrich, Germany	≥98.5%	1-10% (in 1 microliter paraffin oil) (Koschier *et al*., 2000)
Phenylethanol	$C_8H_{10}O$	60-12-8	Alcohol	Sigma-Aldrich, Germany	≥ 99%	*T. tabaci* (Teulon *et al*., 2007)
Methyl-isonicotinate (Lurem-TR)	$C_7H_7NO_2$	2459-09-8	Pyridine	Pherobank, The Netherlands.	-	Several thrips species such as *F. occidentalis*, *T. tabaci* (Davidson *et al*., 2007), *F. schultzei*, *Hydatothrips adolfifriderici* and *Megalurothrips sjostedti* (Muvea *et al*., 2014)

Source:http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=KE&language

Crop

Cowpea, *Vigna unguiculata* L. Walp variety Ken-Kunde1, was planted in 80 m^2 plots with inter- and intra-row spacing of 10 and 45 cm, respectively, at Mbita research station during March 2014, early in the rainy season. The field experiment with selected attractants was conducted during the flowering stage of the crop (45 days after planting). No fertilizers, organic matter or chemical insecticides were applied during the experiment.

Fungal culture

Metarhizium anisopliae isolate ICIPE 69 was used as well. The production protocol of the isolate was described in chapter 3 (see 3.2).

4.2.1 Effect of thrips attractants on conidial viability of *Metarhizium anisopliae*

The *M. anosopliae* conidial suspension was titrated to 1×10^7 conidia ml^{-1}. The spores were retained on a nitrocellulose filter membrane (diameter 47 mm, pore size 0.45 μm, Sigma Chemicals) by pouring 10 ml suspension through a filter holder unit (MFS) under an aspirator vacuum (Maniania, 1994). The nitrocellulose filter membranes were dried for 30 min under a laminar flow cabinet. Five nitrocellulose filter membranes were transferred to single glass desiccator (2.5 liters) for exposure to the attractant volatile. Cotton wicks were soaked in 0.5 ml suspensions of each attractant diluted in paraffin oil and placed in desiccators to allow volatile diffusion. Cotton wicks were used as dispensers (Burks *et al*., 2009; Sidahmed *et al*., 2014). Fungus-treated nitrocellulose membranes were exposed to the respective thrips attractants for 1, 2, 3, 6 and 8 days, respectively. After these exposure times, one fungus-treated nitrocellulose membrane was removed fom the glass dessicator and transferred to 10ml sterile distilled water

containing 0.05% Triton X-100. It was cultured on Sabouraud Dextrose Agar (SDA) media for assessing the effect of the attractants on fungal viability and germ tube length. An untreated control without thrips attractant was included as well as a commercial thrips attractant, Lurem-TR which was included as a check. Treatments were randomized and the experiment was repeated three times over time.

4.2.2 Effect of thrips attractants on germ tube length of *Metarhizium anisopliae*

To determine conidial germination, nitrocellulose filter membranes containing conidia were removed from the desiccators and transferred into 10 ml sterile distilled water containing 0.05% Triton X-100 and vortexed for 3 min to dislodge conidia. A 0.1 ml suspension of titrated to 3 x 10^6 conidia ml^{-1} was spread-plated on SDA plates. Plates were incubated at 26 ± 2 ˚C, 12:12 L:D photoperiod and examined after 18-24 hours for conidial germination and to determine germ tube length. Samples that could not be processed the same day were fixed by pouring a drop of lactophenol cotton blue onto the plate to stop further growth. Percentage germination was determined by counting approximatively 100 spores per plate under a microscope (Leica DMLB) at 40 X magnification. The length of germ tubes was measured using a Leica Application Suite (LAS EZ V1.5.0). Average germ tube lengths were calculated from five spores taken at random from each of three cover slips (22 x 22 mm).

4.2.3 Effect of selected thrips attractants on the attraction of *Megalurothrips sjostedti*

Attractants that were found compatible with *M. anisopliae* from the screening experiment were used to evaluate the attraction of bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) in field experiments with cowpea. The experiment consisted of four

blocks, each consisting of the four treatments, *viz*. *cis*-jasmone, methyl anthranilate, Lurem-TR (included as a reference) and the control treatment (unbaited with no attractant), arranged in a complete randomized block design with four replications. The lures were used in combination with the blue sticky cards (one sticky card per treatment/block). Attractants were diluted in paraffin oil to the recommended 10% solution of pure product. Each attractant suspension was separately poured into 5 ml Eppendorf tubes covered with cotton wicks and suspended on the middle surface of the blue sticky card (10 × 25 cm)(Figure 4.1). The blue sticky cards and the attractant were placed at 30 cm above ground level. The blue sticky cards were placed at the same position throughout the experiment and were separated by 10 m to avoid interference. The experiment was conducted during flowering and podding stages of cowpea. *Megalurothrips sjostedti* populations are high during these reproductive stages (Ezueh, 1981; Niassy *et al*., 2015). Cards were replaced every three days for a period of fifteen days. Number of adult *M. sjostedti* were recorded on each card.

Figure 4.1: *Megalurothrips sjostedti* attracted to the blue sticky card baited with attractant suspension in a 5 ml Eppendorf tube.

4.2.4 Statistical analysis

Data on conidial germination of *M. anisopliae* were normalized by arcsine transformation before it was subjected to a linear mixed model. Data on *M. anisopliae* conidial germ tube length and *M. sjostedti* catches were also analyzed by means of a linear mixed model. Means were separated by Student–Newman–Keuls (SNK) test. The relationship between conidial viability and germ tube length was determined by means of a Pearson correlation analysis. All data analyses were performed using R statistical (R, 2014). The level of significance was maintained at 95%.

4.3 Results

4.3.1 Effect of thrips attractants on conidial viability of *Metarhizium anisopliae*

The effect of thrips attractants on germination of conidia of *M. anisopliae* differed significantly between the attractants ($F = 22.1$; $df = 9,268$; $P<0.0001$) (Table 4.2). There was a significant interaction between exposure period and the attractants ($F = 3.8$; $df = 9,268$; $P<0.0001$) (Table 4.3). The time of exposure had a significant effect on conidial viability, except after 1 day exposure when no significant effect was observed ($F = 1.5$; $df = 9,45$; $P = 0.2$) (Table 4.3). Significant reduction in conidial germination was observed in all the treatments from day 2, 3, 6 and 8 days post-exposure (Table 4.3). The range of viability was between 20.1±4.0 to 62.4±3.5%. The conidial germination was higher in the control (62.5 ± 3.5%), followed by *cis*-jasmone (44.8 ± 16.6%), solvent (paraffin oil) (42.8 ± 11.0%), methyl anthranilate (36.6 ± 8.0%) and trans-caryophyllene (31.3 ± 16.8%) treatments after 8 days of exposure (Table 4.3). No conidial germination was observed in the Lurem-TR treatment after 8 days of exposure (Table 4.3).

Table 4.2: Effect of thrips attractants on mean percentage conidial germination of *Metarhizium anisopliae* and germ tube length (µm) 8 days after exposure.

Treatments	**Mean conidial germination (transformed) ±SE(%)**	**Mean germ tube length ±SE(µm)**
Control	62.4±3.5a	130.5±10.0a
Methyl anthranilate	53.7±3.5ab	103.8±10.0ab
trans-Caryophyllene	51.2±3.5ab	96.3±10.0ab
cis-jasmone	50.9±3.5ab	93.8±10.0ab
Solvent (paraffin oil)	48.4±3.5b	90.9±10.0b
Linalool	33.3±3.5bc	67.1±10.0c
Phenylethanol	32.4±3.5c	50.9±10.0c
4-Anisaldehyde	30.8±3.5c	45.5±10.0c
Lurem-TR	24.4±3.5c	37.1±11.7c
Ethyl benzoate	20.1±4.0c	36.1±10.0c

Means (±SE) followed by the same letters within the column are not significantly different according to Student–Newman–Keuls test (SNK).

Table 4.3: Effect of thrips attractants on *Metarhizium anisopliae* conidial germination (%) over time.

Treatments	Mean conidial germination ±SE (%)					ANOVA
	Days after exposure					
	1	2	3	6	8	
Control	91.3±3.0aA	85.4±3.7aAB	78.3±4.0aB	65.5±10.0aC	62.5±10.0aC	$F_{4,22}$=14.65; P<0.0001
4-anisaldehye	85.7±2.0aA	48.7±14.3bcdB	9.2±7.9dC	0.1±0.1cC	0.1±0.1cC	$F_{4,22}$=38.2, P<0.0001
Ethyl Benzoate	79.8±6.8aA	44.1±14.1abcB	17.5±6.1cdBC	11.9±4.4bcBC	7.5±3.4bcC	$F_{4,22}$=35.8, P<0.0001
cis-jasmone	83.8±4.0aA	74.6±7.5abcA	61.1±12.9abAB	44.6±15.5abB	44.8±16.6aB	$F_{4,22}$=6.1; P=0.001
Linalool	75.5±4.3aA	63±3abcB	50.6±6.2abcC	2.5±1.4cD	0.5±0.5cD	$F_{4,22}$=195.5; P<0001
Methyl anthranilate	85.8±3.6aA	78.8±2.7abA	60.7±2.3abBC	56.8±4.6aB	36.6±8.0aC	$F_{4,22}$=28.2; P<0.0001
Phenylethanol	76.1±8.5aA	50.1±14.6bcdB	36.5±15.3bcdB	18.2±11.5bcC	5.5±4.1bcC	$F_{4,22}$=21.5; P<0.0001
trans-caryophyllene	80.0±5.0aA	74.5±abc7.0A	53.8±16.6abcAB	49.3±16.4abAB	31.3±16.8abB	$F_{4,22}$=6.2; P=0.001
Lurem-TR	75.7±4.2aA	20.6±3.7dB	13.6±3.1cdC	0.03±0.0cD	0.0±0.0cD	$F_{4,13}$=199.8; P<0.0001
Solvent (paraffin oil)	82.0±3.0aA	72.7±5.2abcAB	58.6±7.1abB	43.9±11.0abC	42.8±11.0aC	$F_{4,22}$=14.7; P<0.0001
ANOVA	$F_{9,45}$=1.5;P=0.2	$F_{9,45}$=6.1;P<0.0001	$F_{9,45}$=6.8;P<0.0001	$F_{9,45}$=8.3;P<0.0001	$F_{9,45}$=8.7;P<0.0001	

Means (±SE) followed by the same lower case letters within the column are not significantly different according to Student–Newman–Keuls test (SNK).

Means (±SE) followed by the same upper case letters within the row are not significantly different according to Student–Newman–Keuls test (SNK)

4.3.2 Effect of thrips attractants on germ tube length of *Metarhizium anisopliae*

The effect of thrips attractants on germ tube length followed the same trend as with conidial germination where treatments differed significantly ($F = 12.6$; $df = 9,268$; $P<0.0001$) (Table 4.2). There was no significant day x attractant interaction ($F = 1.0$; $df = 9,268$; $P = 0.5$). Exposure time did, however have a significant effect on the length of the germ tube of *M. anisopliae* at exposure day 1, day 2, day 3, day 6 and day 8 (Table 4.4). The longest germ tube was recorded in the control treatment (89.1±32.4µm) followed by methyl anthranilate (69.6±12.9µm) and solvent (paraffin oil) (54.7 ± 16.4µm) and was not significantly different 8 days after exposure (Table 4.4).

A significant correlation was found between conidial germination and germ tube length of *M. anisopliae* ($r = 0.76$; $P<0.0001$) (Figure 4.2).

Table 4.4: Effect of thrips attractants on *Metarhizium anisopliae* mean conidial germ tube length (µm) over time after exposure inside dessicators.

