Integration of soil-applied azadirachtin with predators, entomopathogens and optical/chemical traps for the management of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae)

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Dedication

This thesis is dedicated to God, my father, the late Mr. Charles Otieno (who never lived to read this work), my mother Nyar gem, for offering the best in life, my husband Jared and children Alpha, Prince and Tunu, for your unwavering support and sacrifice.

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Abstract

To date the main management strategy for the control of western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) employs frequent application of chemical pesticides. These are not only expensive but their heavy use has resulted in the development of pesticide resistant thrips populations. Furthermore, with increasing concern about the overuse of pesticides and consumers demand for residue free produces, farmers are seeking more environmentally benign methods for controlling arthropod pests and biological control has received more attention in the recent past. However, the overall efficacy of single antagonistic guild is often not sufficient for an economically relevant control especially in crops of low damage thresholds. A study indicated that, combination of azadirachtin substrate treatment with foliage and soil dwelling predators improved the reliability and efficacy of WFT control considerably. However, little is known about the effect of further combination of these products with entomopathogenic nematodes (EPN) and entomopathogenic fungi (EPF) in the same micro environment (soil). Moreover, the use of a predator, entomopathogens and a botanical in combinations requires a detailed knowledge about possible interactions to avoid inter and intra guild effects and to achieve synergistic effects. This study first optimized control of soil-dwelling thrips stages by evaluating the potential of combining the entomopathogens and neem (azadirachtin). Selected treatments were then combined with canopy dwelling *Orius laevigatus* (Fieber). Finally, monitoring of thrips is an essential prerequisite for targeted biocontrol, thrips monitoring was improved by constructing and testing the efficacy of an LED-blue sticky trap which was additionally baited with Lurem-TR lure.

Performance of soil application of azadirachtin products with entomopathogens was evaluated as single treatments, in multiple combinations and in different concentrations against the soil stages of WFT in French beans *Phaseolus vulgaris* L. Treatments consisted of NeemAzal-T solution, Neem pellets, *Steinernema carpocapsae* (Weiser) Nemastar®, isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin (IPP2539 & ICIPE-69) and *Beauveria bassiana* (Balsamo)-Naturalis®. Among the single treatments, NeemAzal-T solution proved to be the most efficient (60% reduction in emergence). However, this was surpassed when the late effect due to mycosis in EPF-based treatments was considered, resulting in over 87% reduction. Combined treatments with Steinernema, Metarhizium (ICIPE-69), NeemAzal-T and Neem pellets

resulted in total reduction in adult emergence of 95-97% when late mortality by mycosis was considered. Interactions resulted in two synergistic, four additive and one antagonistic response. Very low concentration of EPN (100 IJ/cm^2) with NeemAzal-T or alternatively using neem at reduced rates (0.25%, 0.5%) with EPN could be equally or even more effective than the operational doses. The highest concentration of *M. anisopliae* (10^8 conidia) combined with EPN resulted in the best performance in the bioassays with 74% reduction in adult emergence.

Selected treatments from the "soil package" developed above were combined with the foliage-dwelling predator *Orius laevigatus* (Re-natur) for a final holistic IPM scheme. The selected soil treatments were *Steinernema carpocapsae* (E-nema), *Metarhizium anisopliae* isolate ICIPE-69 and NeemAzal-T (Trifolio). Efficacy against WFT was significantly improved by combined treatments (83-97% reduction in WFT emergence) as compared to 45-74% in single treatments, and interactions resulted in two synergistic and eight additive responses. *Metarhizium* based treatments reduced WFT survival by 93-99% when late mortality by mycosis was considered. Halving the number of released predators in various combinations did not significantly reduce its efficacy. Releasing *Orius* to target L1 of WFT was significantly more successful (96-98% reduction in adult emergence) than targeting L2 of WFT (71-89% reduction in adult emergence). Releasing *Orius* to target L1 combined with soil application of neem and/or entomopathogens was considered the most successful and reliable biocontrol strategy for WFT.

Lastly, monitoring of WFT should be improved for the fine tuning of biological control measures. Efficient and reliable monitoring technique will provide an early warning system and more accurate account of population sizes. A prompt monitoring system will guide growers on the best time to intervene with a control measure, or allow a very sensitive evaluation of the efficacies of the existing crop protection measures. A new colour/chemical trap version was constructed by combining LED light sources with Lurem-TR lure. First the differential sensitivity of WFT for LED light was explored. The most efficient blue LED at an appropriate intensity was further combined with an attractive lure (Lurem-TR) in flight cages under greenhouse condition. In the release-recapture studies, the selected deep blue LEDs at 445 nm were clearly preferred over conventional blue sticky traps. Up to 2.7 and 2.1 fold more thrips were recaptured by

LED-blue sticky traps as compared to conventional blue sticky traps in choice and no choice experiments, respectively. Lurem-TR improved performance of blue sticky traps and LED-blue sticky traps by a 2.3 and 2.0-fold increase of trapping efficacy, respectively, as compared to those without Lurem-TR. Therefore, the use of LEDs and an attractant lure is useful in increasing the trapping efficiency of the blue sticky traps and therefore thrips monitoring.

Key words: *Frankliniella occidentalis,* Soil application, Biological control, Entomopathogenic fungi, Entomopathogenic nematodes, *Orius laevigatus*, synergism, additivity, Blue sticky traps, Light-emitting diodes, Lurem-TR

Zusammenfassung

Bis heute werden zur Kontrolle des Kalifornischen Blütenthripses (WFT) *Frankliniella occidentalis* (Pergande) chemische Pestizide eingesetzt, und für eine effiziente Kontrolle sind multiple Applikationen erforderlich. Dies ist nicht nur ein hoher Kostenaufwand für den Produzenten, sondern führt zur Selektion von resistenten Thrips Populationen. Die verstärkten Zweifel der Anbauer bezüglich einer häufigen Anwendung von Pestiziden und eine zunehmende Forderung der Konsumenten nach rückstandsfreien Produkten führen zu der Suche nach umweltfreundlicheren Bekämpfungsmaßnahmen gegenüber herbivoren Schädlingen und konsequenterweise hat der biologische Pflanzenschutz mehr Aufmerksamkeit bekommen. Hierbei ist die Effizienz von einzelnen natürlichen Gegenspieler-Gilden, insbesondere für eine ökonomische Kontrolle in Pflanzenbeständen mit niedrigen Schadschwellen, nicht ausreichend. Eine Studie hat gezeigt, dass eine Kombination von Substratbehandlungen mit dem natürlichen Wirkstoff Azadirachtin und natürlichen Gegenspielern im Pflanzenbestand zu einer deutlichen Steigerung der Effizienz und Zuverlässigkeit bei der Bekämpfung des Kalifornischen Blütenthrips geführt hat. Es ist bis heute aber nicht detailliert untersucht ob dieses Produkt und räuberische Makroorganismen auch mit anderen biologischen Agenzien wie entomopathogenen Nematoden (EPN) und entomopathogenen Pilzen (EPF), appliziert in das Substrat, kombiniert werden können. Für eine potentielle Nutzung von Prädatoren, EPN und EPF in Kombination mit Pflanzenextrakten ist detailliertes Wissen nötig, um mögliche Interaktionen aufzuklären, intra- und interspezifische Effekte zu vermeiden und synergistische Effekte zu erreichen. In dieser Studie soll zunächst die Bekämpfung der Bodenstadien von *Frankliniella occidentalis* durch eine Kombination von Entomopathogenen mit Neem (Azadirachtin) analysiert und verbessert werden. Ausgewählte Behandlungsvarianten sollen später mit dem im Pflanzenbestand aktiven Prädator *Orius laevigatus* (Fieber) kombiniert werden. Schließlich ist die Überwachung (Monitoring) von Thripsen eine essentielle Voraussetzung für einen gezielten biologischen Pflanzenschutz. Das Monitoring von *F. occidentalis* wurde in dieser Studie durch die Konstruktion und Testung einer LED-Blautafel mit zusätzlichem Lockstoff Lurem-TR verbessert.

Die Wirksamkeiten von boden-applizierten Azadirachtin Produkten, EPNs und EPFs wurden als einzelne Behandlungen, in verschiedenen Kombinationen und in

unterschiedlichen Konzentrationen gegen die Bodenstadien von *Frankliniella occidentalis* an Einzelpflanzen von Gartenbohnen *Phaseolus vulgaris* (L.) getestet. Zum Einsatz kamen NeemAzal-T Lösung, Neem-Azal Granulat, *Steinernema carpocapsae* (Weiser) Nemastar®, Isolate von *Metarhizium anisopliae* (Metschnikoff) Sorokin (IPP2539 & ICIPE-69) und *Beauveria bassiana* (Balsamo)-Naturalis®. Bei den einzelnen Behandlungen stellte sich die NeemAzal-T Lösung mit einer Thrips Reduktion von 60% als besonders effizient heraus, wurde aber letztendlich durch EPFs übertroffen (Mortalitäten > 87%), wenn auch die späten Effekte der Pilzinfektionen berücksichtigt wurden. Die Kombination von Steinernema, Metarhizium (ICIPE-69), NeemAzal-T und Neem-Granulat führte zu einer nahezu vollständigen Unterbindung des Schlüpfens von adulten Thripsen aus dem Boden (95-97%), erneut, wenn die späte Mortalität der Pilzinfektion mit einbezogen wurde. Die Interaktionen führten in zwei Kombinationen zu synergistischen, in vier zu additiven und in einer zu antagonistischen Antworten. Sehr niedrige Konzentrationen von EPNs (100 IJ/cm²) mit NeemAzal-T oder alternativ die Benutzung von reduzierten Neem Konzentrationen (0.25%, 0.5%) mit EPNs können demnach gleiche oder sogar höhere Effizienzen erzielen wie die üblichen Dosierungen bei Einzelbehandlungen. Die höchste benutzte Konzentration von *M. anisopliae* (10^8 Konidien) in Kombination mit EPNs führte zu einer guten Effizienz mit einer Schlupfreduktion der adulten Thripsen aus dem Boden von 74%. Ausgewählte Behandlungen aus dem „Bodenpaket“ wurden anschließend mit dem im Pflanzenbestand aktivem Prädator *Orius laevigatus* (Re-natur) kombiniert, um ein ganzheitliches IPM Szenario zu entwickeln. Die ausgewählten Bodenbehandlungen waren *Steinernema carpocapsae* (E-nema), *Metarhizium anisopliae* Isolat ICIPE-69 und NeemAzal-T (Trifolio). Die Effizienz gegenüber WFT war bei kombinierten Behandlungen (71-89% Reduktion im Schlüpfen von Adulten aus dem Boden) signifikant erhöht verglichen mit einfachen Behandlungen (45-74%). In zwei Kombinationen konnten erneut synergistische und in acht Fällen additive Antworten ermittelt werden. *Metarhizium*-Behandlungen führten zu einer 93-99 prozentigen Reduktion der Thripse, wenn auch die späte Mortalität durch die Pilzinfektion berücksichtigt wurde. Eine Halbierung der Anzahl an Prädatoren führte zu keiner signifikanten Verminderung der Effizienz. Eine Freilassung von *Orius* synchron mit den L1-Larven auf der Pflanze ergab Reduktionen von 96-98% der adulten F1 Generation und damit eine signifikant höhere Effizienz als spätere Freilassungen von Orius, wenn die Thripse bereits das L2-Satdium erreicht hatten (71-89% Reduktion

des Schlüpfens von Adulten). Eine Freilassung von Orius synchron mit WFT L1-Larven in Kombination mit der Bodenapplikation von Neem und/oder Entomopathogenen erwies sich als die erfolgreichste und zuverlässigste biologische Kontroll-Strategie für WFT.

Zum Ende sollte das Monitoring von WFT verbessert werden, um eine Feinabstimmung der biologischen Kontrollmöglichkeiten zu erlauben. Eine effiziente und zuverlässige Monitoring Technik stellt ein Frühwarnsystem dar und erlaubt eine sorgfältigere Ermittlung der Populationsgrößen von Thripsen im Pflanzenbestand. Ein zuverlässiges Monitoring System zeigt den Produzenten von Pflanzen den besten Zeitpunkt für weitere Kontrollmaßnahmen an und erlaubt eine sehr sensitive Evaluation der Effizienz von Kontrollmaßnahmen. Im letzten Teil dieser Studie wurde eine neue Farb-Duftstoff-Falle durch die Kombination von LEDs und Lurem-TR Duftstoff entwickelt. Zuerst wurde die differenzierte Sensitivität von WFT auf verschiedene LED-Lichtfarben untersucht. Die attraktivste blau-emittierende LED wurde mit geeigneter Intensität mit Lurem-TR in Flugkäfigen unter Gewächshausbedingungen getestet. In den Freilassungs-Wiederfang-Studien wurden tiefblaue LEDs mit 445 nm klar vor konventionellen Blautafeln präferiert. In Wahl- und nicht Wahlversuchen wurden auf LED-Blautafeln um den Faktor 2.7 und 2.1 signifikant mehr Thripse gefangen, als auf konventionellen Blautafeln. Lurem-TR verbesserte die Fangeigenschaften von blauen Klebtafeln und blauen LED-Klebtafeln um den Faktor 2.3 bzw. 2.0 verglichen mit Versuchen ohne Lurem-TR. Folglich erhöht die Kombination von blauen LEDs mit Klebtafeln und einem Lockstoff (Lurem-TR) die Fangeffizienz von *F. occidentalis* und ermöglicht ein sehr effizientes Monitoring.

Schlagworte:

Frankliniella occidentalis, Bodenapplikation, Biologischer Pflanzenschutz, entomopathogene Pilze, entomopathogene Nematoden, *Orius laevigatus*, Synergismus, Additivität, blaue Klebtafeln, Licht-emittierende Dioden, Lurem-TR

Abbreviations

%	Percent
°C	Degree Celsius
AZA	Azadirachtin
CS	Commercial substrate
EPF	Entomopathogenic fungi
EPN(s)	Entomopathogenic nematode (s)
Fig.	Figure
GLM	General linear model
h	Hour
HP	High power
ICIPE	International centre for insect physiology and ecology
IHPS	Institute of horticultural production systems
IJ(s)	Infective juvenile(s)
INSV	Impatiens necrotic spot virus
IPM	Integrated pest management
IPP	Institute of plant diseases and plant protection
L.	Linnaeus
L: D	Relationship of light and darkness
L1	First larval instar
L2	Second larval instar
LED	Light emitting diode
P	*P*-value (statistical significance level)
PDA	Potato dextrose agar
RH	Relative humidity
SD	Standard deviation
SDW	Sterilized distilled water
TSWV	Tomato spotted wilt virus
US$	US dollar
UV	Ultra violet
WFT	Western flower thrips

Note: For all measurements the SI units and their derivatives were used

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1.0 General introduction

1.1 Western flower thrips

1.1.1 Pest identification/morphology

Western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is 2 mm long, slender and narrow cigar-shaped insect with piercing and sucking mouth parts (Cloyd, 2009). Adults are macropterous (i.e. they have fully developed wings). They have two sets of narrow, clear, nearly vein-less wings. The wing-margins have long, fine microscopic fringes of setae (hair). The wings are held parallel along the back when at rest. The abdomen is pointed at the end (Fig.1.1). The adult male WFT is about 1 mm long; the female is slightly longer about 1.4 mm in length and more robust with rather rounded abdomen (OEPP/EPPO, 2002). Most WFT are females and they reproduce by arrhenotokous parthenogenesis. Male WFT are always pale yellow with a rather pointed abdomen, while females vary in colour from pale yellow, yellow with brown splotches, to dark brown or black, though some colour types are more abundant in certain seasons and geographical locations. For example, winter or mountain populations are darker in colour (Tommasini and Maini, 1995). It seems that this dark form is better adapted to survive low temperatures, but males are rarely dark. The eggs are about 0.2 mm long and are delicate, cylindrical/oval, slightly kidney-shaped, smooth, translucent white. The eggs cannot be seen since they are laid into the plant tissues. The first stage larvae are very tiny, spindle-shaped, almost worm-like insects that are translucent white or yellowish. Second stage larvae are also translucent white, but are similar to the adult in size and shape. They crawl and jump quickly on the surface of leaves. Both instars have red eyes. Pre-pupae are similar to second stage larvae except that the short wing buds and antennae protrude forward from the head. Western flower thrips pupate in flowers or in soil. Pupae do not feed and have long wing buds extending more than half-way along the abdomen and the antennae are folded back over the head. Both pupal stages are usually white to cream coloured.

Fig. 1.1 Western flower thrips adult (Photo by Jack Kelly Clark, courtesy of Diane Ullman)

1.1.2 Taxonomy

Thrips is the common name given to the insects of the order *Thysanoptera* (Tommasini, 2003). Some thrips species are predatory but the majority are phytophagous hence some species are always associated with special host plants and environmental conditions. The order is divided into two suborders (*Terebrantia* and *Tubulifera*) and eight families including approximately 5,500 described species (Morse and Hoddle, 2006). The WFT belongs to the family *Thripidae* in the suborder *Terebrantia* that is the largest of both suborders. The genus *Frankliniella* is the second largest in the family *Thripidae*, with about 160 species, most of them native to America (Kirk, 2002). The WFT was first described as a member of the genus *Euthrips* by Theodore Pergande in 1895 from specimens collected in California-USA where its widespread distribution and abundance among many flowering plants was noted (Pergande, 1895). This genus includes many relevant agricultural pests such as *F. bispinosa*, *F. fusca*, *F. schultzei*, *F. intonsa* and *F. tritici*. These species are often polyphagous and WFT is considered the most economically important among them (Kirk, 2002).

1.1.3 Origin and distribution

The Western flower thrips is a native insect of the western part of north America; where it was restricted for a long time (Kirk and Terry, 2003). However, in the last two decades, it has rapidly spread to become a pest of near worldwide occurrence (Kirk, 2002). It was first reported in California in 1895. For most of the early 20th century, WFT remained a localized problem in California and other areas of western USA and Canada, with sporadic problems reported from southern Texas to British Columbia

(Reitz and Funderburk, 2012). Western flower thrips has spread to other continents including Europe, Australia, and South America via transport of infested plant material. This has been greatly influenced by the increasing international movement of plant materials, such as cuttings, seedlings and potted plants (Kirk and Terry, 2003). Global trade in ornamental greenhouse plants at the beginning of 1980s, contributed to the rapid spread of the WFT around the world (Morse and Hoddle, 2006). Its initial spread in Europe was relatively slow because plant movement was more restricted in Europe before the single European market was set up in 1992 (Kirk and Terry, 2003). Currently, many countries have imposed quarantine measures in order to intercept invasion of WFT among other adventive pests. But due to its small size, cryptic habits, high intrinsic rate of increase by parthenogenesis and lack of obligate diapauses, WFT has continued distributing to many countries (Kirk and Terry, 2003). As a result, WFT is reported by Tommasini (2003) as one of the most economically important pests in USA, South America, Asia, Africa, Australia and Europe. In Europe, it is the most important plant damaging thrips especially in greenhouse plant production, although it can survive and overwinter outdoor only in warmer climates and not in the temperate regions of Western Europe (Kirk and Terry, 2003).

1.1.4 Biology and lifecycle

Western flower thrips adults are difficult to find because they are very small and feed in protected areas such as inside flowers, terminal leaf clusters and open buds. Thrips have six developmental stages: egg, two larval stages, pre-pupae, pupae, and adult. The female WFT has an external ovipositor with two opposable serrated saw-like blades that are used to cut through the plant epidermis and deposit eggs in the mesophyll layer of plant tissues. Eggs are deposited in leaves, buds, bracts and petals and hatch in 2 to 4 days. The eggs are fairly well protected and few pesticides are effective against them. Each female may lay 40 to over 100 eggs in the tissues of the plant, often in the flower, but also in the fruit or foliage. Eggs hatch into two feeding life stages first larval stage lasts 1-2 days and the second instar 2-4 days at 25°C. The two feeding larval instars are found in flowers, calyx of fruits or within developing terminal foliage, depending on the host plant. Towards the end of the second larval stage, WFT stops feeding and drops or enters the soil or leaf litter or another protected area on the plant and becomes a pre-pupa, which is a non-feeding stage that lasts 1-2 days followed by a pupal stage that lasts 1-3 days (Fig.1.2) (Tommasini and Maini, 1995;

Jensen, 2000; OEPP/EPPO, 2002). Although capable of movement, neither of the pupal stage moves about unless disturbed. Despite many alternative sites available for pupation, Berndt *et al*., (2004) reported that 98% of the larvae preferred to pupate in the soil environment. Because the pre-pupae and pupae are mostly in the soil, these stages are not affected by insecticides applied to the foliage. Winged adults then emerge from the pupal stage in 1-3 days, depending upon temperature. Adults resume feeding on flowers, buds, and terminal foliage. Developmental times depend on temperature and host plant on which the thrips are feeding. The entire life cycle from oviposition to adult emergence generally can take 12 days in hot weather to 44 days in cool weather. At 20-25°C, it usually takes about 2-3 weeks to develop from egg to adult (Jensen, 2000). Developmental rates, fecundity and longevity of WFT are affected by temperature, day length, plant species it is feeding on and presence of pollen as an important additional protein source.

1.1.5 Reproduction

WFT are facultative parthenogenetic. The female thrips are diploid and the males are haploid, which means that females develop from fertilized eggs, and males develop from unfertilized eggs (Moritz *et al*., 2004). Unmated females can produce only sons partheno–genetically whereas mated females can deliver sons and daughters depending on selected egg fertilization (Cloyd, 2009). The sex ratio, females: males depend on the age of the mother, population density and temperature. Males are more prevalent in low population densities whereas females are more abundant at high densities. Increased population of WFT in greenhouses enhances chances of females encountering and mating with males immediately after emergence from pupal stage. High population densities create an age structure consisting of young, fecund females producing pre-dominance of daughters. However, as females age they produce more males. The fecundity may be dependent on temperature but it's more affected by host plant and especially by availability of pollen. Pollen contains high amounts of proteins providing a high-quality food source affecting oviposition, development rate and larval growth (Cloyd, 2009).

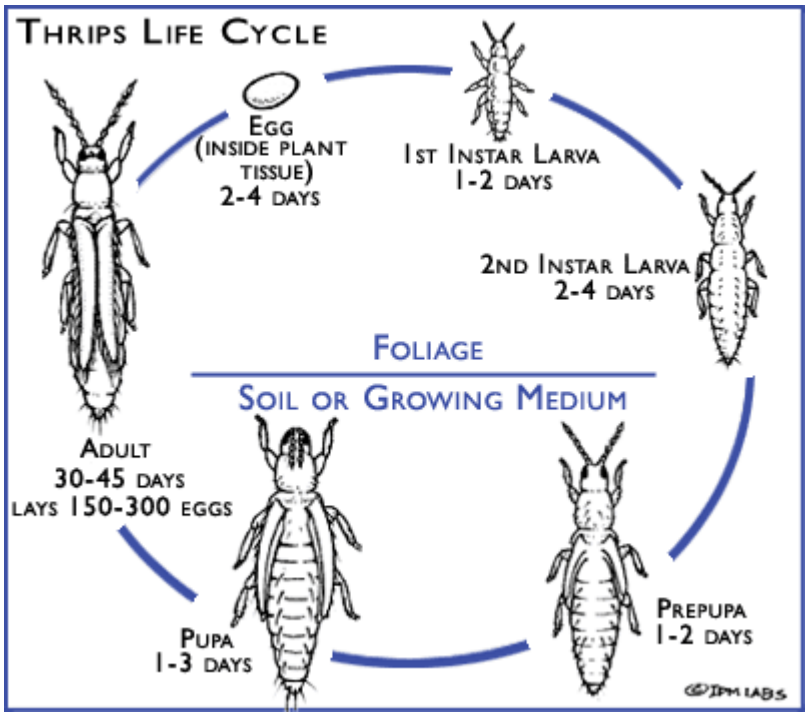

Fig. 1.2 Life cycle of Western flower thrips (Photo by Erin Ashley) https://de.pinterest.com/pin/ (accessed on 10/03/2016)

1.1.6 Pest damage

Over the last decades, WFT has become widespread in greenhouses, it is among the most important greenhouse pests in Europe causing severe damage to food and ornamental crops (Pizzol *et al*., 2010). It is highly polyphagous, with over 500 host plants, including a large number of fruit, vegetable, and ornamental crops. Thrips are gregarious with large numbers often concentrated on the same leaf or flower. Silvery leaf scars and specks of black faeces are a good way of diagnosing the presence of WFT on plants. Western flower thrips have piercing-sucking mouthparts, and they feed on the mesophyll and epidermal cells of leaf tissues using a single stylet (the modified left mandible) to lacerate and damage the parenchyma cells of leaf or flower and sucking out/imbibe the fluids from the cell (Weintraub, 2007). They inject toxic salivary secretions into the plant tissue as they feed. The damage to plant tissues resulting from this feeding is a superficial necrosis which produces grey or silvery chlorotic spots on infested plant parts (Fig.1.3). This reduces photosynthetic activities of the tissues or distorts growth when feeding occurs within meristematic tissues or simply reduces aesthetic value of the tissues affected. Moreover, wounds created during feeding or

oviposition may serve as entry sites for plant pathogens like fungi (Tommasini and Maini, 1995).

Fig. 1.3 Damage symptoms of *Frankliniella occidentalis*. Top Panels; left (Cucumber-leaves); right (Pea-pods), Bottom panels; left (Sweet pepper-fruit) right (Tomato- fruit) (Credit. Cristina Castane, COST Action FA1105, Israel, 2015)

1.1.7 Virus transmission

In addition to direct feeding injury through feeding and egg laying, WFT is also an important vector for plant diseases. Therefore, it is perceived as quarantine risks that negatively impact trades with host plants. Tospoviruses group belonging to the family *Bunyaviridae* causes devastating diseases in many plants. WFT has been described as one of the most efficient vectors of Tospoviruses such as *Tomato spotted wilt virus* (TSWV), or *Impatiens necrotic spot virus* (INSV) (Webster *et al*., 2011). Western flower thrips transmits both types of viruses in greenhouse and field crops. Almost all greenhouse crops are susceptible except poinsettias and roses. TSWV is considered as one of the most important plant viruses worldwide and has a known host range of 1009 species in 85 plant families (Jones, 2005). The TSWV is a problem mostly on vegetable plants such as tomatoes, peppers and peanuts while INSV is the predominant Tospovirus found in ornamentals especially Chrysanthemum and Gloxinia. In North America, INSV is closely associated with ornamentals and more predominant while in Europe, TSWV which is associated with vegetables is more predominant. Common symptoms include leaf spots, necrotic areas, blackening stem, distorted growth, mottling and ring spots on leaves. Young plants are usually more

vulnerable to infection. There is no cure for these viruses. As soon as INSV/TSWV is detected, removing the infected plant material must be combined with strict thrips management to prevent serious losses.

1.1.8 Economic importance

Actual economic losses attributed to any pest are difficult to determine and a few national estimates of damage have been produced. This is because growers are reluctant to publicize that they have a pest problem or that they have suffered a large economic loss. However, crop losses attributed to WFT were estimated to range between 50-90% (Kirk and Terry, 2003). Goldbach and Peters, (1994) estimated that Tomato spotted wilt virus alone caused over US$1 billion losses annually on a global basis. This estimate did not include direct damage caused by WFT, and presently the losses are high because WFT has continued to spread throughout the world (Kirk and Terry, 2003; Reitz *et al*., 2011). In USA, thrips losses averaged US$30.58 million per year, while loses attributed to Tomato spotted wilt virus was estimated at $1.4 billion over 10 years (Riley *et al*., 2011). In addition, losses in 2006, to ornamentals caused primarily by WFT damage exceeded US$ 15 million. In Netherlands, the annual cost was estimated at US$30 million and a further US$19 million from TSWV, this included crop loss and cost of treatment (Roosjen *et al*., 1998). These economic assessments show that WFT is one of the most destructive agricultural pests globally (Reitz and Funderburk, 2012).

1.2 Management strategies

Controlling WFT is difficult because of their small size, feeding habit, broad host range, high mobility, cryptic habitat (unopened flower buds), short generation time (egg to adult) combined with high fertility and soil dwelling life stages (Jensen, 2000; Thoeming *et al*., 2003). Thus the only way to deal with WFT in the green house production systems is by taking a “holistic” approach of implementing a variety of strategies including scouting, cultural and physical, chemical and biological management (Jensen, 2000).

1.2.1 Cultural control

This is the manipulation of crop production operations to favour the crops and put pests at a disadvantage. This approach utilizes multiple agronomic techniques such as trap

cropping, inter-cropping, crop rotation, sanitation, use of sterilized soils/media, tools and removal of alternative host plants including weeds. It reduces the potential for WFT problems by starting with a WFT-free crop. Remove any plant stock material that is infested, inspect all incoming plant materials and cuttings if possible and place incoming plant material in a holding area with sticky cards for approximately 5 days. Maintain a weed-free environment because weeds can provide refuge for WFT, both INSV and TSWV diseases. Remove all plant debris to prevent WFT from migrating back onto the main crop from infested plant parts after pruning. In addition, heating the greenhouse for 4-5 days at 30°C and then washing with disinfectants cleared WFT away (Lewis, 1997).

1.2.2 Physical control

This is the use of physical barriers to prevent contact between the pest and the crops. It uses plastic or glass barriers in greenhouses, screening greenhouse doors/vents with thrips proof nets to exclude thrips. Recently, ultraviolet (UV) absorbing plastic films are being used to control WFT since it affects flight orientation and host searching behaviour (Kigathi and Poehling, 2012). Others also use aluminized reflective fabrics which inhibit or repel WFT from entering greenhouses. Overhead irrigation or misting has been used in controlling not only WFT and aphids species but also two-spotted spider mite, broad mite, cyclamen mite (Cloyd, 2007). It creates a non-conducive environment for their development.