Treatments	Mean conidial germ tube length ± SE (µm)					ANOVA
	Days after exposure					
	1	2	3	6	8	
Control	190.7±2A	146.9±25.4aB	123.2±28.2aBC	102.4±28.9aBC	89.1±32.4aC	$F_{4,22}$=8.3; P=0.003
4-anisaldehye	102.5±19bcA	74.6±12.4bcA	27.7±16.2cB	11.5±0.0cB	11.5±0.0cB	$F_{4,22}$=12.6; P<0.0001
Ethyl benzoate	77.8±20.4cA	40.9±13.4cB	26.8±5.4cB	20.5±4.6cB	14.4±2.0cB	$F_{4,22}$=7.2; P=0.0007
cis-jasmone	185.4±42.6aA	103.8±22.1abB	90.6±21.9abBC	47.3±16.3bcC	41.9±16.7bcC	$F_{4,22}$=13.5; P<0.0001
Linalool	122±26.4abcA	94.3±15.5abA	83.9±12.8abA	22.2±5.7cB	13.4±1.9cB	$F_{4,22}$=17.58; P<0.0001
Methyl anthranilate	134.4±22.6abcA	120.5±20.4abA	108.6±17aAB	86.1±11.4abBC	69.6±12.9abC	$F_{4,22}$=8.4; P=0.0002
Phenylethanol	102.9±25.7bcA	71.9±21.6bcAB	45.9±18.2bcBC	20.4±5.9cC	13.4±1.9cC	$F_{4,22}$=7.1; P=0.0007
trans-caryophyllene	179.3±49.1abA	112.9±34.1abB	81.0±21.6abBC	66.2±21.8abcBC	42.1±13.9bcC	$F_{4,22}$=9.9; P<0.0001
Lurem-TR	75.7±22.6bcA	20.6±6.1cB	13.6±3.3cB	15.4±7.9 cB	11.5±0.0 cB	$F_{4,13}$=14.3; P=0.0001
Solvent (paraffin oil)	139.3±19.9abcA	107.9±21.4abB	87.7±19.0abBC	64.7±18.0abcBC	54.7±16.4abC	$F_{4,22}$=8.3; P=0.0003
ANOVA	$F_{9,45}$=4.3;P=0.003	$F_{9,45}$=6.7;P<0.0001	$F_{9,45}$=6.9;P<0.0001	$F_{9,45}$=6.5; P<0.0001	$F_{9,45}$=5.6;P<0.0001	

Means (±SE) followed by the same lower case letters within the column are not significantly different according to Student–Newman–Keuls test (SNK).

Means (±SE) followed by the same upper case letters within the row are not significantly different according to Student–Newman–Keuls test (SNK).

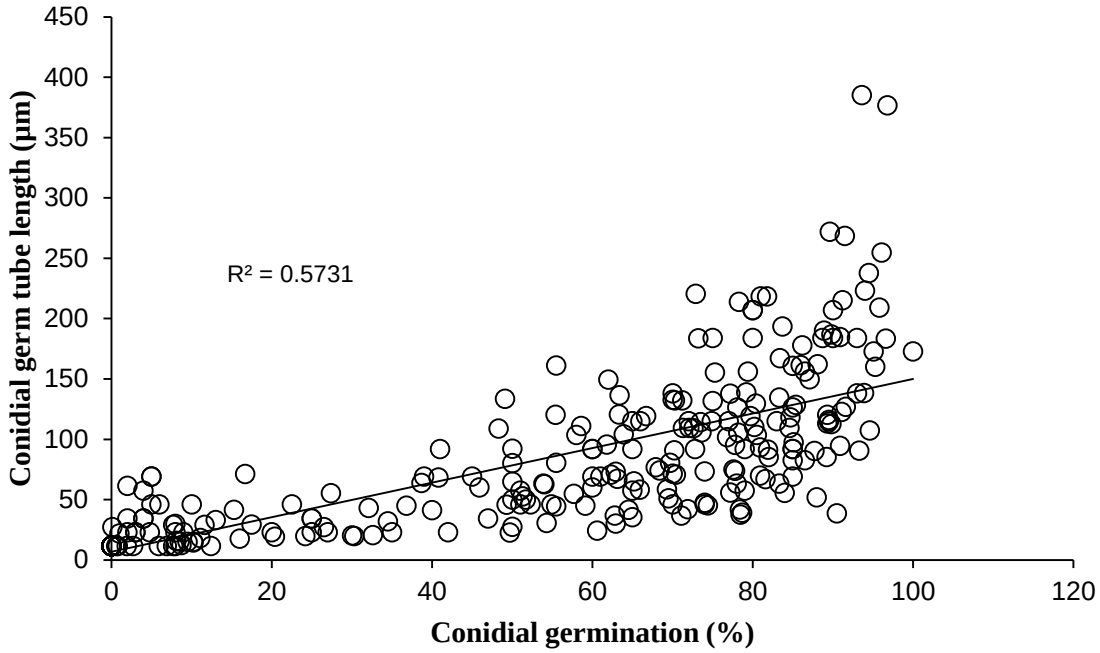

Figure 4.2: Relationship between *Metarhizium anisopliae* conidial germination and germ tube length.

4.3.3 Effect of selected thrips attractants on the attraction of *Megalurothrips sjostedti*

The number of adult *M. sjostedti* caught on the baited sticky cards differed significantly ($F = 7.9$; $df = 3,28$; $P<0.0001$) between the treatments. More adult *M. sjostedti* were caught on sticky cards treated with Lurem-TR and methyl anthranilate compared to cards treated with *cis*-jasmone (Figure 4.3). No significant difference in thrips catches was found between the Lurem-TR and methyl anthranilate lures, while there was also no significant difference between cis-jasmone and control treatments (Figure 4.3).

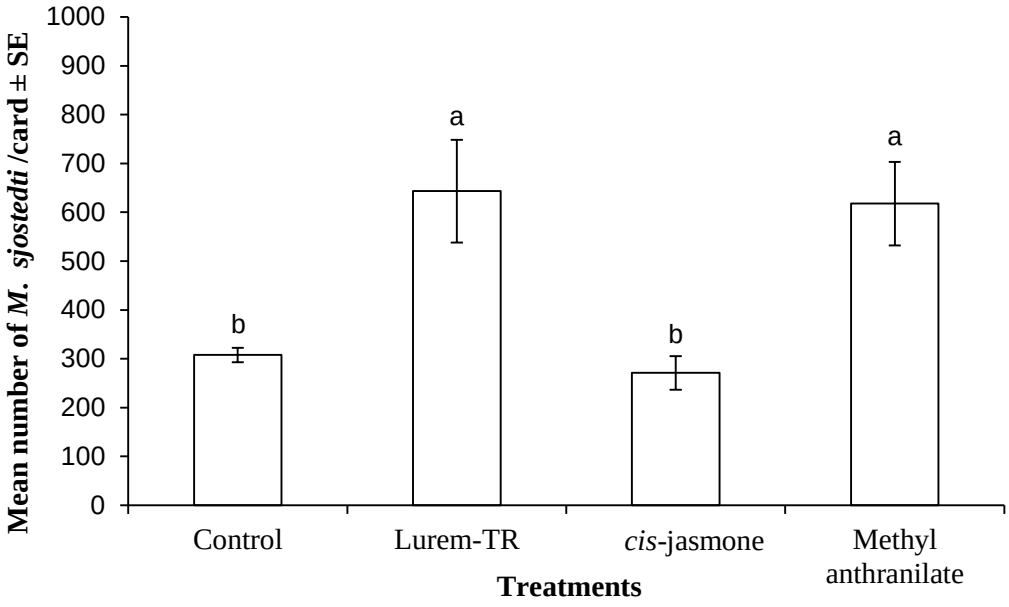

Figure 4.3: Mean number of *Megalurothrips sjostedti* attracted to blue sticky cards baited with methyl anthranilate, *cis*-jasmone, Lurem-TR and control. Means with the same letters are not significantly different according to the Student–Newman–Keuls test (SNK).

4.4 Discussion

Most studies on compatibility of *M. anisopliae* have focused on agrochemicals and botanicals (Nana *et al*., 2012; Niassy *et al*., 2012b) overlooking the potential of semiochemical attractants in insect pest management (IPM). However, a study on compatibility between attractants and EPF is required before their integration in an IPM strategy. Thrips attractants evaluated in this study affected conidial germination and germ tube length differently, with time of exposure being the determining factor. Percentage conidial germination of *M. anisopliae* with three of the eight attractants tested namely methyl anthranilate, *cis*-jasmone and *trans*-caryophyllene was not significantly different from the control treatment after eight days of exposure, but only methyl anthranilate did not inhibit germ tube length. Methyl anthranilate (MA), *cis*-jasmone and *trans*-caryophyllene have been reported in other studies to have antifungal effects (Halim *et al.,* 2006; Erdemgil *et al*., 2007; Chambers *et al*., 2013). Methyl anthranilate has been reported to significantly reduce the growth of strawberry pathogens such as *Botrytis cinerea* (Helotiales: Scerotiniaceae), *Colletotrichum gloeosporioides* (Glomerellale: Glomerellaceae) and *C. acutatum* (Glomerellale: Glomerellaceae). In addition, medium supplemented with methyl anthranilate resulted in complete cessation of growth in the abovementioned pathogens (Chambers *et al*., 2013). The toxicity of MA depends to the level of concentration. For instance, Edgington *et al*. (2000) reported MA at a concentration of 0.1 – 2.5% to be toxic to *Beauveria bassiana*, but at lower concentrations less than 10%, it is not toxic to some other fungus. Several fungal species are, however, known to produce methyl anthranilate e.g. *Pycnoporous cinnabarinus* (Lomascolo *et al*., 1999). The relatively low toxicity observed in this study could be due to vapor toxicity, which could have been different from the agar diffusion toxicity. It is also suggested that jasmonic acid is involved in the induction of genes that act primarily in defense

against plant pathogens rather than insects (Vijayan *et al.*, 1998; Halim *et al.*, 2006). Jasmonic acid is part of the plant's alarm system and defence mechanism. It is a volatile (gas phase of *cis*-jasmone) which is released during insect attack and controls the response to damage (Menzel *et al*., 2014). Essential oil from *Perovskia atriplicifolia* Benth (Lamiales: Liamiaceae) containing 9.30% of *trans*-caryophyllene has been reported to have antimicrobial activity against fungal strains (Erdemgil *et al*., 2007). The difference in antifungal property of MA reported in literature and that found in this study on compatibility of MA could be explained by the attractants which were used as volatiles in this study while they were used as oil supplements in culture media in previous studies.

This study also confirmed previous findings on the antifungal effect of Lurem-TR on conidial germination (Niassy *et al*., 2012a). Direct exposure of fungus to Lurem-TR recorded the lowest conidial germination when compared to placing of the fungus at distances away from the attractant (Mfuti *et al*., 2016). The fungal persistence increased with distance of separation of Lurem-TR (Mfuti *et al*., 2016).

Higher conidial germination and longer germ tube length directly relates to fungal pathogenesis to insects. The role of germ tube formation in pathogenesis is well established (Ortiz-Ribbing and Williams, 2006). Fargues *et al*. (1994) compared four different growth stages namely conidia, germinated conidia with either one or two germ tubes and hyphal bodies of *Isaria fumosorosea* (=*Paecilomyces fumosoroseus*) (Eurotiales: Trichocomaceae) for their infection potential to the first-instar larvae of *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae). He found germinated conidia and hyphal bodies to be more aggressive than ungerminated

conidia. This is in agreement with the findings of Faria et *al*. (2015) who reported that a lower proportion and longer duration of germination of conidia are indicative of lower virulence to insects.

The catches of *Megalurothrips sjostedti* were significantly higher on blue sticky cards baited with methyl anthranilate and Lurem-TR than the control and cis-jasmone baited cards. The increased attraction of *M. sjostedi* to Lurem-TR and blue sticky traps was previously reported by Muvea *et al*. (2014). No difference in *M. sjostedti* attraction was found between methyl anthranilate and Lurem-TR. Methyl anthrinalate has been reported to be attractive to four species of flower thrips (Thysanoptera: Thripidae), *Thrips hawaiiensis*, *Thrips coloratus*, *Thrips flavus*, and *M. distalis*, irrespective of sex (Murai *et al.*, 2000; Imai *et al.*, 2001). This is the first report on *M. sjostedti* response to methtyl anthranilate. *Cis*-jasmone has also been found to be less attractive to *F. occidentalis* and other thrips compared to methyl anthranilate and methyl isonicotinate (Teulon *et al*., 2014).

4.5 Conclusion

This study has identified methyl anthranilate as an effective attractant for *M. sjostedi* and also that it is compatible with conidia of *M. anisopliae*. It can therefore be considered for a "lure and kill" management strategy for *M. sjostedi*. The "lure and kill" strategy could be adopted either in the form of an autodissemination device or a spot spray. Further studies are needed to validate this proof of concept.

4.6 References

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CHAPTER 5: FIELD EVALUATION OF METHYL ANTHRANILATE AS BAIT FOR *MEGALUROTHRIPS SJOSTEDTI* IN AUTOINOCULATION DEVICES

Abstract

The performance of methyl anthranilate (MA) for bean flower thrips (BFT), *Megalurothrips sjostedti* control was compared to the commercial attractant Lurem-TR in autoinoculation devices treated with the entomopathogenic fungus *M. anisopliae* under field conditions over two seasons. Plots with semiochemical-baited autoinoculation devices had generally less *M. sjostedi* than the control treatment plots during both experimental seasons (season I: $F = 7.4$; df = 2, 94; $P<0.001$; season II: $F = 5.2$; df = 2, 94; $P<0.01$). *Megalurothrips sjostedti* densities did not differ significantly between plots with MA-baited autoinoculation devices and plots with Lurem-TR-baited devices during both seasons. However, in the second season, density of *M. sjostedti* in plots with Lurem-TR-baited devices did not differ significantly from the control. The density of *M. sjostedti* varied significantly over time during the first and second season, with the control having the highest number of *M. sjostedti*. Conidial viability of *M. anisopliae* was significantly higher in semiochemical-free devices (control) than in the semiochemical-baited device treatments in both seasons. Conidial viability of *M. anisopliae* decreased over time in all the treatments evaluated, but more than 45% of *M. anisopliae* conidia remained viable 12-15 days post-exposure. The average number of conidia acquired varied between 2.0 and 10.0 x 10^3 conidia per *M. sjostedti* in both Lurem-TR-baited and MA-baited device treatments during both seasons. There was no significant difference in *M. anisopliae* conidial acquisition per single *M. sjostedti* during the flowering stage of both seasons in MA-baited and Lurem-TR-baited autoinoculation devices. However, significantly more *M. anisopliae* conidia were acquired per single *M. sjostedti* during the podding stage of the second season from MA-baited

autoinoculation devices. Mortality of *M. sjostedti* collected from field plots with Lurem-TR-baited and MA-baited autoinoculation devices and maintained in the laboratory, was significantly higher than thrips collected from the control plots in both seasons. The higher yield in MA-baited inoculation treatments may be due to better conidial acquisition as compared to the Lurem-TR-baited device resulting in reduced biotic pressure by *M. sjostedti* in the treatment plot. The use of MA in an autoinoculation device for the management of *M. sjostedti* on grain legumes is therefore recommended

5.1 Introduction

Cowpea, *Vigna unguiculata* L. Walp (Fabales: Fabaceae), is one of the most important protein rich grain legumes. The leaves, green pods and dry grains are consumed (Saidi et *al.,* 2010). Production is, however, insufficient to meet the demand of particularly dry grains (Kimani *et al*., 1994; Mergeai *et al*., 2001). The crop is grown under low-input conditions and grain yields are low, averaging less than 500 kg ha^{-1} (Adipala *et al*., 1999). This is attributed to many factors, of which the most important are heavy biotic pressures from insects and other pests (Rachie, 1985; Jackai and Daoust, 1986; Abate and Ampofo, 1996). The bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae), is the dominant thrips species on cowpea and cause severe damage to the crop. This species is found throughout tropical Africa (Fritsche and Tamo, 2000; Moritz *et al*., 2013). Growers apply chemical insecticides intensively to control *M. sjostedi* outbreaks (Oparaeke, 2006). The misuse of synthetic chemical insecticides has a detrimental effect on the environment and non target insects. Moreover, thrips have developed resistance against many of these chemical insecticides (Espinosa *et al*., 2002; Jensen, 2004).

Entomopathogenic fungi (EPF) are among the alternatives being considered (Ekesi *et al*., 2002). EPF are generally applied through inundative sprays similar to other chemical insecticides (Jaronski, 2010). However, this approach has a number of disadvantages including short persistence of the inoculum due to detrimental effects of solar radiation and high costs as a result of repeated applications (Fargues *et al*., 1996; Inglis *et al*., 2000; Leland and Behle, 2004; Jaronski, 2010).

Semiochemicals such as kairomones (Muvea *et al*., 2014) or aggregation pheromones (Krueger *et al*., 2015; Niassy *et al*., 2015) are known to attract bean flower thrips. Since insect behaviour is mediated by semiochemicals (Hamilton *et al*., 2005), it is possible to use them to develop other application strategies, thereby improving the efficacy of application of entomopathogens (Niassy *et al*., 2012; Mfuti *et al*., 2016). The combination of semiochemicals with entomopathogens is largely explored in a "lure and infect" or "lure and kill"strategy (Vega *et al*., 2007). However, the success of this technology depends on the compatibility of the attractants with the entomopathogens. For instance, Niassy *et al*. (2012) reported the incompatibility of the thrips commercial attractant Lurem-TR and entomopathogenic fungi *Metarhizium anisopliae* isolate ICIPE 69 in an autoinoculation device. This incompatibility was solved by spatial separation between the two agents (Mfuti *et al*., 2016) (Chapter 3). Potential thrips attractants were also screened for their compatibility with *M. anisopliae.* The thrips attractant, methyl anthranilate was evaluated in both laboratory and field trials for its compatibility and attractiveness to *M. sjostedti* (Chapter 4).

The aim of this study was therefore to evaluate the efficacy of methyl anthranilate as an attractant for an autoinoculation device under field conditions. Indicators used were a reduction in *M. sjostedti* numbers per plant, acquisition and persistence of conidia, *M. sjostedti* mortality and cowpea yield.

5.2 Materials and methods

Study site

A field experiment was conducted in western Kenya at Mbita Thomas Odhiambo Campus (0˚26' 06.19"S, 34˚ 12' 53.13"E; 1,137 m above sea level) of the International Centre of Insect Physiology and Ecology (ICIPE), over two seasons. The first experiment was conducted during the cold dry season (June 2014) while the second one was conducted during the short rainy season (August 2014).

Mass production of the fungus

Metarhizium anisopliae isolate ICIPE 69 was used in this study. The production of conidia is described in chapter 3, section 3.2.

Autoinoculation device

The autoinoculation device used in the current study is a modification of the device described by Niassy *et al*. (2012) (Figure 5.1). This device was adapted to address the incompatibility between conidia of *M. anisopliae* and the semiochemical Lurem-TR when put together (Mfuti *et al*., 2016).

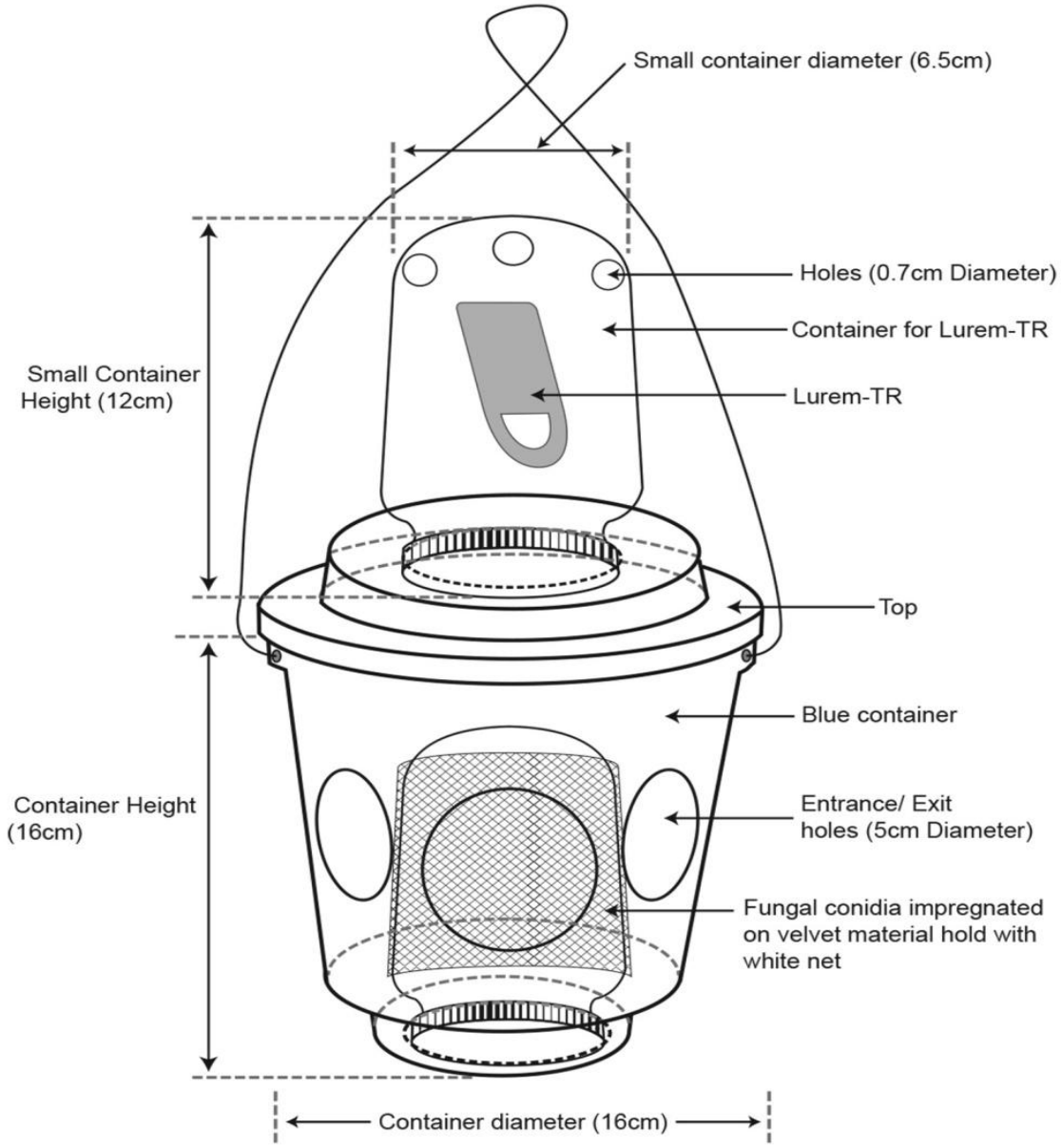

Figure 5.1: Autodissemination device for spatial separation of the semiochemical and entomopathogenic fungi.

Semiochemicals

The semiochemicals used in this study were Lurem-TR and methyl anthranilate. Their chemical composition was described in chapter 3 (section 3.2) and chapter 4 (section 4.2).

Experimental crop

A cowpea field was divided into four blocks and each block subdivided into three 8 x 10 m experimental plots. Blocks were separated by 5 m while plots were separated by 2 m. Cowpea, variety Ken-Kunde 1, was planted with intra-row and inter-row spacing of 50 cm and 20 cm, respectively. Irrigation was applied once a week and weeding was initially conducted fortnightly, but later in the season only when weeds were abundant. No fertilizers, organic matter or chemical insecticides were applied during the experiment.

5.2.1 Effect of semiochemical-baited autoinoculation devices treated with *Metarhizium anisopliae* on *Megalurothrips sjostedti* density

The treatments were fungus treated autoinoculation devices baited with: (i) methyl anthranilate contained in a 2 ml cryogenic vial was placed inside the device (direct contact with conidia), (ii) fungus-treated autoinoculation devices baited with Lurem-TR spatially separated from the conidial source (Figure 5.1) and (iii) a control treatment with no fungus and only a blue sticky card to monitor *M. sjostedi*. One device was placed per treatment plot, within each block and at 12 m apart to avoid interference between treatments. Treatments were arranged in a complete randomized block design. The fungus was placed in the devices in the field at the beginning of the flowering stage (which ran from day 0-15 of the experiment) and the replaced at the beginning of the podding stage (which ran from day 15-30 of the experiment). Therefore, *M.*

sjostedti density was assessed for a period of two weeks during the flowering and podding stages. To assess the number of *M. sjostedti* in the respective treatment plots, five cowpea plants were randomly sampled, every three days. The number of thrips was recorded using a whole plant tapping technique. Plants were tapped with the palm of a hand (five taps) on a white barber tray (25 x 45 cm) which was held underneath the selected plant and the thrips counted (Pearsall and Myers, 2000). Thrips collected in the trap were identified according to Moritz *et al*. (2013).

5.2.2 Effect of semiochemical-baited autoinoculation devices treated with *Metarhizium anisopliae* on conidial persistence of *Metarhizium anisopliae*

The persistence of conidia of *M. anisopliae* was evaluated for a period of two weeks during the flowering and podding stages of the cowpea crop. Samples of conidia were collected from the autoinoculation devices from each treatment with three–day intervals, as described in chapter 3 (section 3.2.2). A semiochemical-free device was included as a treatment and placed at least 15m away from the treatment devices as a check for evaluating the persistence of conidia in the autoinoculation devices used as control.

5.2.3 Effect of semiochemical-baited autoinoculation devices treated with *Metarhizium anisopliae* on conidial acquisition and mortality of *Megalurothrips sjostedti*

To determine the number of conidia acquired by single *M. sjostedti* adults in the semiochemical-baited autoinoculation devices, five cowpea plants were sampled, every three days for a period of two weeks, in a 2 m radius around semiochemical-baited autoinoculation devices in each treatment block. Twenty *M. sjostedti* adults were aspirated from the sampled plants and transferred to 10-ml glass containers. The latter was labelled and placed in the fridge to chill the

insects. Thrips were transferred individually into 2 ml cryogenic tubes containing 1 ml of sterile 0.05% Triton X-100. The tube was then vortexed for 2–3 min to dislodge conidia from the insect and the concentration of conidia was determined using a Neubauer haemocytometer.