1.2.3 Chemical control

This is actually the method of choice for controlling thrips in greenhouses. Careful attention should be paid to pesticide choice, coverage, phytotoxicity and resistance. Small droplets sprays are important to ensure good coverage and better penetration into plant parts where thrips feed. Adults and larvae that emerge after insecticide application are less likely to come into contact with spray residues. Therefore, repeated spray application or use of pesticides with residual effect may increase chances of contact with the thrips. Because most WFT pupate in the soil/media an alternative to foliar spray is soil application of systemic pesticides. However, at one time only a small percentage of the WFT will be in the soil, unless frequently repeated this may not be effective. Also, no pesticides are currently labelled for use in the soil (Ansari *et al*., 2008a; Cloyd, 2009). Control of this pest today relies heavily on frequent applications

of broad-spectrum insecticides whose use is restricted due to environmental pollution and human health concerns (Gao *et al*., 2012a). A further limitation to effective suppression of WFT with chemical pesticides is the development of resistance strains. This has been reported for pyrethroids, organophosphates and carbamates (Gao *et al*., 2012a). Recently populations resistant to the widely used imidacloprid and fipronil (Herron and James, 2005) and spinosad (Bielza *et al*., 2007) has been detected. However, implementing resistance management strategies based on accurate pest monitoring to ensure the need for insecticide application, alternation of pesticide groups with different mode of actions and education to assure proper implementation of this program was more effective and delayed resistance to the pesticides (Herron and Cook, 2002; Gao *et al*., 2012a). The problem is expected to increase since many effective pesticides have been withdrawn from the market on safety grounds based on the EU (Directive 2009/128/EC) highlighting the need to develop alternative control methods for this pest species. Recently, the horticultural sector has come under great pressure to reduce pesticide residue levels in the production of these crops, thus resulting in a shift of emphasis towards biological control (Abdullah *et al*., 2015). Successful control with insecticide is only possible when used as part of an overall pest management program that also include cultural and biological control options.

1.2.4 Biological control

This involves the use of natural enemies (predators and parasitoids) or use of natural compounds from plants or micro-organisms (viruses, fungi or bacteria) with insecticidal activity. For WFT mainly mass reared natural enemies which are applied in large quantities to control an already rising pest population are used. Efforts are made thereafter to provide conducive environment to conserve them or enhance their performance. This can be done by providing refugia/flowering plants to maintain their population when the target pest is absent. In addition, the use of “soft” pesticides would ensure better establishment of the natural enemies. Interest in effective biological control strategies for thrips management has increased in response to decreasing effectiveness of insecticides, risks posed to workers and the environment and consumers’ demand for chemical residue free food and sustainable production systems. Natural enemies which have been used to suppress WFT Populations in greenhouse production include predators such as predatory bugs, mites, and nematodes. Moreover, entomopathogenic fungi are also applied as beneficial

micro-organism. Finally, treatments with the so called biopesticides, plant derived insecticidal compounds, are subsumed under the header "biopesticide". The main groups of natural enemies used in this study are predators, entomopathogenic nematodes, fungi and biopesticide which are discussed below.

1.2.4 1 Use of predators for controlling western flower thrips

Predators naturally present in the ecosystems are often insufficient to maintain the density of thrips below the economic injury level, hence mass release is needed to obtain sufficient low pest populations (Bueno *et al*., 2006). The efficacy of thrips predators can be limited by abiotic factors like temperature, humidity, day length (induces diapause), insufficient prey and alternative food source (van Houten, 2005).
The list of predators recommended to manage WFT populations include anthocorid bugs of the genus *Orius spp*. (Hemiptera: Anthocoridae) and phytoseiid mites such as *Amblyseius spp*. (Acarina: Phytoseiidae) that feed on the foliar stages of WFT, or soil-dwelling predatory mites like *Hypoaspis aculeifer* (Canestrini) and *Hypoaspis miles* (Berlese) (Berndt, 2003; Ebssa, 2006). *N. cucumeris* and *A. barkery* are the most available commercial agents for thrips biocontrol. *N. cucumeris* has been successfully used in greenhouse crops to control WFT (Zilahi-Balogh *et al*., 2007). However, it preys only on eggs and first larval stages of thrips and when prey density is low or in absence of the prey it can also feed on pollen. It has difficulties to capture and kill the older larval stages and adult thrips, which can easily escape since they can jump or fly (Ebssa, 2006). This gap can be closed by using a complementary method that can kill all stages of WFT.

Orius laevigatus

It is a minute, omnivorous pirate bug (Hemiptera: Anthocoridae). It is found throughout Mediterranean basin, from Atlantic region of western Europe to the eastern Mediterranean including Israel. Adults are 1.5–2.5 mm long with piercing–sucking mouthparts and two pairs of wings, the front pair being partially rigid. The adult is brown-black with grey spots. Both sexes are distinguished by the tip of the abdomen, which is pointed in the female due to the ovipositor. It kills its prey by piercing and sucking out the body fluids (Fig.1.4). All their life stages are predacious and can consume all thrips life stages (eggs, larvae and adults). It also feeds on other pest species such as aphids, mites, caterpillars and moth eggs as well as pollen and vascular sap. They are also able to build up their population before pests arrive using

alternative prey and host plants as alternative food source. It has gained worldwide importance in biocontrol because it is easier to rear and it develops quickly. They reproduce best at high humidity and during long days. It aggregates around and within flowers, concealed in the same habitat as thrips resulting in the good performance (Silveira *et al*., 2004). They are also able to spread further than most biocontrol agents, they can develop and reproduce on pollen. It's use to control thrips has been successful in several countries in Europe (Tommasini, 2003). It's capacity to control WFT in greenhouse crops has been demonstrated in sweet pepper cucumber, melon, strawberry (Arnó *et al*., 2008).

Two main factors that contribute to variation in its efficacy include availability of alternative food such as pollen and some species also enter diapause (a state of physiological arrestment with no feeding or egg-laying). Diapause is not a problem in greenhouse production systems due to supplementary lighting which extend the photoperiod (16:8 h light: dark) which is conducive for the predator. However, additional supply of pollen may have negative effect on biological control. For example, overall predation of thrips by *O. laevigatus* is greater in absence of pollen. The predator spends less time searching for prey when pollen is available or they become satiated more easily when offered multiple types of food (Shakya *et al*., 2009). Another drawback to the success of the predator is cannibalism especially in low prey density. This has limited possibility of early massive release of the predator (Tommasini *et al*., 2002). It also tends to leave the greenhouse when prey density is low (Berndt, 2003). In addition, its inability to forage in the soil thus is leaving the soil stages as reservoir for further infestation. Further research on testing *O. laevigatus* combination with other biocontrol agents is a viable course. This predatory antagonist when used together with entomopathogenic fungi or nematodes could help to obtain synergistic effects by combining thrips control in the soil systems with predation in the crop canopy, covering gaps of efficacy and reliability on single antagonist species (Silveira *et al*., 2004).

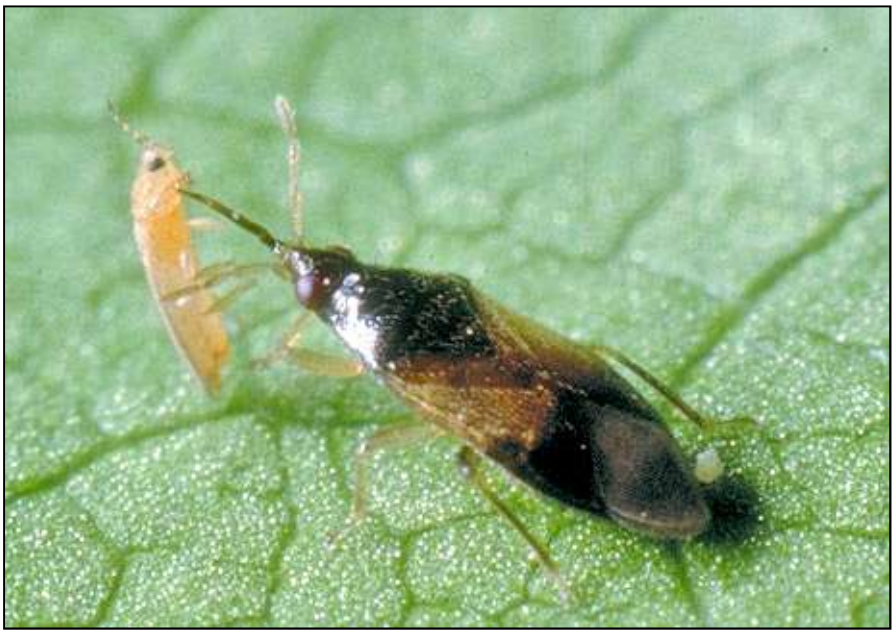

Fig. 1.4 Adult predatory bug *Orius laevigatus* preying on Western flower thrips http://www.hortidaily.com/IBMA-profiles-Orius-laevigatus/the-Thrypidae-hunter (accessed on 30/02/2016)

1.2.4.2 Entomopathogenic nematodes (EPN)

Over the last two to three decades, insect-infecting/parasitic (entomopathogenic) nematodes have become increasingly popular as biocontrol agents, especially against soil inhabiting pests (Berndt, 2003). They are tiny, less than 1 mm in length, very slender, non-segmented roundworms often tapering at the head and tail end. They are obligate or facultative parasites of insects.

Biology and ecology

They occur naturally in the soil environments and possess durable, motile infective stage that can actively seek out, locate and infect a broad range of insect hosts in response to carbon dioxide, vibration and other chemical cues (Kaya and Gaugler, 1993). They have limited dispersal capacity and thus must be drenched into the growing media at high rates. The EPNs belonging taxonomically to the order Rhabditida and families *Steinernematidae* and *Heterorhabditidae* are obligate parasites of large number of insect species. Nematodes of these families form a mutualistic symbiosis with entomopathogenic bacteria of the *Enterobacteriaceae* family. Steinernematids are associated with *Xenorhabdus spp*. and Heterorhabditids with *Photorhabdus spp*. (Forst and Clarke, 2002; Ferreira and Malan, 2014). The relationship between the nematode and bacteria is an obligate mutualism because the

bacteria need the nematode to carry it to the insect body cavity. The nematode also needs the bacteria to create conditions in the insect suitable for its reproduction and growth, and as food. The bacteria have never been found freely living in the soil. The bacteria produce pigments, so that insect cadavers infected with Heterorhabditis turn brick-red/maroon, and those infected by Steinernematids turn brown or tan.

The bacteria are carried in the gut of the third juvenile invasive stage of the nematodes (Ferreira and Malan, 2014). In the soil, the free–living infective juvenile (IJ) seeks out for the host insects. Once they come into contact with the host, they gain entrance to the insect haemocoel via natural openings (spiracles, mouth, anus or through the intersegmental membrane of the cuticle). Having reached the insect body, the nematode releases the bacteria into the haemocoel where they proliferate, break down the tissues into food and cause the septicemic death (haemolymph poisoning) of the host between 24 and 48 hours. After the death of the host, they continue to feed on the host tissue, mature and reproduce. Eventually large numbers of infective juveniles are released into the environment to infect other hosts (Kaya and Gaugler, 1993) (Fig. 1.5).

Searching behavior

The EPNs are classified based on their foraging behaviour; this is defined by their ability to move during foraging and response to host cues. They can get attached to either sedentary or mobile hosts. The EPN species are classified as ambushers, cruisers or intermediate (Lewis, 2002). The ambushers have a sit-and–wait strategy, standing on its tail in upright position near the soil surface or waiting to attach to a mobile host, for example *Steinernema carpocapsae*. It is most effective when applied to highly mobile surface-adapted insects. The cruisers have an active searching strategy by responding to chemical cues from hosts or plant exudes and attacking less mobile or even sedentary hosts. They can infect insects that live deep in the soil, for example the *Heterorhabditis spp*. and *S. glaseri* (Lewis, 2002). Other species like *S. feltiae* and *S. riobrave* use intermediate foraging strategy (combination of ambush and cruiser) to find their host.

Their performance in above ground targets is however constrained unless they are formulated for that use. This is due to their sensitivity to desiccation and UV radiation, the optimal temperature is rather narrow (20°C and 30°C), they are also affected by sub-optimal soil type (they work best in sandy soil with pH between 4 and 8) and irrigation frequency (Koppenhöfer and Grewal, 2005). They are actually semi-aquatic, living in the water film that surrounds the soil particle and therefore soil application is considered most cost-effective (Ebssa *et al*., 2001; Buitenhuis and Shipp, 2005). Despite the high virulence shown by some EPN strains, they are not able alone to reduce the populations of WFT to the required levels because of thrips rapid development and overlapping generations. For example, Arthurs and Heinz, (2006) used high concentration and frequent application *S*. *feltiae* and *Thripinema nicklewoodi* (Siddiqi) aimed at foliar and soil dwelling stages of WFT, but they could not get plants below acceptable damage. The dose rates currently needed for sufficient control are not economical (Premachandra *et al*., 2003a), thus the need to combine the EPN with other biocontrol agents that target the feeding life stages of thrips. The EPN applied to the soil can be combined without any detrimental interaction with other biocontrol agents, in particular foliage-dwelling predators of thrips. Premachandra, (2003a) reported that combined release of *Hypoaspis aculeifer*, a soil dwelling predatory mite and EPN to control soil dwelling instar of WFT, significantly lowered the number of thrips adults emerging from the soil. Other attributes to the EPNs that makes them compatible in IPM programs include, target (pest) specificity, non-toxicity to humans, beneficials or environment and they can be applied with the conventional pesticide equipment (Shapiro-Ilan *et al*., 2006).

Steinernema carpocapsae

It is the most studied of all the EPNs, it is widely available. It's attributes include ease of mass production and ability to be formulated into partially desiccated state that provides several months of room-temperature shelf-life (Shapiro-Ilan *et al*., 2010). It is the nematode of choice for controlling highly mobile insects like armyworms and cutworms. This is due to its sit-and-wait (ambush) forage strategy (see above). It is active at many temperature ranges but most effective against many insect pests at temperatures ranging from 22 to 28°C. Their infective juveniles (IJ) always carry symbiotic bacteria *Xenorhabdus nematophila* in their gut which is used to kill their host as explained above. It highly responsive to carbon dioxide cues once a host has been

contacted thus the spiracle are key portals of host entry. It's efficacy in controlling the soil-dwelling stages of WFT was reported by Premachandra *et al*., (2003b).