To evaluate the efficacy of *M. anisopliae* in semiochemical-baited autoinoculation devices, thrips were collected from five randomly sampled plants within a 2 m radius around each device. Twenty *M. sjostedti* were transferred into 10 ml sterile cylindrical tubes (2 x 10 cm) with pods and flowers and maintained under laboratory conditions. Mortality was recorded daily for 7 days.

5.2.4 Cowpea yield

Cowpea pods in each treatment were harvested approximatively 90 days after planting. The seed was harvested from pods which were dried for three weeks and weighed with a Mettler PM 15 balance. Yield was expressed in kg/ha.

5.2.5 Statistical analysis

Data on *Megalurothrips sjostedti* densities were checked for normality and homogeneity of variance using Shapiro–Wilk and Bartelett tests. Data on *M. anisopliae* conidial viability were arcsine transformed and subjected to repeated measures ANOVAs. Multiple comparisons of means were made with Tukey HSD and Levene tests. Repeated measures ANOVAs were also used to analyse the mean number of conidia acquired by a single thrips. *Megalurothrips sjostedti* mortality and cowpea yield were analysed by means of one way ANOVA. All data analyses were performed individually for each season using R statistical (R Development Core Team, 2014). The level of significance was maintained at 95%.

5.3 Results

5.3.1 Effect of semiochemical-baited autoinoculation devices treated with *Metarhizium anisopliae* on *Megalurothrips sjostedti* density

Plants in plots with semiochemical-baited autoinoculation devices had generally less *Megalurothrips sjostedti* than plants in the control treatments during both experimental seasons (season I: $F = 7.4$; $df = 2, 94$; $P<0.001$; season II: $F = 5.2$; $df = 2, 94$; $P<0.01$) (Table 5.1). No significant differences in *M. sjostedti* densities were observed between plants in treatment plots with MA-baited autoinoculation devices and plants in plots with Lurem-TR-baited devices during both seasons. In the second season, *M. sjostedti* numbers in the Lurem-TR-baited device did not differ significantly from the control (Table 5.1). The density of *M. sjostedti* varied significantly over time during the first ($F = 31.9$; $df = 8, 94$; $P <0.001$) (Figure 5.2 a) and second seasons ($F = 9.6$; $df = 8, 50$; $P<0.001$) (Figure 5.2 b) with the control having the highest number of *M. sjostedti*. Significant differences were recorded between MA-baited autoinoculation device and Lurem-TR-baited device on day 21, 24 and day 30 (Figure 5.2 b).

Table 5.1: Mean number of *Megalurothrips sjostedti* in plots with methyl anthranilate-baited and Lurem-TR-baited inoculation devices in the two planting seasons.

Treatments	**Mean number of *M. sjostedi* per treatment plot ± SE**	
	Season I	**Season II**
Control (Blue card alone)	17.9±3.1a	61.5±17.3a
ADD[a]+LUREM	14.5±4.1b	55.1±22.3ab
ADD+MA	15.4±4.1b	49.8±20.2b
	F= 7.4; df= 2, 94; P<0.001	F= 5.2; df= 2, 94; P<0.01

[a]ADD = Autoinoculation/autodissemination device

Means (±SE) followed by the same lower case letters within the column are not significantly different according to Tukey's HSD

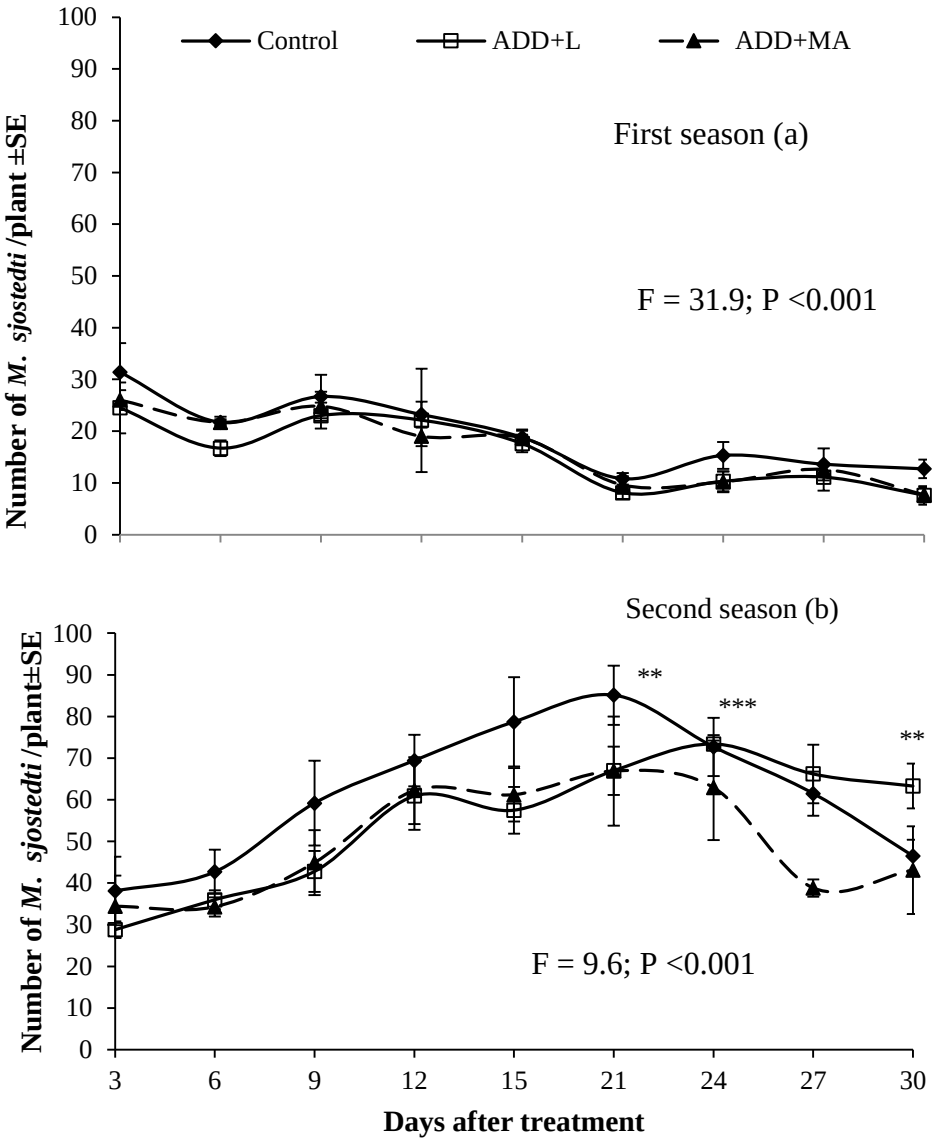

Figure 5.2: Mean number of *Megalurothrips sjostedi* per plant during the flowering (from day 3 to day 15) and podding (from day 21 to day 30) stages in plots with methyl anthranilate-baited (ADD-MA), Lurem-TR-baited (ADD-L) and control autoinoculation devices over two seasons. * Significance using Levene's test.

5.3.2 Effect of semiochemical-baited autoinoculation devices treated with *Metarhizium anisopliae* on conidial viability of *Metarhizium anisopliae*

Conidia of *M. anisopliae* was significantly more viable in semiochemical-free devices (control) than in semiochemical-baited device treatments in both seasons (season I: $F= 35.9$; $df = 2,141$; $P<0.001$; season II: $F= 18.2$; $df = 2,141$; $P<0.01$), except during the flowering stage in season II where no significant differences were found between treatments (Table 5.2). Conidial germination decreased from 93.4% on day 0 to 54.9, 53.1 and 46.6% on day 15 in control, Lurem-TR-baited and MA-baited devices, respectively, during the flowering stage in season I. However, a drastic decrease was recorded on day 3 in both semiochemical-baited treatments (Figure 5.3). A similar trend was also observed during the podding stage in the first season. During the flowering stage in the second season, conidial germination decreased from 84.6% on day 0 to 52.0-54.9% on day 15 and there were no significant differences between the treatments. The trend was similar during the podding stage, except that there was a significant decrease in conidial germination on day 3 in Lurem-TR-baited devices (60.7%) and MA-baited devices (57.3%) as compared to the unbaited control devices (76.2%) (Figure 5.3).

Table 5.2: Effect of semiochemical-baited autoinoculation devices treated with *Metarhizium anisopliae* on conidial persistence of *M. anisopliae* during flowering and podding stages of the two planting seasons.

Treatments	**Mean percentage germination ±SE**			
	Season I		**Season II**	
	Flowering	Podding	Flowering	Podding e
Control	65.2±3.1a	64.5±3.1a	64.1±13.2a	64.5±4.5a
ADD[a]+LUREM	57.2±4.1b	50.8±4.2b	57.2±16.8a	54.1±5.0b
ADD+MA	55.0±4.1b	51.1±4.1b	51.6±15.3a	55.0±5.1b
	F= 60.0;df= 2,70; P<0.001	F=60.2;df= 2,62; P<0.001	F= 1.7;df= 2,70; P = 0.2	F= 9.5;df= 2,62; P<0.001

[a]ADD = Autoinoculation device

Means (±SE) followed by the same letters within the row are not significantly different according to Tukey's HSD

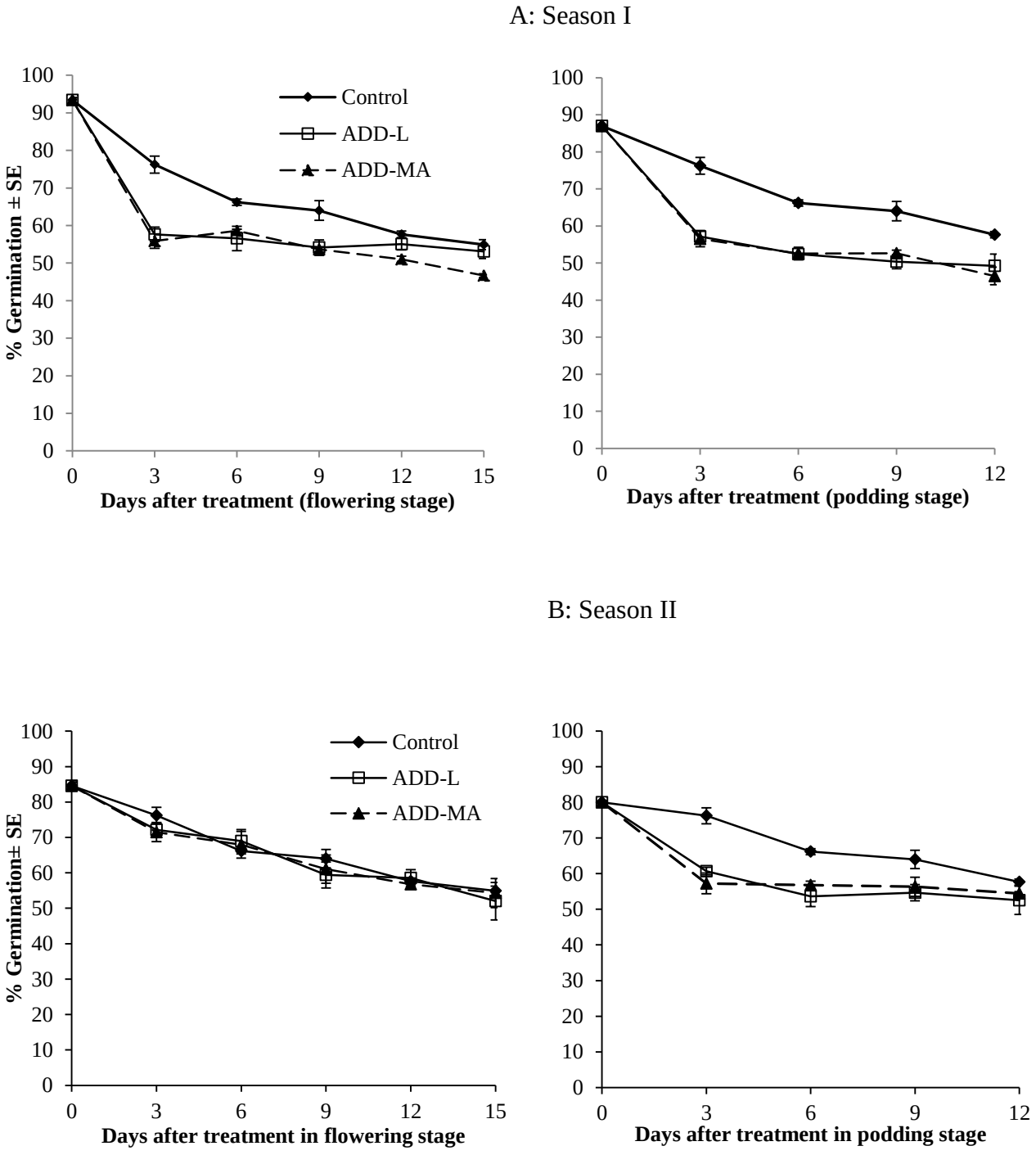

Figure 5.3: Effect of semiochemical-baited autoinoculation devices on conidial persistence of *Metarhizium anisopliae* during the flowering and podding stages of cowpea over seasons.

5.3.3 Effect of semiochemical baited autoinoculation devices treated with *Metarhizium anisopliae* on conidial acquisition by and mortality of *Megalurothrips sjostedti*

The mean number of conidia acquired varied between 2.0 and 10.0 x 10^3 conidia per *M. sjostedti* in both Lurem-TR-baited and MA-baited device treatments during both seasons. There was no significant difference in *M. anisopliae* conidial acquisition per single *M. sjostedti* during the flowering period of both seasons in MA-baited and Lurem-TR-baited autoinoculation devices. However, significantly more *M. anisopliae* conidia were acquired per single *M. sjostedti* during the podding stage of the second season from MA-baited autoinoculation devices ($F = 4.4$; $df = 1, 15$; $P = 0.05$) (Table 5.3).

Mortality of *M. sjostedi* collected from field plots with Lurem-TR-baited and MA-baited autoinoculation devices and maintained in the laboratory, was significantly higher than thrips collected from the control plots in both seasons (Season I: $F = 15.6$; $df = 2,78$; $P<0.001$; Season II: $F= 10.8$; $df = 2,78$; $P<0.001$). Mortality from field plots with baited devices ranged from 40 – 46% during the two seasons. No significant difference in *M. sjostedi* mortality was found between plots with the two semiochemical-baited autoinoculation devices (Table 5.4).

5.3.4 Cowpea yield

Cowpea yield varied significantly between treatments ($F = 5.4$; $df = 2,8$; $P<0.03$), with the highest yield recorded in plots with the MA-baited device (Table 5.3). No significant difference in the yield was observed between the control and Lurem-TR-baited device (Table 5.3).

Table 5.3: *Metarhizium anisopliae* conidial acquisition by single thrips from MA-baited and Lurem-TR-baited autoinoculation devices at flowering and podding stages.

Season	Crop stage	Days after treatment	Number of conidia/individual thrips	
			ADD[a]+Lurem-TR(Conidia/individual)	**ADD+MA** (Conidia/individual)
Season I	**Flowering**	6	$4.0\pm0.2x10^3$	$1.0\pm0.1\ x10^3$
		9	$5.0\pm0.2\ x10^3$	$8.0\pm0.3\ x10^3$
		12	$10.0\pm0.5\ x10^3$	$4.0\pm0.2\ x10^3$
		15	$6.0\pm0.3\ x10^3$	$8.0\pm0.3\ x10^3$
		F = 0.3; df = 1, 21; P = 0.6		
	Podding	6	$3.0\pm0.2\ x10^3$	$10.0\pm0.4\ x10^3$
		9	$3.0\pm0.3\ x10^3$	$8.0\pm0.3\ x10^3$
		12	$1.0\pm0.1\ x10^3$	$1.0\pm0.1\ x10^3$
		F = 3.3; df = 1, 15; P = 0.1		
Season II	**Flowering**	6	$6.0\pm0.5\ x10^3$	$6.0\pm0.6\ x10^3$
		9	$1.0\pm0.1\ x10^3$	$1.0\pm0.1\ x10^3$
		12	$1.0\pm0.1\ x10^3$	$0.0\pm0.0\ x10^3$
		15	$0.0\pm0.0\ x10^3$	$1.0\pm0.1\ x10^3$
		F = 0.0; df = 1, 21; P = 1.0		
	Podding	6	$0.0\pm0.0\ x10^3$	$5.0\pm0.2\ x10^3$
		9	$1.0\pm0.1\ x10^3$	$8.0\pm0.3\ x10^3$
		12	$5.0\pm0.2\ x10^3$	$6.0\pm0.4\ x10^3$
		F = 4.4; df = 1, 15; P = 0.05		

[a] ADD = Autoinoculation device

Means (±SE) followed by the same letters within the row are not significantly different according to Tukey's HSD.

Table 5.4: *Megalurothrips sjostedti* mortality from plots with MA-baited and Lurem-TR-baited autoinoculation devices as well as the control after seven days during the podding stage of the two seasons.

Treatments	**Mean mortality ± SE (%)**	
	Podding stage/Season I	**Podding stage/Season II**
Control (Blue card)	24.7±1.3b	25.0±1.3b
ADD[a]+Lurem-TR	46.0±4.0a	40.0±8.0a
ADD+MA	45.0±5.2a	42.8±5.5a
	F = 15.6; df= 2,78; P<0.001	F = 10.8; df= 2,78; P<0.001

[a]ADD = Autoinoculation device

Means (±SE) followed by the same letters within a column are not significantly different according to Tukey's HSD test.

Table 5.5: Mean cowpea yield (kg/ha) of plots with MA- baited and Lurem-TR-baited autoinoculation devices as well as the control during the second season.

Treatments	**Mean yield ± SE (kg/ha)**
Control (Blue card)	978.6±345.1b
ADD[a]+Lurem-TR	1041.7±66.3b
ADD+MA	1427.5±370.2a
	F = 5.4; df= 2,8; P<0.03

[a]ADD = Autoinoculation device

Means (±SE) followed by the same letters within a column are not significantly different according to Tukey's HSD test.

5.4 Discussion

The field performance of methyl anthranilate (MA) which was found to be attractive and compatible with *M. anisopliae* was studied and found to reduce the number of *M. sjostedi* in plots in which MA-baited devices, as well as Lurem-TR-baited devices was placed. The decrease in *M. sjostedi* was most evident during the second season (short rainy season) when higher *M. sjostedi* infestation was observed. Decreased infestation levels of *M. sjostedi* in the first season may be due to the cold weather widely prevalent in the months of June – August. This is in agreement with Ogah, (2011) who reported decrease in *M. sjostedi* population due to the weather conditions. The results on the effectiveness of semiochemical-baited devices treated with EPF are in agreement with previous reports on the effectiveness of this strategy against target insect pest populations (Dimbi *et al*., 2003; Niassy *et al*., 2012; Mfuti *et al*., 2016).

Conidial viability of *M. anisopliae* decreased over time in all the treatments. However, in all autoinoculation devices, at least 45% of *M. anisopliae* conidia remained viable 12-15 days post-exposure. However, Ekesi *et al.* (2001) reported persistence of about 3-4 days of this fungal isolate on cowpea and Daoust and Pereira (1986) reported persistence of 1-2 days on cowpea leaves for *M. anisopliae* and *B. bassiana.* The radiation in the ultraviolet region is considered as the major factor affecting the survival of conidia on plant foliage (Smits *et al*., 1996). In the present study, conidia were not directly exposed to solar radiations as they were applied in autoinoculation device. This may therefore explain the observed persistence of >45% after 12-15 days post-exposure. In field cage studies, Niassy *et al.* (2012) reported that conidial viability was not affected in autoinoculation device without a semiochemical, 7 days post-treatment, but in the Lurem-TR-

baited autoinoculation device, conidial viability decreased from 80 to 6% at 2 and 7 days post-inoculation, respectively. The difference between the two studies is that conidia were directly exposed to Lurem-TR (Niassy *et al.*, 2012) while they were spatially separated in the present study thereby enhancing compatibility (see Mfuti *et al*., 2016). Maniania (2002) reported that conidia of *M. anisopliae* could retain up to 60% of their viability after a 31-day exposure in a contamination device.

The mean number of conidia acquired by a single thrips in both semiochemical-baited devices varied between 2.0 x 10^3 and 10.0 x 10^3 conidia and was lower than what was reported by Niassy *et al.* (2012) for *F. occidentalis* (Pergande) (Thysanoptera: Thripidae). The study conducted by Niassy *et al.* (2012) was, however, conducted in experimental cages where multiple infections are possible compared to the present study which was conducted under field conditions. These results have shown that MA is as effective as Lurem-TR in terms of conidial acquisition.

Mortality of *M. sjostedi* in the two semiochemical-baited treatments ranged between 40-46%. However, in the presence of the semiochemical, Dimbi *et al*. (2003) reported mortality between 70 and 93% of fruit flies *Ceratitis rosa* (Karsch) and *C. fasciventris* (Bezzi) (Diptera : Tephritidae) after being attracted to *M. anisopliae*-treated autoinoculators baited with brewer's yeast in a field cage. In field cage studies, Niassy *et al*. (2012) reported thrips mortality ranging between 41.7 - 59.3% in autoinoculation device with or without semiochemicals. Similarly to conidial acquisition, differences in mortality among the different studies can be attributed to differences in experimental conditions (field cage vs open field) and difference in target insects.

Cowpea yield was higher in plots with the MA autoinoculation baited device compared to the Lurem-TR baited autoinoculation device and the control device. Bud formation and flowering phase of the crops are considered as critical crop growth stages for *M. sjostedi* management. Alghali (1991) reported that bean flower thrips can cause a yield reduction of 44% on cowpea at bud formation and flowering stages. Ezueh, (1981) reported that yield loss of 100% can occur on cowpea production if no control measures are taken. The higher yield in treated plots observed in this study can be linked to the higher mortality of *M. sjostedi* in treated plots as compared to the control. Among the treatments the increase in yield was more perceptible in the treatment MA-baited inoculation device as compared to the inoculation device baited with Lurem-TR. This could be attributed to the better conidial acquisition in MA treatments, which could have resulted in better secondary spread of the entomopathogen. The underlying factors for greater efficiency of MA as compared to Lurem-TR need further scrutiny.

5.5 Conclusion

The current study demonstrated the field efficacy of methyl anthranilate that is compatible with *M. anisopliae* and effective in attracting and infecting *M. sjostedi*. Hence, it could be used as alternative attractant to commercial semiochemical Lurem-TR in an autoinoculation device for *M. sjostedi* management instead of cover spray applications of biopesticides.

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CHAPTER 6: IMPROVING APPLICATION OF FUNGUS-BASED BIOPESTICIDE IN COMBINATION WITH SEMIOCHEMICAL FOR THE MANAGEMENT OF BEAN FLOWER THRIPS ON COWPEA

Abstract

The efficacy of spot spray and cover spray applications of *Metarhizium anisopliae* (Metsch.) Sorok. in combination with the thrips attractant Lurem-TR (methyl-isonicotinate), was compared in field experiments for the management of the bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) on a cowpea crop over two seasons. Treatments were applied five days after the placement of Lurem-TR in the field. During the first season, *M. sjostedti* densities were lower in spot spray (10.1±4.3 thrips) and cover spray (11.5±4.8 thrips) treatments than in the control treatment (41.8±15.2 thrips). In the second season, the lowest *M. sjostedti* density of 32.5±6.0 thrips were recorded in the spot spray treatment, followed by cover spray with 40.9±7.0 thrips recorded. The highest *M. sjostedti* density of 67.4±10.3 thrips was recorded in the control treatment. Fungal viability and thrips conidial acquisition did not differ between the two application methods. Both application strategies resulted in a yield increase of 34.1 and 46.2% compared to the control with the spot and cover spray treatments, respectively. The cost benefit analysis indicated more profits with the spot spray than cover spray application due to the reduction in labour and the quantity of inoculum used. Spot spray application of biopesticides could therefore be a more viable option for small-scale farmers for the management of *M. sjostedti* on cowpea.

6.1 Introduction

Cowpea, *Vigna unguiculata* L. Walp. (Fabales: Fabaceae), is an important food and cash crop in different parts of the tropics (Quin *et al*., 1997). It occupies a vital place in human nutrition as sources of protein, vitamins and minerals. In Kenya, cowpea is among the most consumed grain legumes, but the recorded yield is low and the demand can therefore not be satisfied (Mergeai *et al*., 2001). Annual production of cowpea in Kenya declined from about 83,000 MT in 2007 to about 48,000 MT in 2008, despite an increase in area planted from around 130,000 in 2007 to about 148,000 hectares over the same period (Belmain *et al*., 2013; Kiprotich *et al*., 2015). Insect pests are the main factor responsible for the low grain legume production (Abate *et al*., 2012; Ajeigbe *et al*., 2012). The bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae), is considered as major pest attacking the reproductive structures of cowpeas during plant development (Ezueh, 1981). *Megalurothrips sjostedti* can cause yield losses ranging from 20 to 100% (Singh and Allen, 1980).