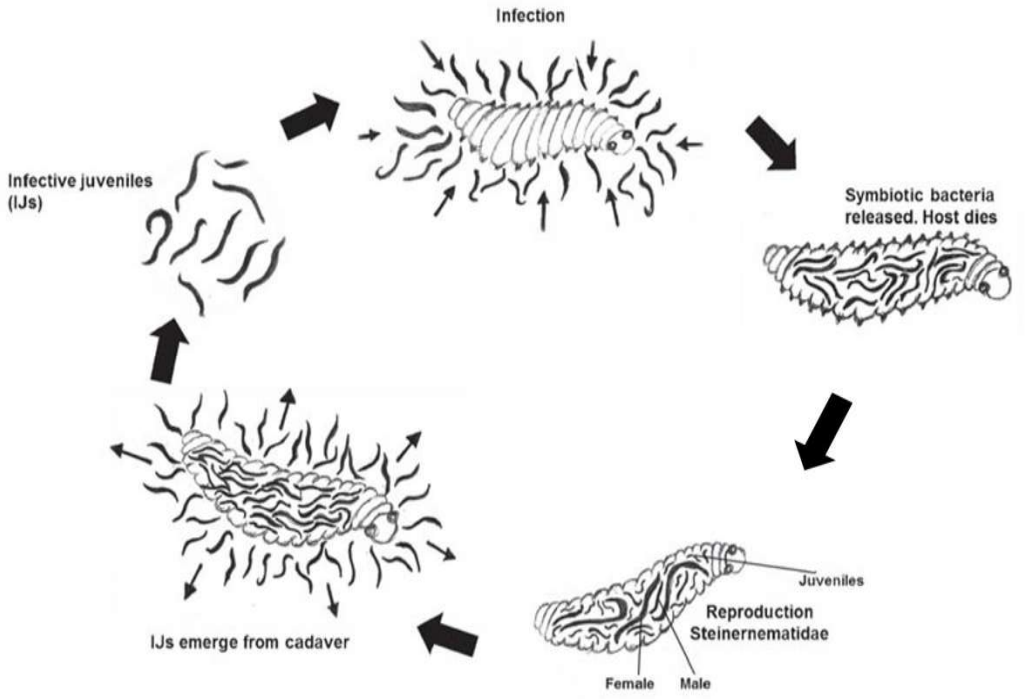

Fig. 1.5 Life cycle of entomopathogenic nematodes (Credit: Dr. Tshima Ramakuwela, 2015).

1.2.4.3 Entomopathogenic fungi (EPF)

Entomopathogenic fungi (EPF) are soil-borne organisms that control pests of plants and vertebrates. They do not need to be ingested to be infective. They actively infect insects via the cuticle, penetrating the insect integument, infecting it and eventually killing it (Shahid *et al*., 2012). The "active ingredients" in fungal preparations are spores, also called conidia. These must contact the host surface (cuticle) to be effective, either directly at the time of spray or later as the host moves over the treated foliage or soil. An intensive contact with spores is essential to achieve high efficacy. Once the spores are attached to the insect which is favoured by their sticky surface, they germinate and penetrate the insect's body wall. The fungus then multiplies within the body, causing the insect to stop feeding and die a few days later. Finally, mycelia grow out of the host body and produces spores for secondary spread of the pathogens (Fig.1.6).

More than 700 species of EPF are known to be pathogens of many insect species. However, they need very specific relative humidity (RH) and temperature conditions

for the germination and penetration phase and this may limit their use in many cropping systems if applied to the crop canopy (Ekesi *et al*., 2000). The soil where thrips mostly pupate offers a convenient microclimate for fungal infection. Several authors have reported high susceptibility of thrips pupae to soil treatment with *Metarhizium anisopliae* (Metchnikoff) (Brownbridge, 2006; Skinner *et al*., 2012). Fungal infection is also dependent on spore concentration, and they are sometimes slow, this speed of killing increases the need for frequent application and thorough coverage of plant parts. In addition, adult insects are more susceptible to fungal infection than nymphs. Apart from the nymphs' thicker cuticle that can delay fungal penetration into their body cavity, they also shed off their cuticle during ecdysis thus losing the spore infection.

Generally, the genera *Metarhizium*, *Beauveria*, *Paecilomyces* and *Verticilium* in the class *Deuteromycetes* have been greatly exploited as microbial control agents of WFT, showing considerable impact in the field and laboratory studies (Vestergaard *et al*., 1995). *Beauveria bassiana* when combined with *N. cucumeris* successfully controlled WFT in cucumber. However, it was shown to be more successful when the population density was high but did not completely eliminate the thrips when the population was low (Jacobson *et al*., 2001). On the other hand, the efficacy of the treatment of WFT with *M. anisopliae* was higher at low population densities (Azaizeh *et al*., 2002). *Verticilium lecanii* was successfully used to control WFT in Chrysanthemums, infecting even the pupae in the soil but the persistence was poor (Sermann and Welsch, 1998). The entomopathogenic fungi are host specific and this reduces hazards to non-target organisms making them ideal for IPM program with natural enemies. They are active over wide range of environmental conditions all year round and are unaffected by day lengths which inhibit performance of some natural enemies. Direct exposure to UV destroys the fungal spores. Extreme temperatures, low humidity can affect their efficacy. Combined use of the EPF with other biocontrol agents has been shown to enhance its efficacy. For example, synergistic/additive effects were obtained with combined use of *B. bassiana* and *S*. *carpocarpsae* (Williams *et al*., 2013) and *M*. *anisopliae* with four EPN species (Ansari *et al*., 2008b). Interactions with other control methods must be understood if they are to be integrated into an IPM program.

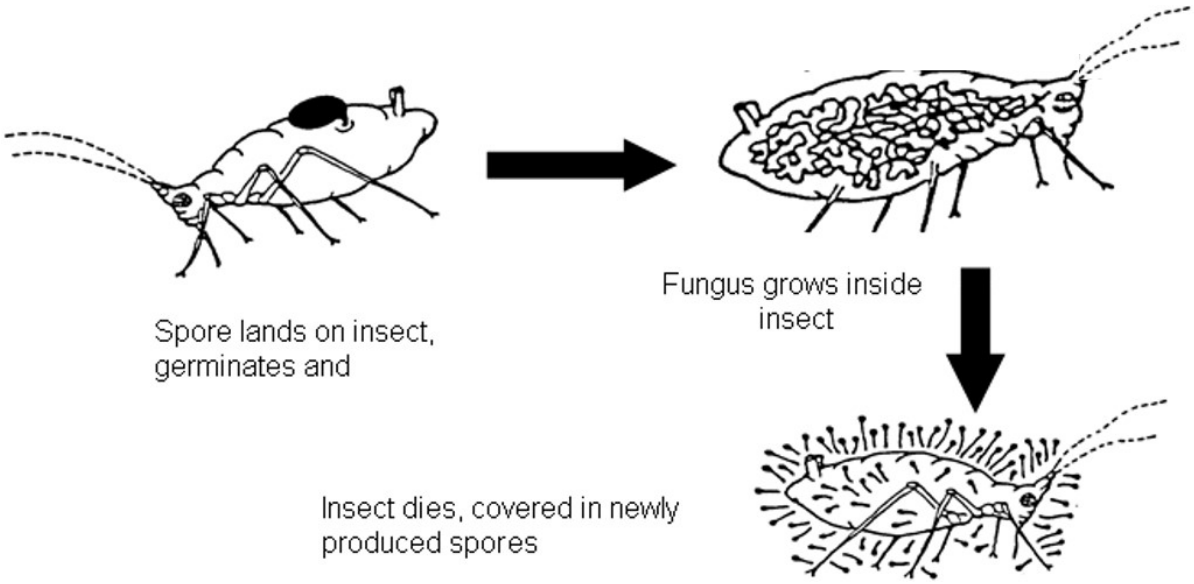

Fig. 1.6 Mode of action of Entomopathogenic fungi (Credit: Dudutech, Kenya)

***Metarhizium anisopliae* (Metchnikoff) Sorokin**

Metarhizium anisopliae (Hypocreales: Clavicipitacea) was the first fungus to be mass produced and used in pest control world-wide (Roberts and St. Leger, 2004). It is widely distributed and found in every habitat. The conidia are green, slightly oblong or bean shaped, they form large aggregates which stay in or on the soil rather than becoming airborne (Fig.1.7). It is characterized by broadly intertwined conidiophores. It is a pathogen mainly for soil insects, causing the so called green muscardine disease due to the green colour of the spores. The conidia penetrate the insect body, germinate inside the insect. Victims are killed after few days. The lethal effect is due to insecticidal cyclic peptides. The cuticle of the cadavers turns red and under high humidity a white mould grows on the cadavers that soon turn green. Brazil is the largest user, where it is used to control spittlebugs in sugarcane and grasslands. It is also used for the control of locusts and grass-hopper pests in Africa and Australia (Lomer *et al*., 2001; Li *et al*., 2010). Their efficacy against WFT has been reported by Azaizeh *et al*., (2002).

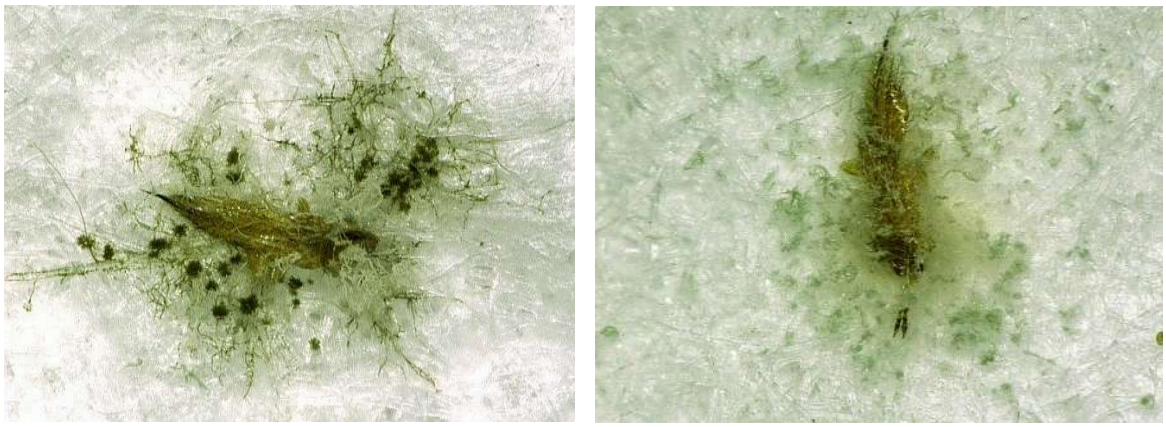

Fig. 1.7 Thrips cadavers covered by *M. anisopliae* conidia

***Beauveria bassiana* (Balsamo) Vuillemin**

Beauveria bassiana (Hypocreales: Clavicipitacea) was the first EPF to be discovered in 18th and 19th century (Rehner, 2005). It is the most broadly distributed species in this genus as it occurs in temperate and tropical soils. It is a cosmopolitan soil born pathogen which affects over 700 species of insects but survives as a saprophyte. It has also been reported as an endophyte. The most important compound produced by this fungus is beauvericin. It causes the white muscardine disease on arthropods. Conidia of *B. bassiana* are small (<3.5 µm diameter). They produce a white mold with distinctive white spore balls with rachis forming a zig-zag (Fig.1.8). Spores are dispersed in dry and single forms, they are therefore easily carried by wind for biocontrol. Two *B. bassiana*-based products, BotaniGard® and Naturalis®, are recommended for thrips management.

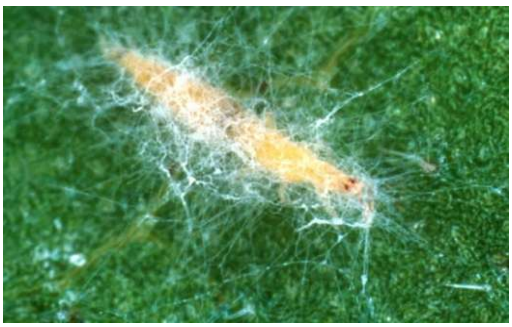

Fig. 1.8 The fungal pathogen *Beauveria bassiana* (Credit: Mark Hoddle University of California, Riverside (biocontrol.ucr.edu)

1.2.4.4 Biopesticide-Neem (Azadirachtin)

In addition to natural enemies, biopesticides have been tested to control thrips with low impact (risk) to growers, consumers and environment. Biopesticides are derived from natural sources like plants or organism in the soil like bacteria or fungi. A common biopesticide used for thrips control is Spinosad, which is derived from soil-dwelling bacteria *Saccharopolyspora spinosa.* It contains spinosyns as active ingredient with a unique mode of action. The second biopesticide is neem which is derived from *Azadirachtin indica* (A) Juss (Meliaceae), its active ingredient is azadirachtin (Fig.1.9). Recently, there is increased interest in the use of plant-based materials, such as neem-oil as a biorational approach (Rossel *et al*., 2008). These products are commercially available and are adopted by growers.

Neem (azadirachtin), extract from the neem tree is reported to effectively control over 600 species of insect pests (Schmutterer, 1997). The physiological effects of azadirachtin on growth, moulting and reproduction are consistent for many important pests of different crops. It shows growth regulatory and sterilant effects caused by alteration of ecdysteroid (ecdysone disruptor) and juvenile hormone titres. In addition, it is a chitin synthesis inhibitor. It also deters oviposition and feeding which depends on both neural input from chemical senses (taste receptors on tarsi, mouthparts and oral cavity) and central nervous integration of these sensory codes. Azadirachtin deters cells in chemoreceptors and also blocks the firing of ‘sugar’ receptors cells, which normally stimulate feeding. The result is starvation and death by feeding deterrence. It also has direct sub-lethal effects upon muscles and gut which leads to general loss of fitness. Neem products are fast and completely degradable when applied to the soil, low risk to human and non-target organism and so far, no selection of resistant target organisms has been observed (Schmutterer, 1997). Additional advantage of neem extracts is their systemic activity with a fast uptake by roots and bottom–up translocation to other plant parts (Thoeming *et al*., 2003). However, azadirachtin molecule is susceptible to photo-degradation by UV radiation, and therefore soil application is recommended because it minimizes exposure to sunlight. In addition, pests with cryptic feeding behaviour such as thrips are often poorly controlled by foliar application of neem extracts, even if treatments are frequently repeated, because of incomplete contamination and slow inter-laminar distribution when sprayed on the plant cuticle protected surface. Spraying in the crop canopy also might harm natural enemies foraging in this habitat thus limiting the potential combination of neem extracts

and beneficials in IPM. However, such constraints can be avoided by applying neem to the soil. Soil drenching can control many soil born pests like nematodes as well as the soil dwelling life stages of thrips (Thoeming *et al*., 2003. Bonsignore and Vacante, (2012) reported that azadirachtin had no effect on *O. laevigatus* making it a useful tool for integration with predators in an IPM approach. Furthermore, Mohan *et al*., (2007), Islam *et al*., (2010) asserted that joint efficacy between azadirachtin and entomopathogens could potentially benefit from synergistic interaction.

NeemAzal-T solution and NeemAzal granules (pellets)

NeemAzal-T solution is a water based formulation mostly suited for hydroponics and soil applications alongside insecticides and organic fertilizers and NeemAzal granules contains the active ingredient in an inert carrier material, it is a slow release formulation. Effectiveness of neem applied as drenching solutions against foliar and soil stages of WFT was reported by Thoeming *et al*., (2006). However, most commercial neem products for example, NeemAzal-T/S are oil-based formulations (oil 51% and tenside 45%). These additives can accumulate in the substrate thereby resulting in detrimental effects to the root systems and plant growth besides the soil fauna (Thoeming and Poehling, 2006). Therefore, a water-based neem product could be a better option for soil drenching. Although they have high risk to leaching before they are absorbed by the plant roots and this may affect its persistence calling for frequent application. Pellet formulation is considered least harmful to non-target organisms. It also ensures long-term and stable control by its slow and continuous release of the active ingredient in the soil substrate where WFT pupates.

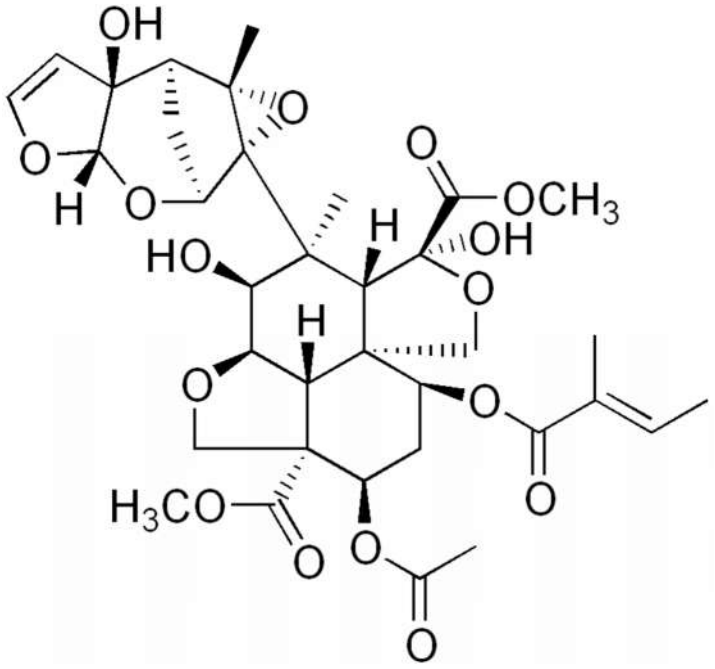

Fig. 1.9 Azadirachtin molecule (Mordue and Nisbet, 2000)

1.3 Thrips monitoring

Integrated pest management (IPM) approach combines various crop protection practices with careful monitoring of pests and their natural enemies. However, adoption of IPM is an increase of workload to growers. It requires a more detailed monitoring of pests, their natural enemies in addition to knowledge about the biology of the pest and their natural enemies as well as the use of selective pesticides. Therefore, growers need decision support tools to indicate when it is beneficial to apply pesticides and other control measures. This includes calculations of economic/action threshold based on stringent pest population monitoring scheme. The key to implementing a successful biological control program is often to release natural enemies early enough in the cropping cycle and spatially close to the hot spots. Releases must be initiated prior to WFT entering terminal or flower buds. Monitoring can also detect seasonal trends in WFT population throughout the year and assesses the effectiveness of management strategies implemented. Because WFT have high reproduction rate combined with short generation time (egg to adult), their population increases fast, often faster than for instance applied predators (see above), Most predators will not control an already established or “high” WFT population because most predators have a certain lag phase before achieving efficient numerical as well as functional responses to lower WFT numbers below damaging levels. Hence for this timing, early detection and control is very essential. Action thresholds may be affected by; plant attractiveness and

susceptibility to viruses, presence of flowers, placement of sticky cards, age structure of WFT population and their ability to migration into greenhouses and crop growth stage. Generally, action threshold varies from 10 to 40 WFT adults/ sticky card/ week depending on the above factors.

1.3.1 Optical trapping

Thrips use colour and scent for detection and orientation to their host plants (de Kogel and Koschier, 2002). They employ both volatile chemical signal and visual cues to locate host plants. Most herbivore insects with optical orientation possess photoreceptors in their compound eyes that are sensitive to limited intercepts (colours) of the wavelength spectrum of incident solar radiation, including UV, blue, and green. Among these, UV perception in the range of 350 nm-390 is most important for insect orientation and host location (Raviv and Antignus, 2004; Kumar and Poehling, 2006; Döring and Chittka, 2007; Nguyen *et al*., 2009). According to (Matteson *et al*., 1992) electrophysiological studies, WFT have a di-chromatic visual system with peak sensitivity around 540 nm (green-yellow) and 365 nm (UV range).

Initially, visual inspection by looking into open flowers and or shaking open flowers over a white sheet of paper may help to scout for nymphs and adults. However, the main techniques include the use of blue or yellow sticky traps above the crop canopy, although there is a disagreement on which colour is the most attractive to WFT (Cloyd, 2009). Sticky traps consist of plastic sheets coated with non-drying glue used to trap winged insects. Although blue sticky traps are more effective for catching thrips, many growers use yellow traps because they can be used to scout also for other important pests such as winged aphids, adult whiteflies, leaf miners, leafhoppers, fungus gnats, and shore flies. In addition, thrips are generally easier to see on yellow sticky cards than blue ones. Sticky traps are unlikely to give complete control since immature stages are not trapped (Chandler e*t al.,* 2011).

Recently, light-emitting diodes (LEDs) which can emit narrow-banded light and allow an easy combination of distinct colours were used to improve attractiveness of traps for thrips and whiteflies (Chen *et al*., 2004). First studies reveal that using these actively radiating light sources (LEDs) in combination with reflecting colour targets may improve trapping efficacy (Chen *et al*., 2004; Chu *et al*., 2006).

1.3.2 Kairomone trapping

Most of the new traps, often in addition to colours, lure the targeted insect with pheromones, which imitate the odour of the insect's mate, or use plant based attractive volatiles for the same purpose. Pheromones contain the odour of unmated females and attract only males, this may reduce female mating success thus reducing pest damage. Kairomone elicits the odour of host plant and traps both males and females. Both are used to help indicate that pest control measures are applied at the most effective time. Research on thrips attracting or repelling semiochemicals has recently become an attractive field of research and first compounds for thrips controlling "push and pull" strategies were described by van Tol *et al*., (2007). Odour that attracts thrips is used to pull them out of their refuge which can be either sex-pheromone, aggregation pheromone, host plant or food scent (Hamilton *et al*., 2005). A commercial aggregation pheromone, Thripline (Syngenta Bioline) was added to blue sticky traps and increased WFT catches by up to 3 times compared to traps without the pheromone (Davidson *et al*., 2007).

Synthetic kairomone (Lurem-TR): The active substance, methyl-isonicotinate is contained in a dispenser with perforated membrane for slow release. Once the aluminium foil at the back is opened, the active ingredient makes thrips more mobile. They appear from their shelter and are more attracted to the sticky traps (van Tol *et al*., 2012). This enhances the attraction of the blue sticky traps. Mechanical and biological control has been applied together using *Metarhizium anisopliae* in a trap device together with Lurem-TR to control WFT in French beans (Niassy *et al*., 2012a). It would be interesting to combine such lures with optical signals to improve thrips monitoring at low population densities for optimization of biological/biotechnical control measures.

1.4 Justification of the study

Over-reliance on chemical pesticides for the control of western flower thrips (WFT) *Frankliniella occidentalis* has resulted in development of resistant strains and health hazards due to toxic residues especially for the highly valued horticultural crops (Jensen, 2000). Although consumer awareness and legislation on pesticide residues in horticultural products are not very high in some parts of the world, pesticide management specialists are concerned about the risk posed by pesticide residue to the consumers. This concern is especially due to the suspected indiscriminate use of chemical pesticides in controlling this pest. This highly invasive pest has great propensity for developing resistance because of its biological attributes, this has made chemical control of this pest quite expensive (Jensen, 2000). Bielza, (2008) reported that, WFT has developed resistance against major groups of insecticides and hence insecticides are often not effective against this pest. Augmentative biological control has become a viable option and it has been adopted by a high proportion of growers throughout Europe, because of the advantages it offers over the use of pesticides (Sanchez *et al*., 2000; van Lenteren, 2000).

Studies have shown that a predator like *Orius spp*. can attack the foliar feeding stages of WFT, while entomopathogenic nematodes can prey on the soil dwelling thrips stages, and both groups can significantly reduce thrips populations (Berndt, 2003; Ebssa, 2006). However, the overall efficacy of single guild is often not sufficient for an economically relevant control. Predators in the crop canopy show preferences for different life stages of WFT. They often fail to follow the prey inside cryptic habitats such as buds, or they have problems to establish permanent populations in the crop when lacking additional nutritional components like pollen. Predators on the soil surface can attack the downward moving second larval stages but not the deeper burrowing pre-pupae and pupae stages. Nematodes on the other hand, are highly susceptible to environmental factors such as temperature and humidity limiting their efficacy if optimal conditions are not given (Ebssa, 2006). Moreover, entomopathogenic fungi (EPF) are generally viewed as less effective, less reliable, slow-acting, and more difficult to apply and they have poorer shelf-life than chemical compounds. Panyasiri *et al*., (2007) showed that high mortality of WFT could be obtained with among others *B. bassiana* and *M. anisopliae*. The most virulent isolate of *M. anisopliae* (275) caused at least 94% mortality 7-days post treatment. Therefore,

extensive studies and application schemes should be developed fitting to IPM programs.

The combined use of entomopathogens and predators however requires detailed knowledge of biotic interactions in order to avoid inter and intra guild effects. This can establish an ecologically sustainable crop protection system and if possible achieve synergistic effects. Studies on systemic effect of neem showed that soil application of neem formulation affected plant sucking life stages of WFT systemically, soil dwelling pupal stages directly, and migrating late second instars in a repellent way (Thoeming *et al*., 2003). Thoeming and Poehling, (2006) indicated that, combination of azadirachtin substrate treatment with foliage and soil dwelling predators improved the reliability and efficacy of WFT control up to 99%, with minor side effect of neem product on *Hypoaspis aculeifer*. However, little was known about the possibility of further combining these products with the EPN and EPF released in the same micro environment (soil). This study aimed at first optimizing thrips control via the soil systems, hence evaluated the potential of combining the entomopathogens such as selected strains of EPN, *Steinernema carpocapsae* (Weiser) Nemastar®, and EPF; *Beauveria bassiana* (Balsamo)-Naturalis® and isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin (IPP 2539 & ICIPE-69) with soil applied Neem (azadirachtin) treatments such as NeemAzal-T water-based solution and Neem pellets. Emphasis was laid on the interactive effects (synergistic, additive and antagonistic). Second the “soil package” was combined with the predator *Orius laevigatus* released in the crop canopy for a final holistic IPM scheme. Third an optical/chemical trap was designed for sensitive monitoring of the WFT under greenhouse conditions to trigger the biological control measures. Basically spectral responses of thrips were determined by screening with a set of small bandwidth LED monitoring screens, and the conventional blue sticky traps were equipped with selected light emitting diodes (LEDs) and evaluated for trapping efficacy in small and large cage experiments in the greenhouse. Moreover, the effect of the amendment with a suitable attractant lure (Lurem-TR) to the optimal visual trap set up was further evaluated with and without the presence of host plants.

1.5 Overall objectives

The main objective is to improve biocontrol of WFT by combining the use of predators and entomopathogens, with soil application of specifically designed neem formulations and evaluating their interaction effects as well as designing an optical/chemical trap design for monitoring the WFT.

1.5.1 Specific objectives

1) To evaluate the interactive effects (synergism, additive and antagonistic) of combined use of entomopathogenic nematodes (*Steinernema carpocapsae*) and entomopathogenic fungi EPF *Metarhizium anisopliae* (IPP 2539 & ICIPE-69) and *Beauveria bassiana* with neem formulations (NeemAzal-T solution, Neem pellets) in controlling the soil stages of WFT
2) To assess the effects of combined releases of predator *Orius laevigatus with* the “soil package” developed in (1)
3) To evaluate the use of Light Emitting Diodes (LED) traps impregnated with thrips attractive lures (LUREM-TR) as compared to the conventional blue sticky traps

1.6 Hypotheses

The following hypotheses were tested in this study:

1) Soil treatment using Neem formulations together with the EPN and EPF will significantly reduce the population of WFT
2) Interactive effects of entomopathogens and neem will be mostly additive or even synergistic
3) Combining control of thrips soil stages with release of natural enemy in the crop canopy will significantly improve efficacy of overall thrips control.
4) Specific LED traps with narrow bandwidth radiation optimized for thrips optical sensitivity combined with reflective colour traps and amendment with an attractive lure will improve sensitivity for thrips monitoring at low population densities.,

2.0 The combined effect of soil-applied azadirachtin with entomopathogens for integrated management of western flower thrips

Abstract

Performance of soil application of azadirachtin products with entomopathogens was evaluated as single treatments, in multiple combinations, and in different concentrations against the soil stages of western flower Thrips, *Frankliniella occidentalis* (Pergande) in French beans *Phaseolus vulgaris* L. Treatments consisted of NeemAzal-T solution, Neem pellets, *Steinernema carpocapsae* (Weiser) Nemastar®, isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin (IPP 2539 & ICIPE-69) and *Beauveria bassiana* (Balsamo)-Naturalis®. All treatments were analyzed for the number of emerging adults, while emerged adults in fungi-based treatments were analyzed additionally for the retarded development of mycosis as a possible cause of secondary mortality. Possible interactive effects in combined treatments were analyzed using a generalized linear model (GLM) approach, and three levels of dose-response combinations of the selected treatments were further tested. Bioassay results of the single treatments indicated between 43% and 60% reduction in adult emergence with NeemAzal-T solution thus proving to be the most efficient. However, most cadavers with entomopathogenic fungi (EPF) treatments showed the development of mycosis. Therefore, the reduction in adult emergence attributed to the EPF was altogether > 87%. Combined treatments with *Steinernema*, *Metarhizium* (ICIPE-69), NeemAzal-T and Neem pellets resulted in total reduction in adult emergence of 95-97% when late mortality by mycosis was considered. Out of the treatment combinations, two showed synergistic, four additive and one an antagonistic response. Combining low concentration of entomopathogenic nematodes (EPN) (100 IJ/cm^2) with NeemAzal-T resulted in satisfactory control compared to the operational dose of EPN, while the highest concentration of *M*. *anisopliae* (10^8 conidia) combined with *Steinernema* showed the best performance in bioassay with 74% reduction in adult emergence.

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*Tables and figures numbers are related to each chapter and reference list is combined for all chapters

2.1 Introduction

Western flower thrips (WFT) is a polyphagous pest, with over 250 different host plants from more than 60 plant families (Morse and Hoddle, 2006). Direct plant damage results in major yield losses (Shipp *et al*., 2000). Western flower thrips is also the key vector of several destructive plant viruses from the genus *Tospovirus* (Bunyaviridae) (Boonham *et al*., 2002; Pappu *et al*., 2009; Webster *et al*., 2011) such as *Tomato spotted wilt virus* (TSWV) (Reitz, 2009) and the *Impatiens necrotic spot virus* (INSV) (Riley and Pappu, 2004).