The control of *M. sjostedti* relies heavily on the use of synthetic chemical insecticides (Jackai and Daoust, 1986; Abate and Ampofo, 1996), which is associated with numerous problems such as safety of workers health risks to consumers and livestock, and environmental contamination (Nderitu *et al*., 2007). Furthermore, intense applications of chemical insecticides can result in residue problems on harvested beans (Löhr, 1996). The use of entomopathogenic fungi (EPF) in horticulture is gaining momentum in Africa over the last few years (Ekesi *et al*., 2001; Ekesi and Maniania, 2007). Biopesticides are generally applied through inundative sprays, which requires high quantities of inoculum. In addition, their short persistence in the field due to solar radiations

means that repeated applications are often needed, resulting in high costs. Therefore, there is the need to find an alternative application strategy to overcome these limitations.

The use of semiochemicals to lure large number of insects in a trap that can then be inoculated with EPF has been reported in the case of termites (Alves *et al*., 2002). This technique is attractive as it has low cost in comparison to the broadcast application. The integration of pheromones and kairomones in thrips management (Teulon *et al*., 2014; Broughton *et al*., 2015) can offer new perspectives for application of EPF for the control of thrips (Niassy *et al*., 2012; Mfuti *et al*., 2016). It has been reported that the presence of Lurem-TR attracts high numbers of *M. sjostedti* adults in baited traps (Muvea *et al*., 2014). It is therefore hypothesized that spot spray application of EPF in combination with thrips attractant could reduce the quantity of inoculum, thereby the cost of the thrips management. The objective of this study was therefore to evaluate the efficacy and cost benefit analysis of spot spray and cover spray applications of the entomopathogenic fungus *M. anisopliae* through the use of the attractant Lurem-TR for the management of *M. sjostedti* on cowpea crop.

6.2 Materials and methods

Study site

A field experiment was conducted from June to December 2014 at Mbita Thomas Odhiambo Campus, situated in the eastern shores of Lake Victoria (0˚26’ 06.19” S, 34˚12’ 53.13” E; 1,137 m above sea level) in western Kenya. The vegetation type around the campus is mainly savannah grassland with mixed combretum and acacia trees to the north and papyrus along the shores of the lake. The experiment was conducted in two seasons. In the first season, cowpea was planted on 6 June 2014 and the experiment was conducted from July to August 2014. It coincided with

the dry season with low infestation of *M. sjostedti*. However, the season was characterized by high cowpea aphid infestation. In the second season, cowpea was planted on 10 August 2014 and the experiment was conducted from October to December 2014. This coincided with the short rainy season with high *M. sjostedti* infestation.

The fungus

Metarhizium anisopliae isolate ICIPE 69 was obtained from Real IPM Ltd. in Kenya. It is registered in Kenya and other African countries and commercialized under the trade name of Campaign®. Emulsifiable formulation of the fungus (1×10^9 CFU ml^{-1}) was applied at the recommended dose of 200 ml ha^{-1}, corresponding to 2.0×10^{14} conidia ha^{-1}, using a CP15 knapsack sprayer (Cooper Pergler, Sussex, UK) with an output of 350 litres ha^{-1}. Spores were checked for viability before application and conidial germination over 85% was considered acceptable.

Semiochemical

Lurem-TR was used in the present study as previously described in chapter 3, section 3.2.

Experimental design

Cowpea variety Ken-Kunde 1 was planted with an intra-row spacing of 50 cm and inter-row spacing of 20 cm. Irrigation was provided once a week and weeding was done regularly. No fertilizers, organic matter or synthetic chemical insecticides were applied during the experimental periods. The experiment was conducted during the flowering and early podding stages which coincided with higher *M. sjostedti* populations (Ezueh, 1981; Nyasani *et al*., 2013).

The field was divided into four blocks, each with three equal size experimental plots (7 x 7 m^2). Treatments were arranged in these plots in a complete randomized block design. Blocks were separated by 5 m while within blocks, plots were separated by 2 m to avoid interference of treatments.

The treatments were: T_1: Blue card+Lurem-TR (Control); T_2: Blue card+Lurem-TR+*M. anisopliae* applied in spot spray; T_3: Blue card+Lurem-TR+*M. anisopliae* applied in cover spray. For spot spray, the fungus was applied on a 9-m^2 area around the Lurem-TR attractant placed at the centre of each plot within the block. In the cover spray treatment, the fungus was applied as a full cover spray in the plot i.e. 48 m^2. Preliminary data on the attraction of *M. sjostedti* population to Lurem-TR-baited sticky cards indicated that the optimal time to attract ~50% of *M. sjostedti* was between 3 and 5 days after deployment of the baited cards in fields (Niassy et al. unpublished). The fungus was applied five days after deployment of Lurem-TR in the plots at the beginning of flowering (which ran from day 0-9 of the experiment) and then again at the beginning of the podding stage (which ran from day 9-21 of the experiment). No fungus was applied in the control plot. For assessing conidial persistence and acquisition, data from treatments with *M. anisopliae* applied in cover and spot sprays only were analysed.

6.2.1 Effect of fungal application strategy on *Megalurothrips sjostedti* density

The number of *M. sjostedti* per plant was recorded every three days for a period of 9 days during both the flowering and podding stages. Random sampling of five cowpea plants was done in each treatment plot using the whole plant tapping technique. Plants were tapped gently with the

hand, five times, on a white barber tray (25 x 45 cm) held underneath the selected plant (Pearsall and Myers, 2000). The tray was cleaned after each sampling.

6.2.2 Effect of fungal application strategy on *Metarhizium anisopliae* conidial persistence

The persistence of conidia of *M. anisopliae* was evaluated from samples taken on the day of fungal application (day 0), day 1 and 4 after fungal applications. Samples were collected randomly by cutting three cowpea leaves from plants in plots that received fungus treatments. Leaves were cut in small pieces and suspended in 10-ml 0.05% (wt ⁄vol) Triton X-100 and vortexed for 1 min to dislodge the conidia. A sample of 100 µl was spread-plated on SDA plates containing chloramphenicol (500 µg/ml) to inhibit growth of bacterial contaminants (Inglis *et al*., 2012). It was incubated for 16 h at 25 ± 2 ˚C in total darkness. Germination of conidia was determined as described above (see chapter 3, section 3.2). The sampling for cover spray treatments was done at random over the entire plot, while for the spot spray; it was done in the $9m^2$ area around the traps.

6.2.3 Effect of fungal applications strategy on conidial acquisition

Twenty adult *M. sjostedti* were randomly collected from 5 cowpea plants in fungus-treated plots using an aspirator to assess the number of conidia acquired by single insect. *Megalorothrips sjostedti* were transferred to glass containers which were labelled and stored in the fridge to immobilize the insects. They were then transferred individually into 2-ml cryogenic tubes containing 1 ml of sterile 0.05% Triton X-100. The tubes were vortexed for 2–3 min to dislodge conidia from the thrips and the concentration of conidia was determined using a Neubauer haemocytometer.

6.2.4 Cowpea yield

Due to high infestation of cowpea aphid during the first season, the yield could not be assessed. Therefore, cowpea yield was only obtained during the second season. Pods from each plot were harvested 90 days after planting, sundried on a large cement surface for three weeks and weighed with a Mettler PM 15 balance. The yield obtained from the grains was calculated in Kg/ha.

6.2.5 Cost benefit analysis

The net benefit was calculated using the partial budgeting procedure (El-Deep Soha, 2014) which assesses the costs and benefits associated with a specific change in an individual enterprise within the business operation. The procedure focuses specifically on the implications of the intended change in a business operation by comparing the benefits and costs resulting from implementing the alternative with respect to the current practice. Partial budget, like an enterprise budget, is based on a unit (a one crop farm). Only variable input costs are used in a partial budget. The net benefit is the difference between the gross farm gate benefit and total variable input costs. The cost benefit analysis was calculated taking into account the traditional farmer's practices where no blue sticky card and semiochemical attractants are used. Grain yields of crops grown under low-input conditions are low and unstable. Grain yields of between 350 and 540 kg ha^{-1} were reported in Kenya (Ekesi *et al*., 1998, 1999; Katungi *et al*., 2009). The grain yield of 350 kg ha^{-1} was therefore used for the cost benefit analysis calculation.

6.2.6 Statistical analysis

All data were tested for normality and homogeneity of variance using Shapiro–Wilk and Bartelett tests (Shapiro and Wilk, 1965; Snedecor and Cochran, 1989). Due to the over dispersion of *M. sjostedti* between treatments, data were subjected to the negative binomial of generalize linear model. The means were compared with Tukey HSD test at 5% significance level. Repeated measures ANOVAs were used to analyze *M. anisopliae* conidial persistence and conidial acquisition. Yield data were subjected to one way ANOVA and the means compared with Tukey HSD test at 5% significance level. All data analyses were performed using R (Version 3.1.3, 2015) statistical software (R Development core Team 2014).

6.3 Results

6.3.1 Effect of fungal application strategy on *Megalurothrips sjostedti* density

The density of *M. sjostedti* was higher in the control than in the fungus-treated plots in both seasons (season I: $F = 59.5$; $df = 2,61$; $P<0.001$; season II: $F = 85.5$; $df = 2,61$; $P<0.001$). There was no significant difference in *M. sjostedti* density between spot spray and cover spray application plots in season I (Table 6.1). There was, however, a significant difference in *M. sjostedti* density between spot spray and cover spray application plots in season II, with the spot spray treatment having the lowest number of thrips (Table 6.1). A significant difference was detected over time during the two experimental seasons (season I: $F = 4.2$; $df = 6,61$; $P<0.001$; season II: $F = 26.4$; $df = 6,61$; $P<0.001$)(Figure 6.1). *Megalurothrips sjostedti* density was higher in the control than in cover and spot spray treatments at all sampling days in season I (Figure 6.1A) and season II (Figure 6.1B).

Table 6.1: *Megalurothrips sjostedi* density per plant following spot and cover spray applications of *Metarhizium anisopliae* during flowering (from day 0 to day 9) and early podding (from day 15 to day 21) during the two seasons.

Treatments	**Mean *M. sjostedti* density/plant ± SE**	
	Season I	Season II
Control	41.8±15.2a	67.4±10.3a
Cover spray	11.5±4.8b	40.9±7.0b
Spot spray	10.1±4.3b	32.5±6.0c
	F = 59.5; df = 2, 61; P < 0.001	F = 85.5; df = 2, 61; P < 0.001

Means (±SE) followed by the same letters within the column are not significantly different according to Tukey's HSD