Because of the severe threat posed by WFT, there has been a heavy reliance on insecticides for its management. This intensive use of insecticides led to the development of resistance of WFT against various insecticides (pyrethroids, organophosphates and carbamates) (Gao *et al*., 2012a). Western flower thrips have even become resistant to new pesticides such as Spinosad, one of the most effective and integrated pest management (IPM) compatible insecticides (Shan *et al*., 2012). Resistance in WFT has been reported in Australia (Herron and James, 2005) and south-eastern Spain (Bielza *et al*., 2007). Most troubling to resistance management are recent findings that resistance to insecticides like Acrinathrin and Spinosad does not occur with a fitness cost (Bielza *et al*., 2008).

Due to increasing concern about the overuse of pesticides, farmers are seeking more environmentally benign methods for controlling arthropod pests, and biological control has received more attention in the recent past. However, there is no satisfactory and reliable single biological control technique that can effectively control WFT, particularly on high-value crops, because of their low damage threshold levels (DeCourcy Williams, 2001; Herrin and Warnock, 2002). Therefore, using a suite of natural enemies or entomopathogens (Arthurs and Heinz, 2006; Brownbridge *et al*., 2013), which could have additive or synergistic effects, improving the efficacy and reliability of single antagonistic species as well as including low-risk pesticides with selective application seems to offer a sustainable control strategy.

The WFT life cycle includes the foliar-feeding (adult, first and second instars) and soil-dwelling developmental stages (late second instars, pre-pupae and pupae). Most larvae leave plants as late second instars going to the soil to enter into non-feeding

pre-pupa and pupal stages, with up to 98% of thrips preferring to pupate in the soil environment (Berndt *et al*., 2004). An ideal biological control strategy would therefore target both the foliar-feeding and soil-dwelling development stages of the pest (Ansari *et al*., 2008a). Attention has largely focused on the control of adults and larvae in the crop canopy, while few attempts have been made to control soil-dwelling stages of the pest (Berndt, 2003; Belay *et al*., 2005; Ansari *et al*., 2008a), which constitute significant reservoirs for re-infestation. However, given the amount of time spent in the soil, these stages make ideal targets for soil-dwelling antagonists or soil-applicable entomopathogens, preferably when they are used as part of an IPM strategy. The pupal stage in the soil is protected from insecticides sprayed on leaves and stems of the plant, and there are currently no pesticides labelled as drenches to kill pupae in the soil (Ansari *et al*., 2008a; Cloyd, 2009). Contrastingly, these immobile soil-dwelling phases are vulnerable to soil-dwelling predators and pathogens (Ansari *et al*., 2008a; Steiner *et al*., 2011; Holmes *et al*., 2012).

A biorational pesticide potentially suited for an integrated approach to control WFT soil stages is the biopesticide neem. Soil-applied neem has been shown to be effective against *Meloidogyne incognita* (Lee *et al*., 2010), *Liriomyza sativa*e (Hossain *et al*., 2007), *Ceratothripoides claratris* (Thoeming and Poehling, 2006) and WFT (Thoeming *et al*., 2006; Cloyd, 2009). The active ingredient of neem products, azadirachtin (AZA), can affect the behaviour and physiology of different target insects (Mitchel *et al*., 2004; Islam *et al*., 2010). It is registered as an IPM-conforming pesticide, with different commercial products available. It has a low risk of pest resistance due to its complex mode of action and can show low detrimental side effects to a broad range of non-target organisms if properly applied. It is biodegradable in nature (Schmutterer, 1997). However, pests with cryptic feeding behaviour such as thrips are often poorly controlled by foliar application of neem extracts, because of incomplete contamination. At the same time, intensive foliar spraying can increase the risk of toxicity for natural enemies foraging in the crop canopy, thus limiting the potential combination of neem extracts and beneficials in IPM. Soil application can avoid this risk because the systemic properties still affect plant-sucking life stages of WFT as well as soil-dwelling pupal stages directly (Thoeming *et al*., 2003).

Over the last two to three decades, entomopathogenic nematodes (EPN) have become increasingly popular as biocontrol agents, especially against soil-inhabiting pests (Berndt, 2003). They have a wide host range and are able to efficiently locate their victims either actively seeking them out or by ambushing and rapidly killing the host. They are safe to vertebrates and bear a low risk of affecting non-target organisms (Shapiro-Ilan *et al*., 2012). In particular, many products containing *Steinernema carpocapsae* are available in the market, and their basic potential to control WFT soil stages has already been shown (Premachandra *et al*., 2003b; Berndt, 2003).

Entomopathogenic fungi (EPF) are most often studied and used to control foliar pests by applying conidia solutions to the crop canopy. However, they offer an interesting additional option for controlling soil-dwelling pest stages, as the soil is a convenient microclimatic environment for fungal infection. Studies by Ansari *et al*., (2007; 2008a) have already shown the high susceptibility of thrips pupae to infection by *Metarhizium anisopliae* in various media. Therefore, advances have been made to improve their quality and performance to make them more cost-competitive with chemical pesticides (Lacey *et al*., 2001).

An option for improving WFT control efficacy on a biorational basis is the combined application of biological agents. Several studies have demonstrated the high efficacy of a combined use of various entomopathogens with neem products. For example, NeemAzal-formulations have been combined with *Amblyseius cucumeris* and *Hypoaspis aculeifer* (Thoeming, 2005; Thoeming and Poehling, 2006), *Eretmocerus warrae* (Kumar *et al*., 2010), and EPNs (Krishnayya and Grewal, 2002; Meyer *et al*., 2012). However, the use of different predators and pathogens in combinations requires detailed knowledge about possible interactions to avoid inter- and intra-guild effects while achieving synergism. Different authors have reported various interactive effects. For example, synergistic/additive effects were obtained with combined use of *Steinernema feltiae* and a neem seed kernel extract, NeemAzal-T (Neemix) (Mahmoud, 2007); *M. anisopliae* and *S. kraussei* (Georgis, 1997); *Beauveria bassiana* and *S. carpocapsae* (Williams *et al*., 2013) and *M. anisopliae* with four EPN species (Ansari *et al*., 2008b). No study has so far assessed the efficacy of combining EPF and EPN by soil application with specifically designed neem formulations in controlling the soil stages of WFT.

2.2 Materials and methods

2.2.1 Bean plants and western flower thrips

Dwarf French beans (*Phaseolus vulgaris* L.) var. 'Speedy' (Fabaceae) were pre-germinated for three days before transplanting into plastic seedling trays (50 × 30 × 6.5 cm) at a seeding rate of 50 seeds per tray. A commercial substrate (CS) Frühstorfer Erde, type P (Archut GmbH, Lauterbach-Wallenrod, Germany), was used. The seedlings were left to grow for 6 days until the primary leaf stage under glasshouse conditions of 22 ± 2°C temperature, and 65-75% relative humidity (RH) with a 16:8 h light: dark photoperiod. The seedlings were then individually transplanted into 16-cm-diameter plastic pots filled with 600 g of CS and left to grow for another 6 days in the same glasshouse conditions.

To obtain uniformly aged thrips for the experiments, synchronized rearing of WFT was performed on pods of organically grown French beans in 0.75-L glass jars (Leifheit, Nassau, Germany) in a climate chamber 23 ± 2°C, 50-60% (RH) and 16:8 h L: D photoperiod. The rearing procedure was based on the original protocol by Berndt *et al*., (2004).

2.2.2 Biopesticide-Neem

Two formulations were used: NeemAzal-T solution (1% AZA), mostly suited for hydroponics and soil applications, and NeemAzal granules (7% AZA) containing the active ingredient in an inert carrier material (slow release formulation). Both products were obtained from Trifolio-M GmbH (Lanhau, Germany) and dosed to achieve a basic concentration of 10 mg AZA/kg soil.

2.2.3 Entomopathogenic fungi (EPF)

We tested three isolates of EPF: *M*. *anisopliae* 2539 IPP (access DSM-Nr 100117), ICIPE-69 (commercialized strain by International Centre of Insect Physiology and Ecology (ICIPE), for identification characteristics see Niassy *et al*., 2012b) and *B. bassiana* (Naturalis® commercial product; BioGard, Grassobbio, Italy). The latter is an emulsifiable suspension (ES) containing 2.3 x 10^7 conidia/ml. The liquid culture of *M*. *anisopliae* 2539 IPP was stored in sealed vials at -20°C, while the ICIPE-69 culture obtained as dry conidia from ICIPE was stored at 4°C.

For the experiments, both *M. anisopliae* strains were reactivated on adult WFT to recover the pathogenic characteristics for WFT (Goettel and Inglis, 1997). Conidia from infected cadavers were isolated onto potato dextrose agar medium (PDA), supplemented with peptone and yeast extract. This medium ensured fast growth and sporulation of the fungi. The petri-dishes were then stored in an incubator at 25°C. Fungal cultures not older than two weeks and grown on artificial medium less than three times after isolation from WFT were used for the experiments.

The aqueous suspension of *M. anisopliae* conidia was prepared by scraping off dried conidia from the PDA plates into 15-ml test tubes using a sterile spatula. To determine the number of conidia/g of dry powder, a 0.1-g sample was aseptically mixed with 100-ml sterile 0.05% Tween-80 (AppliChem GmbH, Darmstadt, Germany). The solution was vortexed for 5 minutes to break chains or aggregates of conidia to achieve a homogenous suspension. The conidia suspension was adjusted to a final concentration of 10^7 conidia using a haemocytometer (Fuchs Rosenthal Counting Chamber by Marienfeld, Lauda-Königshofen, Germany) (Goettel and Inglis, 1997). To determine the germination rate of conidia, five aliquots of a conidia solution diluted to 10^6 conidia/ml were added to a Petri dish filled with agar, which was incubated at 25°C for 24 h in darkness. Lacto-phenol cotton blue was used for stopping conidial growth and staining conidia. 100 conidia were evaluated under a compound microscope (x400 magnification) and considered germinated if the germ tube was twice the length of the conidia. Assessing conidia viability before the experiments was useful for adjusting the desired concentration of viable conidia/ml for every bioassay.

2.2.4 Entomopathogenic nematodes (EPN)

The *S. carpocapsae* Nemastar® was obtained as a clay formulation from E-nema GmbH (Raisdorf, Germany) and stored for less than two weeks at 4°C until used. Before the experiments, the cold stored nematodes were allowed to acclimatize at ambient room temperature for at least 4 h before exposure to WFT. A gram of the product was dissolved in 50 ml de-ionized water and 1 ml of the suspension was mounted on a microscope slide for counting the nematodes. A final concentration of 80,435 infective juveniles (IJ) in 25 ml of distilled water was prepared by quantification and dilution (Kaya and Stock, 1997; Premachandra *et al*., 2003b).

2.2.5 Experimental set-up (Microcosm)

The potted bean plants were separately enclosed in acrylic glass tubes (diameter 15 cm, length 30 cm) (AK Kunststoff Technik GmbH, Isernhagen, Germany) serving as microcosm. For ventilation, the open end at the top and four additional holes (diameter 3 cm) at the side of the cylinder were closed with thrips-proof nylon gauze (pore size 64 µm; Heidland, Gütersloh, Germany). The microcosms were kept in a climate chamber at 23 ± 2°C, RH 50-60% and 16:8 -h L: D photoperiod.

A cohort of one-week-old adult WFT from synchronized rearing was anesthetized with a low dosage of carbon dioxide for easy handling. Ten females and two males were transferred into a clean Eppendorf tube (2 ml) using a fine hairbrush and sealed. The Eppendorf tube was then hung on the petiole of the bean plant and opened to allow the adults to crawl/fly out of the tube and infest the plant. The edge between cylinder and pot was sealed with parafilm to prevent thrips from escaping. Thrips were allowed to feed and to lay eggs on the plants for three days. Thereafter, the adults were removed from the cylinders by shaking the plant on a white sheet of paper and using an aspirator. Eight days after introduction of the adults when the larval stage (L2) should have been descending to the soil for pupation (Berndt *et al*., 2004), the substrate was treated.

2.2.6 Treatments

2.2.6.1 Single-compound treatments

All dosages used in the treatments are listed in Table 2.1. For the application of NeemAzal-T, 0.6 ml of the basic solution containing 1% AZA was diluted in 100 ml distilled water and drenched, while neem pellets (90 mg) were mixed with the soil and then gently watered. For the EPN, 80,435 IJs suspended in 25 ml of distilled water were drenched evenly on top of the soil by using a Pasteur pipette (Premachandra *et al*., 2003b) giving an amount of 400 IJ/cm^2. Afterwards, the soil surface was further irrigated with 25 ml of distilled water 5 minutes after the EPN application to percolate the EPN into the soil layers where the WFT prefer to pupate. To avoid water logging and enhance percolation, plants were not irrigated 2 days before the EPN application. For both Metarhizium isolates and for *B*. *Bassiana* conidia, solutions containing 10^7 conidia suspended in 100 ml of Tween 0.05% were applied to each pot to ensure a total wetting to field capacity of the upper soil layer which should be passed by the

descending larvae. During application to the soil, the plant was protected by tissue paper to avoid any contamination. A blank treatment with sterile distilled water only was set up as a control as pre-tests with a set of blanks (0, 05% Tween–EPF; Blank NeemAzal T; sterile distilled water - EPN) showed no significant differences between the blanks of the compounds used and the water control.

Twelve days after the introduction of adults, the cylinders were removed and the bean plants cut off. Adult emergence rate from the soil was measured as the main parameter for efficacy of the treatments. Based on the photosensitivity of the emerging adults, an emergence apparatus called Photoeclector was used. This consisted of an inverted pot of the same dimensions as those used in the experiment. A hole was drilled in its base and a 10-ml Pipette tip inserted tightly fitting. A 2-ml Eppendorf tube was placed on top of the pipette tip to capture the emerging adults. For ventilation, four holes, each 2 cm in diameter were drilled on the sides of the pot and covered with thrips-proof nylon screen (64 µm pore size). The two pots were sealed with parafilm to prevent the emerging thrips from escaping.

Daily counts of adults emerging from each pot were recorded for 7 days. Adults from treatments with EPF (most of which died 2 days later) were incubated at 25˚C in 55-mm-diameter Petri dishes filled with 5-mm plaster of Paris-charcoal (9:1) layer and a Whatman filter paper of the same diameter; 1.5 ml of sterile water was added to maintain humidity. The cadavers of these specimens were later examined under a binocular microscope for spores and hyphae on the surface of the insects to confirm mycosis as the cause of death. A completely randomized design with fifteen replicates per treatment was used.

2.2.6.2 Combined treatments

For combined treatments, we selected the EPF with highest performance *M. anisopliae*-ICIPE-69, *S. carpocapsae* (EPN) and both neem formulations, NeemAzal-T solution and Neem Pellets. Double combinations consisted of treatments with EPF/Neem pellets, EPF/NeemAzal-T, EPN/EPF, EPN/Neem pellets and EPN/NeemAzal-T, while triple combinations were composed of EPN/Neem pellets/EPF and EPN/NeemAzal-T/EPF. The experimental design and procedure were

the same as for the single treatments. The mixing ratios of the combinations are described in Table 2.1.

Moreover, we tested dose-response reactions with three concentration levels for *M. anisopliae* (10^6, 10^7 and 10^8 conidia/100ml), *S. carpocapsae* (100, 400 and 800 IJ/cm^2) and NeemAzal-T (basic solution 0.25%, 0.5% and 1% AZA) applied with the same amounts of drenching water as described with the single treatments above.

2.2.7 Statistical analysis

The total numbers of emerging thrips per pot were analyzed using Poisson generalized linear models (GLMs) with logarithmic link function and over-dispersion ('quasi-Poisson') and as explanatory variable the treatment (McCullagh and Nelder, 1989). Means of emergences for single and combined treatments were separated by pairwise comparisons of Poisson means (Tukey's test) at a multiple type I error level of 5%. This test was also used to separate means in the dose-response experiment.
As a consequence of the experimental set-up, it was not possible to determine the numbers of thrips that were actually exposed to the treatments in the soil. Hence to assess interactions of treatments when applied in combinations, again a quasi-Poisson GLM was fit to the counts of emerging adults. The GLM included as explanatory variables the treatment and the date (to acknowledge that the single and combination experiments were run consecutively).

Formal inference about treatment interactions was achieved by testing linear combinations of GLM parameter estimates, using their associated standard errors from the model. The linear combinations were chosen such that they reflected the mixing ratios (Table 2.1), thereby adopting a straightforward notion of additivity: the expected number of emergences (on the log scale) under additivity was calculated as the sum of fractions of log emergences in the involved single-treatment groups, where the fractions corresponded to the mixing ratios. Subtracting the observed from the expected log emergences and then exponentiation of the difference yielded the combination's estimated percentage deviation from additivity.
Whenever a test for a combination produced a p-value below 0.05, the null hypothesis of additivity was rejected and the interaction was deemed synergistic or antagonistic, depending on the direction of deviation from additivity. For p-values greater than 0.05,

the null hypothesis of additivity was retained. All computations were performed in R version 3.1.1 (R Core Team, 2015).

Table 2.1: Applied doses of *M. anisopliae*-ICIPE, NeemAzal-T, neem pellets, and *S. carpocapsae* for the single treatments and combinations, given as amount of active compounds/pot (600g of soil) and percentages (relative to the single treatment doses).

A	Treatment(s)	*M. anisopliae*-ICIPE (EPF)	NeemAzal-T	Neem pellets	*S. carpocapsae* (EPN)
	Single treatments				
	M. anisopliae-ICIPE (EPF)	10^7 conidia	-	-	-
	NeemAzal-T	-	6 mg Azadirachtin A	-	-
	Neem pellets	-	-	6 mg Azadirachtin A	-
	S. carpocapsae (EPN)	-	-	-	80435 IJ (400IJ/ cm^2)
	Double combinations	% of single treatment used-See A			
B	EPF + NeemAzal-T	(40%)	(60%)	-	-
	EPF + Neem pellets	(50%)	-	(50%)	-
	EPF + EPN	(67%)	-	-	(33%)
	NeemAzal-T + EPN	-	(75%)	-	(25%)
	Neem pellets + EPN	-	-	(67%)	(33%)
	Triple combination	% of single treatment used-See A			
C	EPF + NeemAzal-T + EPN	(33%)	(50%)	-	(17%)
	EPF + Neem pellets + EPN	(40%)	-	(40%)	(20%)

2.3 Results

2.3.1 Single treatments

All treatments resulted in considerably fewer thrips emergences compared to the control. However, no significant difference in % reduction in adult emergence among the single treatments was recorded ($p \geq 0.084$). The bioassay results indicated between 43% and 60% reduction in adult emergence, with NeemAzal-T solution proving to be the most efficient (Fig. 2.1, left). The daily trend indicated that the treatments particularly reduced the peak of the emergence course, between days 3 and 4; afterwards, the numbers of emerged adults were very similar in the control and treatments (Fig. 2.1, right). The daily trend also showed that NeemAzal-T outperformed other treatments having the least number of daily survivals of WFT adults. However, when the mean percentages of individuals being killed after emergence by retarded mycosis were considered, the total survivals from the EPF treatments reduced considerably to 12% (*M. anisopliae*-IPP), 11 % (*B. bassiana)* and 6% (*M. anisopliae*-ICIPE-69) (Fig. 2.3, left).

2.3.2 Treatment combinations

Based on the results above, *S. carpocapsae*, *M. anisopliae* ICIPE-69, NeemAzal-T and Neem pellets were further used in various combinations (Table 2.1). In most cases, the combinations of NeemAzal-T and entomopathogens, or both entomopathogens, resulted in fewer emergences compared to single treatments, except in combinations with neem pellets (Fig. 2.2, left). The course of emergence also showed that combinations with NeemAzal-T solution steadily reduced the WFT population from the second day onwards in contrast to combinations with Neem pellets that took longer to achieve similar reductions in adult emergence (Fig. 2.2, right).

The combined application of EPN + EPF + NeemAzal-T (65% mean emergence) (Fig. 2.2, left) was more effective as compared to treatment with EPN + EPF + Neem pellet ($p=0.007$ from Tukey's test) but not significantly different from combined application of EPN + NeemAzal-T ($p=0.998$), EPN + EPF ($p=0.996$) and NeemAzal-T + EPF ($p=0.463$). The combined application of neem pellets + EPF or EPN were significantly different from combined application of EPN + EPF, NeemAzal-T + EPN and EPN + EPF + NeemAzal-T ($p<0.001$). In EPF combinations, total survivals were again considerably reduced by the late mycosis effect in all the relevant treatments to

between 10% and 14% in combinations with Neem pellets, while NeemAzal-T resulted in 3- 5% adult survivals (Fig. 2.3, right).

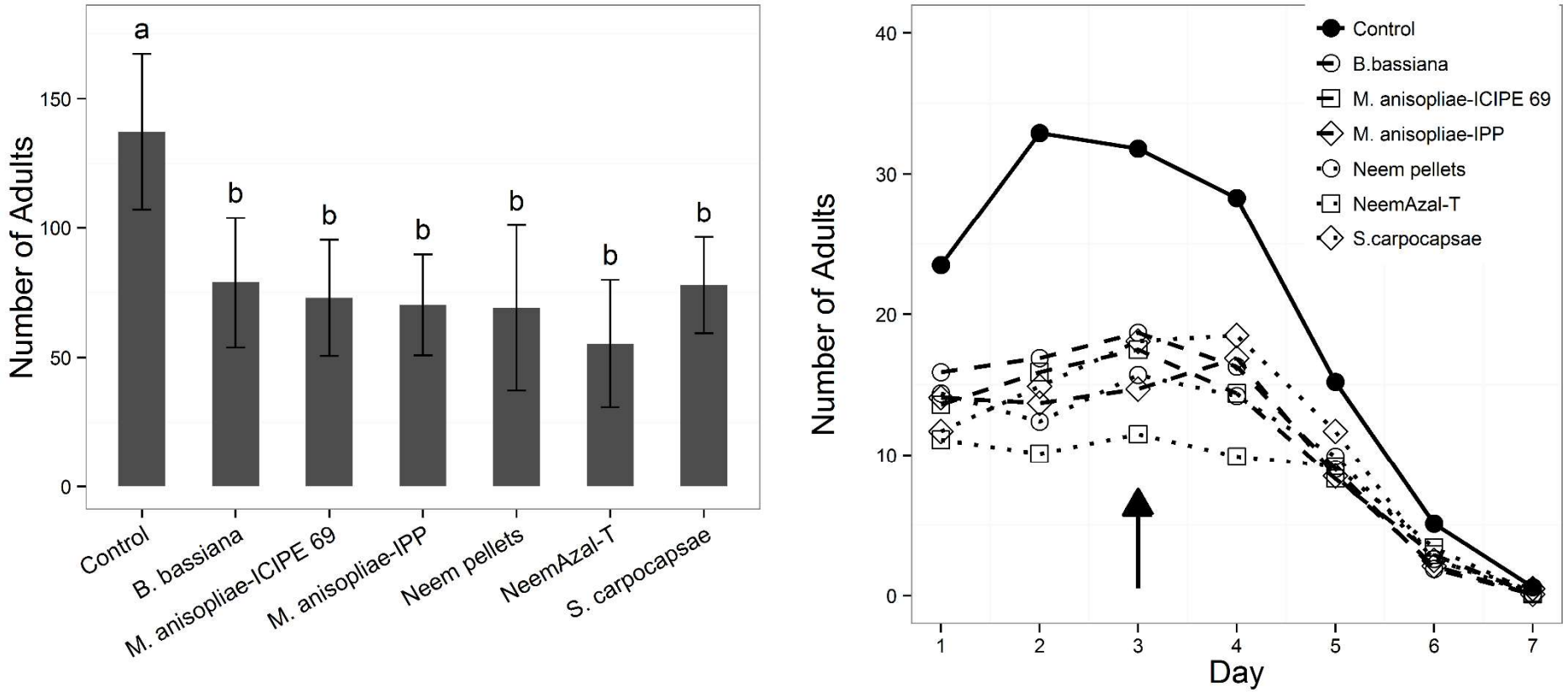

Fig. 2.1 Left - Emergence of western flower thrips (WFT) (mean ± SD) in single treatments of NeemAzal-T solution (1% azadirachtin (AZA) and neem pellets (7% AZA); *Beauveria bassiana, Metarhizium anisopliae* (IPP 2539 & ICIPE-69) and *Steinernema carpocapsae*. Treatments sharing no common letter are significantly different at a multiple type I error level of 5% (Tukey's test).

Right- Trend lines showing daily emergence of WFT within the 7-day period.

Black arrow- Peak of adult emergence course beyond which emergence steadily reduced.

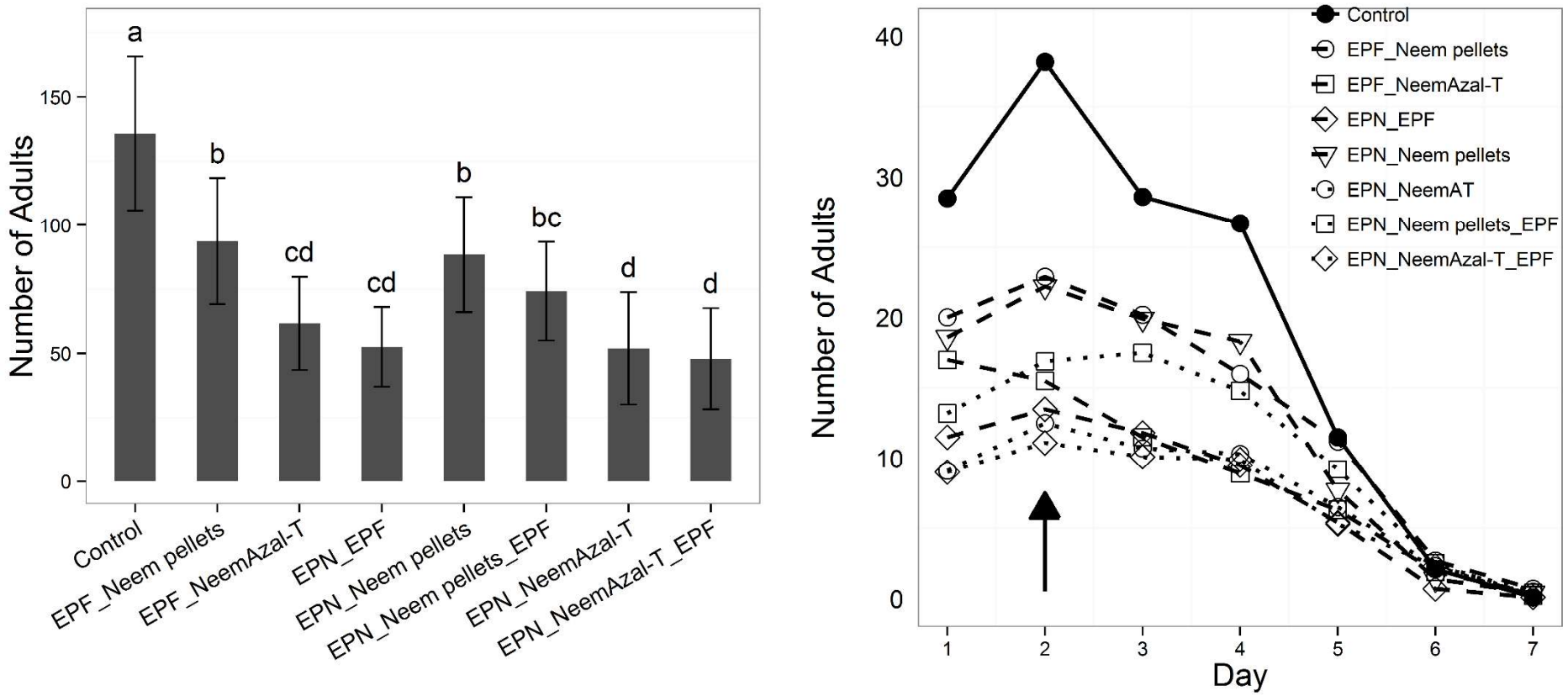

Fig. 2.2 Left -Emergence of western flower thrips (WFT) (mean ± SD) in combined treatments amongst NeemAzal-T solution (1% Azadirachtin (AZA); neem pellets (7% AZA); *Metarhizium anisopliae* ICIPE-69 (entomopathogenic fungi-EPF) and *Steinernema carpocapsae* (entomopathogenic nematode-EPN). Treatments sharing no common letter are significantly different at a multiple type I error level of 5% (Tukey's test). Right- Trend lines showing daily emergence of WFT within the 7-day period

Black arrow- Peak of adult emergence course beyond which emergence steadily reduced.