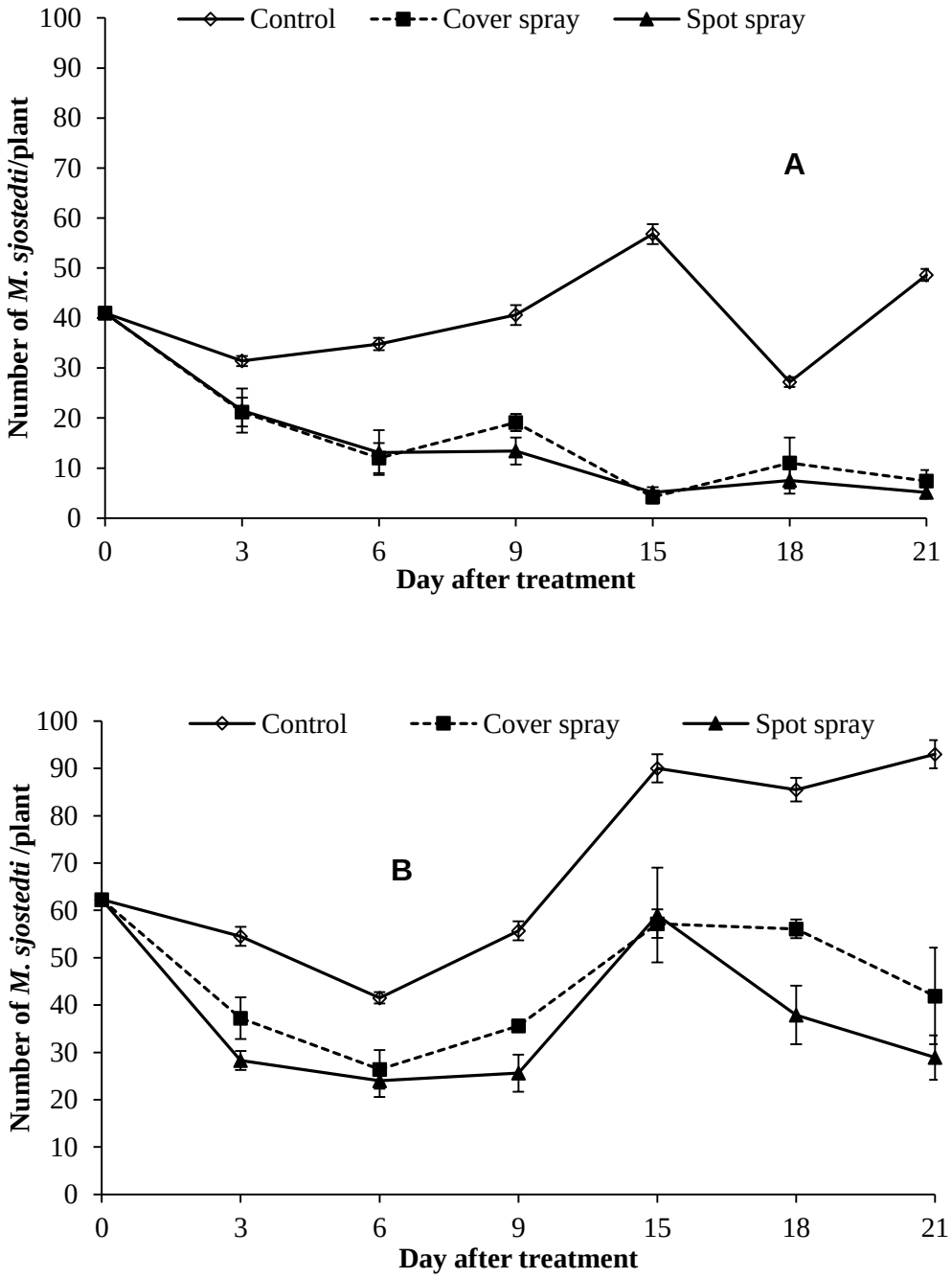

Figure 6.1: *Megalurothrips sjostedti* density per plant following spot and cover spray applications of *Metarhizium anisopliae* during flowering (from day 0 to day 9) and early podding (from day 15 to day 21) during season I (A) and season II (B). Bars denote means ± one standard error at P =0.05 (Tukey HSD test).

6.3.2 Effect of fungal application strategy on *Metarhizium anisopliae* conidial persistence and *Megalurothrips sjostedti* conidial acquisition

There was no significant difference in conidial persistence of *M. anisopliae* between the two fungal application strategies in both seasons. Season I (Flowering: $F = 0.2$; $df = 1, 11$; $P = 0.7$; Podding: $F= 0.0$; $df = 1, 11$; $P = 1.0$), season II (Flowering: $F = 0.1$; $df = 1, 11$; $P = 0.8$; Podding: $F= 0.2$; $df = 1, 11$; $P = 0.7$). There was also no significant treatment x time interaction during flowering ($F = 0.2$; $df = 1, 30$; $P = 0.6$) as well as during podding ($F= 0.0$; $df = 1, 30$; $P = 0.9$) of season I.Conidial viability did, however, decrease significantly over time during both flowering ($F = 40.7$; $df = 1, 30$; $P < 0.0001$) and podding ($F= 515.1$; $df = 1, 30$; $P < 0.0001$) (Figure 6.2 A). For example, conidial germination dropped from 93.4% at day 0 to 58.6 and 47.9% after one day in spot spray and cover spray treatments, respectively, and to 32.0% after 4 days in both treatments during the flowering stage of season 1 (Figure 6.2 A). During the podding stage, conidial germination reduced from 87.0% on day 0 to between 42.2% and 45.6% on day 1 and to 13.7% on day 4 post-treatment in both spray applications (Figure 6.2 A). There was a significant treatment x time interaction during flowering of season II ($F = 6.3$; $df = 1, 30$; $P < 0.01$), but not during the podding stage of season II ($F= 0.0$; $df = 1, 30$; $P = 1.0$). Conidial viability of the fungus, regardless of the method of application decreased significantly over time (Flowering: $F = 16.5$; $df = (1, 30)$; $P < 0.0001$; Podding: $F=93.0$; $df = (1, 30)$; $P < 0.0001$) (Figure 6.2 B). A drastic decrease in conidial germination was observed one day after application of the fungus during the flowering stage of season II with a decreased from 85.0% at day 0 to 26.2% in both treatments. Germination further decreased 4 days after treatment, to 17.0% in the cover spray and 9.4% in spot spray treatments respectively (Figure 6.2 B). During the podding stage, conidial viability decreased from 84.6% on day 0 to 47.0 and 45.0% one day after treatment in spot spray

and cover spray treatments, respectively, and to 6.1%, 4 days post-treatment for both application methods (Figure 6.2 B).

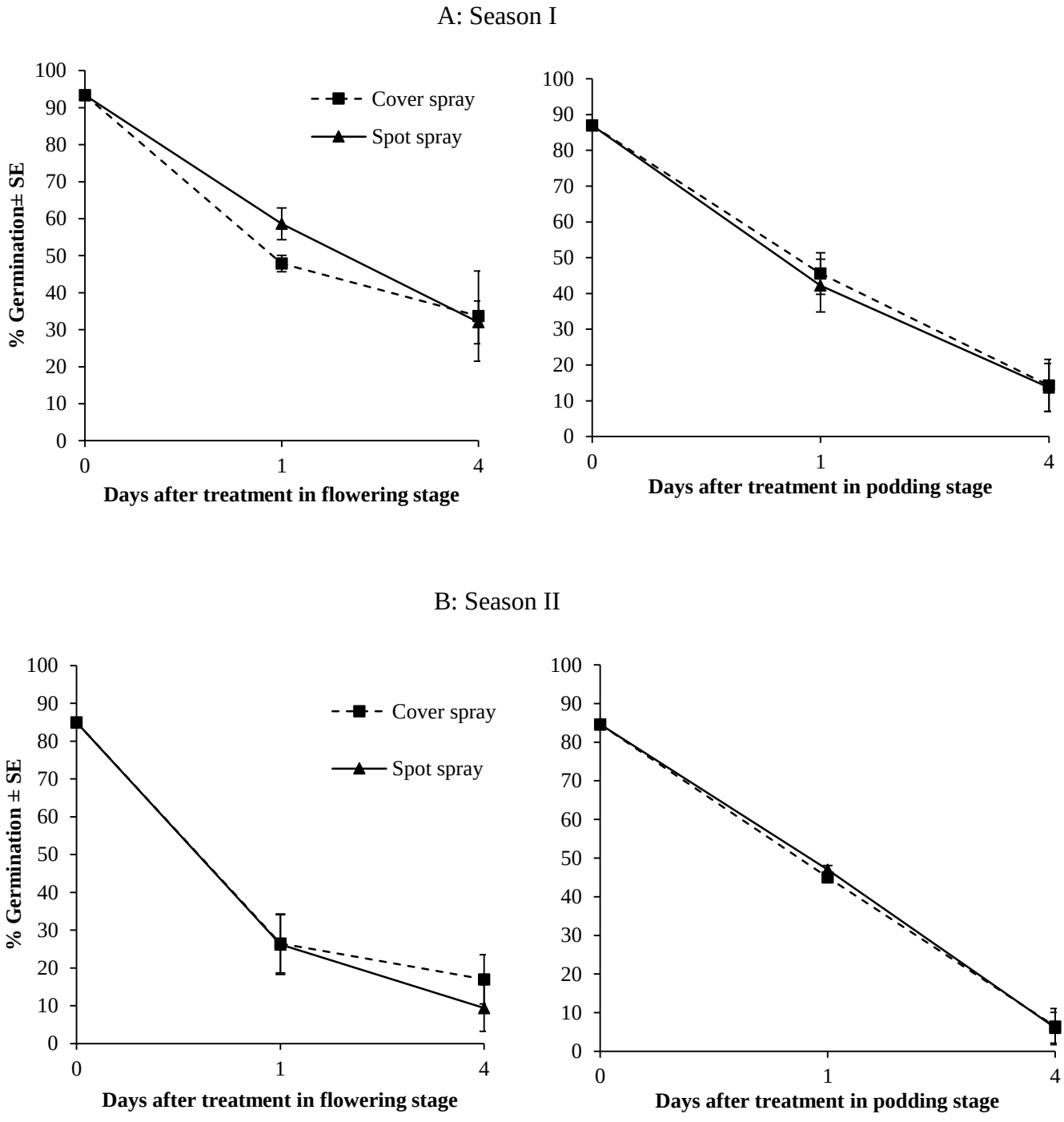

Figure 6.2: Conidial viability of *Metarhizium anisopliae* following spot spray and cover spray applications during the flowering and podding stages of cowpea during season I (A) and season II.

6.3.3 Effect of fungal application strategy on conidial acquisition

No significant differences in the number of conidia acquired by *M. sjostedti* were observed between the two application strategies during the flowering stage of both seasons and the podding stage of season 2 (Table 6.2). There was, however, a significant difference in the number of conidia acquired by *M. sjostedti* between the cover and spot spray application treatments during the podding stage of season 1 (Table 6.2).

Table 6.2: Conidial acquisition by *Megalurothrips sjostedti* following application of *Metarhizium anisopliae* as spot and cover sprays during flowering and podding of cowpea over two seasons.

Season	**Crop stage**	**Days**	**Cover spray (x 10^4conidia)**	**Spot spray (x 10^4 conidia)**
Season I	**Flowering**	1	1.4±0.8	0.5±0.3
		4	0.4±0.2	0.5±0.3
			F = 1.2; df = 1, 11; P = 0.3	
	Podding	1	0.3±0.1	1.1±0.8
		4	0.3±0.1	0.4±0.2
			F = 5.6; df = 1, 11; P < 0.05	
Season II	**Flowering**	1	0.3±0.2	0.5±0.2
		4	1.3±0.8	0.5±0.3
			F = 0.4; df = 1, 11; P = 0.5	
	Podding	1	0.8±0.1	1.0±0.4
		4	0.9±0.7	0.1±0.1
			F = 0.5; df = 1, 11; P = 0.6	

6.3.4 Cowpea yield

The yield was significantly high ($F = 5.8$; $df = 2, 6$; $P < 0.04$) in both cover (1430.7±114.2 kg) and spot spray (1312.1±87.7 kg) treatments than in the control treatment plots (976.8±105.2 kg). There was, however, no significant difference in yield of the fungus treatment plots.

6.3.5 Cost benefit analysis in US$ following application of *M. anisopliae* as spot and cover sprays compared to current farmer's practices

Variables that were considered in the cost benefit analysis are listed in Table 6.3. The total input cost for crops treated with cover and spot sprays is estimated at US$ 674 and US$ 611.4 respectively. The input cost for the control plots is estimated at US$ 597 and US$ 155 for traditional farmer's practices (Table 6.3). The gross income is estimated at US$ 3,148 and US$ 2,887 for crops receiving cover and spot spray, respectively, US$ 2,153 for crops produced in the control plots and US$ 770 for crops produced by using the traditional farmer's practices (Table 6.3). The net benefit is therefore higher in the cover and spot spray treatments than in the control plots (Table 6.3). In comparison with the traditional farmer's practices and with the control, the cost benefit analysis showed that the rate of return was higher for the spot spray treatment than the cover spray (Table 6.3).

Table 6.3: Cost benefit analysis in US$ following application of *Metarhizium anisopliae* as spot and cover spray treatments in comparison with a control treatment and traditional farmer's practices.

Variables		**Cover spray**	**Spot spray**	**Traditional farmer's practices**	**Control**
	Area for calculation	1 ha	1 ha	1 ha	1ha
	Fungal application area	1 ha	0.1875ha		
Gross income					
	Average cowpea yield (kg/ha)	1,430.7	1,312.1	350.0	978.6
	Unit selling price of dry cowpea seeds (US$/kg)	2.2	2.2	2.2	2.2
	Revenue (US$/ha)	3,147.5	2,886.6	770.0	2,153.0
Variable input costs					
Technological cost (US$)					
	Attractant (US$/ha)	383.6	383.6		383.6
	Blue card (US$/ha)	58.4	58.4		58.4
	Cost of oil formulation of fungal spores (US$/ha) x 2 sprays	50.0	9.4		0.0
	Total technological cost (US$/ha)	492.0	451.4	0.0	442.0
	Seed cost (US$/ha)	73.0	73.0	73.0	73.0
	Labor cost(US$/ha)	109.0	87.0	82.0	82.0
	Total variable input costs	674.0	611.4	155.0	597.0
Net benefit (US$)		**2,473.5**	**2,275.2**	**615**	**1,556.0**
Change in net benefit relative to the traditional farmer's practices[#] and to the control*		[#]1,858.5 *917.5	[#]1,660.2 (719.2)		
Change in total variable input cost relative to the traditional farmer's practices[#] and to the control*		[#]519.0 *77.0	[#]456.4 *14.4		
Rate of return to the traditional farmer's practices[#] and to the control*		[#]**3.58** *11.91	[#]**3.64** *49.94		

6.4 Discussion

This study demonstrated that a combination of Lurem-TR with *M. anisopliae* applied as cover and spot sprays significantly reduced *M. sjostedti* densities on cowpea during both seasons. With the exception of the second season during which a greater reduction in *M. sjostedti* numbers occurred in spot spray than in cover spray treatments. Both the fungus treatments reduced the *M. sjostedti* numbers significantly during the first season. The use of spot sprays can therefore overcome the shortcomings of cover spray applications, e.g. high volume of inoculum needed to achieve effective mortality of the target pest (Samuel and Graham, 2003; Jaronski, 2010). This is evidenced by the low input costs (fungus application, total technological and labour costs) recorded in the spot spray treatment compared to that of the cover spray. In comparison with the traditional farmer's practices, the cost benefit analysis showed a rate of return of 358% for the cover and 364% for the spot spray treatments. In other words, an investment of US$1 in fungus application on cowpea recoups the US$1 and gives an additional US$ 3.58 and US$ 3.64 benefit for the cover ans spot sprays, respectively. However, when compared with the control used in this study, US$ 1 recoups the US$ 1 and results in an additional US$ 11.91 and US$ 49.94 respectively, if *M. anisopliae* is applied as cover and spot spray treatments. Despite the fact that the total variable inputs were less in the traditional farmer's practices, the rate of return was much lower due to biotic pressure on cowpea plants, resulting in lower productivity. It is expected that this rate of return will increase over time since some of the materials such as blue cards can be reused, resulting in lower input costs for the farmer.

Fungal conidia acquired by individual thrips were similar in both application methods and are lower than reported in literature. For instance, Mfuti *et al*. (2016) reported conidial acquisition in

the range of 3.0 and 10.0 x 10^4 by single adult *M. sjostedti* depending on distance of separation of Lurem-TR and *M. anisopliae* in autoinoculation devices. Similar results were reported by Ugine *et al*. (2005) with *B. bassiana* against adult *F. occidentalis*. In another study, Niassy *et al*. (2012) observed that the overall mean number of conidia of *M. anisopliae* acquired per single *F. occidentalis* adults were 5.0 ± 0.6 x 10^4 in field cages with Lurem-TR-baited device while 2.2 ± 0.4 x 10^4 conidia in cages without semiochemical 7 days post-inoculation. A correlation between conidial acquisition and mortality was reported by Migiro *et al*. (2010) and Niassy *et al*. (2012). However, Ugine *et al*. (2005) noted that the rate of conidial acquisition on the bodies of *F. occidentalis* decreased significantly as the density of conidia on the leaf disk surface increased. The relatively lower number of conidia acquired by *M. sjostedti* in the present study compared to other studies could also be explained by the flower dwelling behaviour of *M. sjostedti* considering that experiments were conducted during flowering and the early podding stage.

Conidial viability decreased drastically after application in both cover and spot spray treatments. This could be explained by the fact that conidia in both treatments were subjected to the same abiotic factors such as solar radiation which is known to affect their persistence (Jaronski, 2010). Results from this study are in agreement with that of Fargues *et al*. (1996) who reported that survival of conidia of entomopathogenic ascomycetes species decreased with increased exposure to sunlight. For example, exposure for 2 h or more was found to be detrimental to all isolates tested. Under simulated sunlight in the laboratory, the same authors observed that persistence of conidia of *Beauveria bassiana* isolate LRC 26 were reduced by 64% after only 1 h exposure and none survived 4 hours of continuous exposure. Under natural conditions, conidial populations of the same isolate reduced much slower, 99% and 75-90% after 4 days on the top of the canopy of

crested wheatgrass *Triticum aestivum* (Poales: Poaceae) and alfalfa *Medicage sativa* (Fabales: Fabaceae), respectively. Similar results were reported with *M. anisopliae* on cowpea leaves (Ekesi *et al*., 2001). The decrease in viability found in this study, is likely to favour spot spray application as smaller quantities of inoculum is used compared to cover spray applications.

The first season was characterized by a low *M. sjostedti* population but the crop was highly infested by cowpea aphid *Aphis craccivora* (Hemiptera: Aphididae). Since no action was taken to control this insect pest, the grain yield was not recorded. As a result, the cowpea yield was recorded and the cost benefit analysis was calculated for the second season only. The cowpea yield in plots that received the spot and cover spray applications increased respectively with 42.6 and 34.1. The grain yields obtained in fungus treatments in this study are similar to yields reported by Saxena and Kidiavai (1997) following three applications of cypermethrin to the cowpea crop during the long rainy cropping season at the same site.

6.5 Conclusion

The current study showed that spot spray application of EPF in combination with an attractant is as effective as cover sprays in reducing *M. sjostedti* populations on cowpea. It requires less inoculum and labour as compared to cover spray applications. Moreover, spot spray applications recorded higher rates of returns than cover spray and this could increase over time with depreciation of materials used. This approach seems to be cost-effective and could be adopted by small-scale farmers if disseminated at large scale through technology transfer and sensitization campaigns.

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CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1. General discussion

In eastern Africa and in Kenya in particular, chemical control remains the main option for the management of the bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae). However, the frequent use of these synthetic chemical insecticides is associated with several problems such as health risks to the users and consumers, non-target organisms and environmental contamination, in addition to resistance of thrips to these chemical insecticides (Oparaeke, 2006; Alao *et al*., 2011).

Biological control using EPF is among the alternatives being considered (Ekesi and Maniania, 2007). EPF are generally applied using inundative sprays. However, this approach has a number of disadvantages including short persistence of the inoculum due to detrimental effects of solar radiation and high costs as a result of repeated applications (Inglis *et al*., 2000; Leland and Behle, 2004; Jaronski, 2010). In order to overcome these shortcomings, autodissemination devices have been developed for the management of several insects including thrips (Niassy *et al*., 2012). Autodissemination consists of attracting insects, using visual and chemical cues to a killing agent, namely an entomopathogenic fungi in this study. Attracted insects are infected with fungal conidia before returning to the environment where they can infect mates during mating or casual contacts. For thrips, the attractant is both visual (blue color) and may also be through a kairomone such as Lurem-TR, a methyl isonicotinate. However, Lurem-TR was found to negatively affect conidial viability of the *M. anisopliae* isolate ICIPE 69 (Niassy *et al*., 2012). Therefore, there is need to find the most appropriate way to combine the two agents or to identify

attractants that are compatible with *M. anisopliae* and which could be used both in autodissemination and "spot spray" approaches for the management of bean flower thrips (BFT), *Megalurothrips sjostedti* on grain legumes. The objective of this study was to develop efficient, economical and sustainable strategies for the management of *M. sjostedti* on grain legumes using the "lure and infect'' approach.

Influence of spatial separation of the semiochemical on thrips attraction and conidial acquisition by thrips from the autoinoculation device was investigated in the laboratory and in the field (Chapter 3). The results of this study demonstrated that the persistence of conidia of *M. brunneum* and *M. anisopliae* increased with distance of separation from Lurem-TR. Direct exposure of the fungus without separation from Lurem-TR recorded the lowest conidial germination as compared with the other treatments. Attraction of thrips to the device also varied significantly according to distance between the device and the semiochemical, with a higher number of thrips attracted when Lurem-TR was placed in a container below the device and at a 10 cm distance. No significant difference in conidial acquisition by *M. sjostedti* resulted from differences between spatial separation treatments of conidia and Lurem-TR. Positive correlations were found between conidial acquisition and thrips attraction. These results have confirmed the antifungal effect of Lurem-TR on the fungus as reported earlier by Niassy *et al*. (2012). Spatial separation of fungal conidia from Lurem-TR in an autoinoculation device could therefore provide a low-cost strategy for effective management of thrips in grain legume cropping systems.

Seven thrips attractants were screened in the laboratory for their compatibility with *M. anisopliae* isolate ICIPE 69, in terms of conidial germination and germ tube length, before possible

integration in autodissemination devices (Chapter 4). Conidial germination of *M. anisopliae* was significantly higher and germ tube length, significantly longer in the control, followed by methyl anthranilate, *cis*-jasmone and *trans*-caryophellene, and were found to be compatible with *M. anisopliae*. The lowest conidial germination and shortest germ tube length were obtained when conidial spores were exposed to Lurem-TR which further confirmed its antifungal properties (Niassy *et al*., 2012; Mfuti *et al*., 2016). Under field conditions, methyl anthranilate was as attractive as Lurem-TR to *M. sjostedi*. Methyl anthranilate has been reported to be attractive to four other flower thrips species, namely *Thrips hawaiiensis*, *T. coloratus*, *T. flavus*, and *M. distalis*, irrespective of sex (Murai *et al*., 2000; Imai *et al*., 2001), and it could therfore be considered as a potential attractant candidate.

The evaluation of semiochemical baited aoutinoculation devices (methyl anthranilate and Lurem-TR) in a field experiment resulted in a significant reduction in *M. sjostedti* numbers in cowpea plots (Chapter 5). Conidial viability of *M. anisopliae* was, however, significantly higher in semiochemical-free baited devices (control) than in semiochemical-baited devices in both seasons. The average number of conidia acquired by single *M. sjostedti* varied between 2.0 and 10.0 x 10^3 conidia in both semiochemical-baited device treatments during both seasons and was not significantly different, except for the podding stage in the second season where significant differences were found between treatments. Mortality of *M. sjostedti* in the two semiochemical-baited treatments ranged between 40-46%. Dimbi *et al*. (2003) reported *Ceratitis rosa* (Karsch) and *C. fasciventris* (Bezzi) (Diptera: Tephritidae) mortality of 70-93% and Niassy *et al*. (2012) 59% mortality of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in the presence of a semiochemical in field cages. Similarly to conidial acquisition, the lowest level of mortality in

the, current study despite the presence of a semiochemical can be ascribed to infection levels under field conditions as compared to field cages. Cowpea yield varied significantly between treatments, with the highest yield recorded in plots containing a methyl anthranilate-baited device. However, no significant difference in the yield of plots containing semiochemical free and Lurem-TR-baited devices was found. It can therefore be recommended from this study that methyl anthranilate should be used in autoinoculation devices for the management of *M. sjostedti* on grain legumes.

The efficacy of *M. anisopliae* applied in spot and cover sprays in combination with the thrips attractant, Lurem-TR was also evaluated in the field for two seasons (Chapter 6). *Megalurothrips sjostedti* densities were lower in spot and cover spray treatments than in the control treatment in both seasons, resulting in a yield increase. However, the cost benefit analysis following a procedure by El-Deep Soha (2014) procedure suggests that a spot spray application was more profitable due to a reduction in labour cost and the quantity of inoculum used.

7.2. Conclusions

With regard to the results obtained from all the objectives, we concluded that:

(i) Separating fungal conidia from Lurem-TR in an autoinoculation device could provide effective management of thrips in grain legume cropping systems.

(ii) Considering the attraction of other insect pests such as whiteflies, bean flies and leafminer flies to semiochemical-baited autoinoculation devices, the strategy could be an alternative control option not only for thrips but also for other insect pests of grain legumes.

(iii) Methyl anthranilate is as effective as Lurem-TR and can be recommended as an alternative thrips attractant for use in autoinoculation devices and spot spray applications in combination with *M. anisopliae* for the control of *M. sjostedti.*

(iv) Spot spray is a more profitable application strategy than cover spray of *M. anisopliae* due to a reduction in labour cost and the quantity of inoculum used. It should therefore be a more viable option for small-scale farmers and adoption should be facilitated by technology transfer campaigns.

7.3. Recommendations

(i) Since the autoinoculation strategy is effective in controlling thrips and other insect pests of grain legumes, surveys should be carried out to investigate farmers' perception to enable an eventual adoption of the strategy by farmers.

(ii) Methyl anthranilate could be used in spot spray applications of *M. anisopliae* or in the autoinoculation strategy for the control *M. sjostedti*.

(iii) The prototype of an autoinoculation device designed up to now seems to be cumbersome. A need therefore exists to develop a simple design of an autoinoculation device. The semiochemical included in the spot spray strategy is expensive. Potential for its reuse and replacement of the semiochemical with a colour attractant alone need to be investigated.

(iv) Electrostatic formulation of conidia of the fungus should also be considered as it could improve the adherence of conidia to the host cuticle.

7.4. References

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