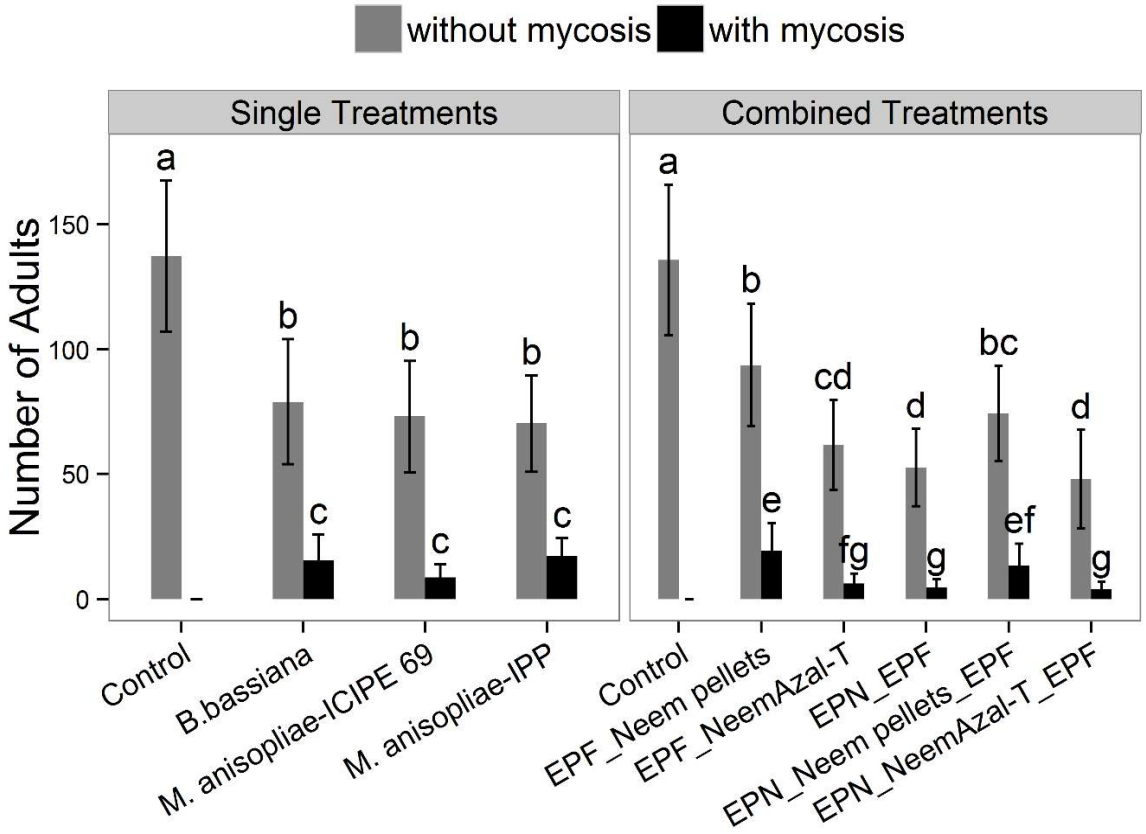

Fig. 2.3 Emergence and survivors of western flower thrips (WFT) (mean ± SD) in entomopathogenic fungi-based single and combined treatments (*Beauveria bassiana, Metarhizium anisopliae* (IPP 2539 & ICIPE-69). The number of adults is given as total number of emergences (light grey) and number of survivors taking late mortality by mycosis into account (dark grey). Treatments sharing no common letter are significantly different at a multiple type I error level of 5% (Tukey's test).

2.3.3 Interaction effects

When the interaction effects were determined, most combined treatments resulted in additive effects (Table 2.2). However, the double (EPN + EPF) and the triple combinations (EPN + NeemAzal-T + EPF) resulted in synergistic responses. On the other hand, combined application of EPN + Neem pellets showed additivity towards antagonism and EPF + Neem pellets gave an antagonistic response (Table 2.2).

Table 2.2: Evaluation of treatment combinations: estimated percent deviations from additivity with 95% confidence intervals, p-values, and conclusions about interactions based on quasi-Poisson GLM analysis.

Treatment combination	Estimated % deviation from additivity	95% confidence interval	p-value	Interaction
EPF + NeemAzal-T	-0.9%	-24.4%; +30.0%	0.949	additive
EPF + Neem pellets	-25.0%	-41.3%; -4.2%	0.022	antagonistic
EPF + EPN	+40.4%	+6.4%; +85.2%	0.016	synergistic
NeemAzal-T + EPN	+14.8%	-14.0%;+ 53.2%	0.351	additive
Neem pellets + EPN	-19.6%	-37.4%;+3.3%	0.088	additive
EPF + NeemAzal-T + EPN	+32.7%	+0.4%; +75.5%	0.047	synergistic
EPF + Neem pellets + EPN	-3.5%	-24.8%;+ 23.7%	0.776	additive

2.3.4 Dose response

For EPF, a clear dose-response relation could be observed for all combinations with NeemAzal-T and the EPN (Fig. 2.4, left). The highest concentration of 10^8 conidia/100ml combined with Steinernema showed the best performance with 74% reduction in adult emergence. Significant differences were recorded between EPF 10^8 conidia/100ml + EPN and combinations with the lowest concentration (p<0.001).

All treatments with NeemAzal-T applied with 0.25 and 0.5 mg/kg and combined either with EPN or EPF had no significant differences in thrips emergence with a mean reduction of approximately 65%. However, when the dosage was increased to 1 mg/kg with both entomopathogens, there were significant differences between NeemAzal-T 1 mg/kg + EPN and NeemAzal-T 0.5 mg/kg + EPF (p=0.0111) and NeemAzal-T 0.25 mg/kg + EPF (p=0.0043). Meanwhile, NeemAzal-T 1 mg/kg + EPF differed significantly from NeemAzal-T 0.5 mg/kg + EPF (p=0.0181) and NeemAzal-T 0.25 mg/kg + EPF (p=0.0073) (Fig. 2.4, middle). Dose-response evaluation of EPN with 100, 400 and 800 IJ/cm^2 and combinations with EPF and NeemAzal-T did not show a consistent and significant tendency, except between 100 IJ/cm^2 + EPF and 800 IJ/cm^2 + NeemAzal-T (p=0.0219) (Fig. 2.4, right).

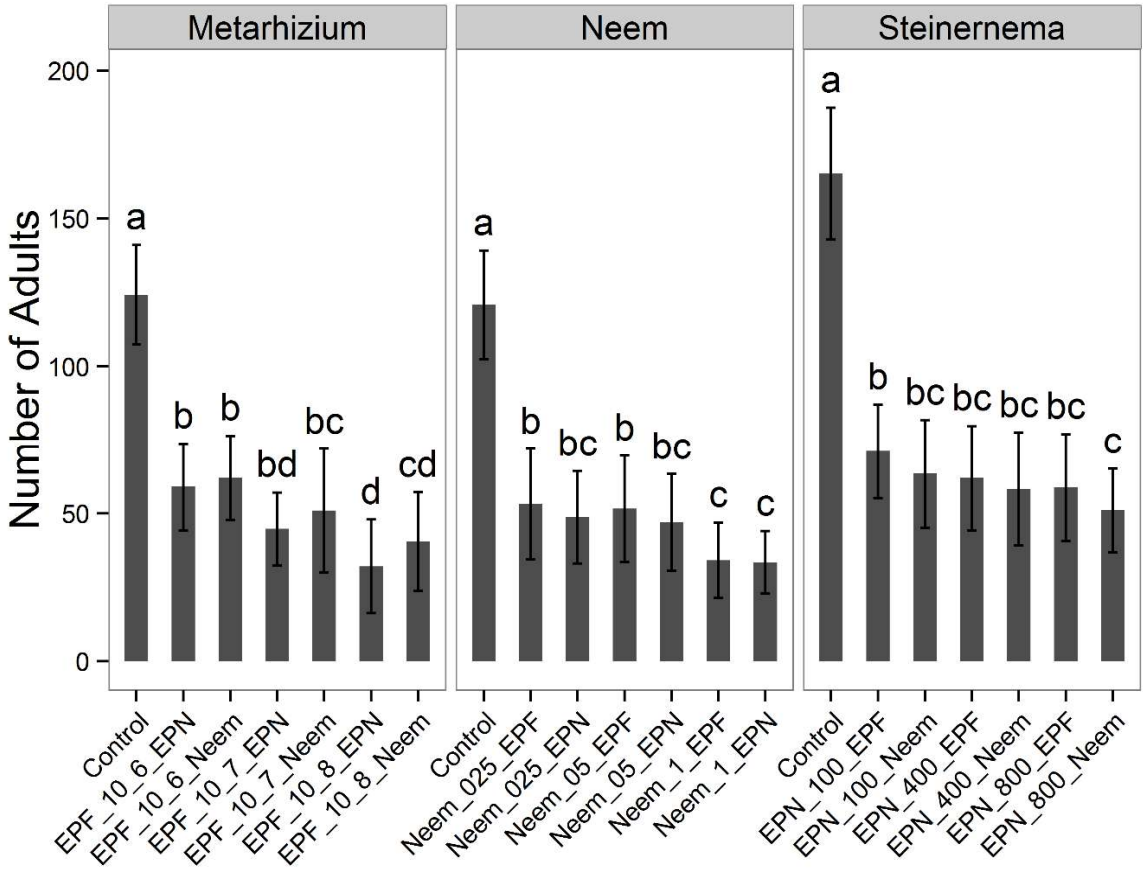

Fig. 2.4 Emergence of western flower thrips (WFT) (mean ± SD) in combinations of three level doses of NeemAzal-T solution (1% azadirachtin (AZA) *Metarhizium anisopliae* (ICIPE-69) and *Steinernema carpocapsae*. Treatments sharing no common letter are significantly different at a multiple type I error level of 5% (Tukey's test).

2.4 Discussion

This study combines EPN and fungi by soil application with specific neem formulations for controlling the soil stages of WFT in glasshouse conditions. NeemAzal formulations and the other antagonists were applied when late L2 was descending to the soil to pupate, mimicking the natural conditions where thrips larvae leave the plants to pupate in the soil (Premachandra *et al*., 2003b).

Regarding the effect of single-compound treatments on *Frankliniella occidentalis*, NeemAzal-T was most efficient. The high efficacy of neem solutions was also shown by the triple treatment combination; it caused the highest % reduction in adult emergence. This effect was only exceeded when secondary mortality effects of the

EPF-based treatments were considered. Contrastingly, the nematodes gave low efficacy despite their active host-finding of IJs being presumed better suited for infecting the cryptic pupal stage of WFT in the soil as opposed to the conidia of EPF which depend on contact by chance either from thrips movement or passive distribution with water in the soil (Williams *et al*., 2013).

Despite having the same active ingredients and concentrations, NeemAzal-T out performed Neem pellets. The better performance of AZA applied as solutions in contrast to the formulation in the pellet carrier material could be explained by the specific release characteristics of the Neem pellets, which are formulated for a slow and continuous release of AZA in the rhizosphere for systemic translocation in the plant but yielding too low concentrations in soil for effective control of thrips stages. A short-term influence but with high concentration, as achieved by the NeemAzal-T solution, should be more effective. It is possible that the efficacy of the pellets could be enhanced by strongly increasing the pellet concentration in the soil.

Among the three EPF, *B*. *bassiana* was out-performed by the two *M*. *anisopliae* strains both in reducing adult emergence and in the late mycosis effect. A poor performance by *B*. *bassiana* was reported by Skinner *et al*., (2012) where BotaniGard (a commercial product of *B*. *bassiana*) resulted in only 15% thrips control. In addition, drenching *M*. *anisopliae* strain V275 yielded higher mortality (84-93%) than *B*. *bassiana* (54-84%) (Ansari *et al*., 2008a). Jacobson *et al*., (2001) used *B*. *bassiana* as a foliar spray and could reduce thrips population by 65% to 87%. The commercial product, Naturalis used here is designated for foliar spray applications and not for soil treatments. Therefore, it could be that an insufficient number of conidia reached and adhered to the soil stages of WFT.

Between the two strains of *M*. *anisopliae*, the ICIPE-69 strain was more efficacious. This could be related to the higher viability of conidia as the ICIPE strain consistently showed a viability of 96% of the conidia compared to only 88% of the IPP strain. The high efficacy of ICIPE-69 strain to 2nd larval instars of WFT was previously reported by Niassy *et al*., (2012b), who compared 10 isolates of *M*. *anisopliae,* 8 isolates of *B*. *bassiana*, and the ICIPE-69 strain that produced the most conidia and therefore proved to be especially virulent.

The soil stages of WFT are most vulnerable for the soil-dwelling nematodes (Buitenhuis and Shipp, 2005). We selected *S*. *carpocapsae* for the studies even though according to Premachandra *et al*., (2003b), *S*. *feltiae* proved more virulent than *S*. *carpocapsae*. The latter is an ambush forager that remains near the point of application and searches for its host mostly on the surface while the former combines both ambush and cruiser (actively searching for the hosts in the soil) modes of hunting.

The highest efficacy with EPN could be obtained, if the nematodes are present in the soil when the L2 is moving into the soil to pupate (Premachandra *et al*., 2003b). This increases the chances of the IJs coming into contact with the thrips, as the nematodes need some time to locate the thrips, and the encounter rates are higher when the thrips are still moving in the soil. The commercial strain of *S*. *carpocapsae* (Nemastar) caused only 43% reduction in thrips emergence with the applied dose rate of 400 IJ/cm^2. This result is comparable to findings by Premachandra *et al*., (2003b) who achieved 40-45% reduction using the same strain at a dose rate of 300 IJ/cm^2 of soil. Further studies (see for instance Premachandra *et al*., 2003b; Ebssa *et al*., 2004) clearly showed that the strain, times of application as well as the concentration of the nematodes are key determinants of efficacy of the EPNs. The 400 IJ/cm^2 application rate used here, has been shown to be the most effective dose rate (Ebssa *et al*., 2001).

The aim of combining different control agents was either to achieve higher efficacies or an increase in reliability. Two control agents applied together may act independently and be directed at different targets in a given host; hence, their effect would be simply additive. However, they may also complementarily improve sensitivity of the target organism and ideally interact synergistically. On the other hand, other competitive interactions are possible leading to antagonistic effects. Our findings showed that combined application of NeemAzal-T may enhance the efficacy of entomopathogens. The combined treatments revealed that the double combinations of the two entomopathogens (EPF + EPN) and the triple combinations of the two entomopathogens with NeemAzal-T (EPF + EPN + NeemAzal-T) led to the most efficient synergistic effects. We hypothesize that neem weakened the WFT by being a physiological stressor or behavioural modifier, thereby predisposing them to the microbes or reducing the defence response of the thrips making them an easier target for nematode penetration and more susceptible to the fungi. Akbar *et al*., (2005) also

hypothesized that the prolonged inter-moult period of insect larvae by the growth-regulating action of AZA may give time for the establishment and penetration of fungal conidia through the insect's cuticle.

Evidence for the synergistic interaction of neem with *B. bassiana* against Army worms, *Spodoptera litura* (Fabricius) has been shown by Mohan *et al*., (2007). Islam *et al*., (2010) observed that combined use of 0.05% neem and 10^7 conidia/ml *B. bassiana* resulted in 97.2% nymphal mortality of *Bemisia tabaci* (Gennadius). This caused 20.5% more nymphal mortality than individual treatments. Nymphal mortality of 90% of whitefly *Bemisia argentifolii* (Bellows & Perring) was obtained when *Paecilomyces fumosoroseus* (Wize) and AZA were combined (James, 2003). Also, of the 25 treatment combination between NeemAzal-T 5% and *S. carpocapsae* by Mahmoud, (2007), 19 of the responses were synergistic, 1 additive, none antagonistic and 5 without response. In this study, combined treatments resulted not only in increased mortality, but also a faster onset of thrips mortality. The number of cadavers that showed mycosis also increased as compared to treatments with single EPF isolates although in combinations, the number of conidia delivered to the soil was lower compared to single treatments with EPF. The larvae migrating in to the soil might have acquired the inoculums but were more vulnerable to the fungi by the additional stress factor. Alternatively, immature stages might have acquired in the combined treatments relatively few conidia but as mortality is dose related, infection took longer to develop; therefore, more individuals survived through to the adult stage but succumbed thereafter (Ansari *et al*., 2008a). Speculating about long-term effects in natural populations, conidia from infected thrips could serve as source of secondary inoculum for the spread of fungal infection in the insect population (Mohan *et al*., 2007). Adding the late effect of the fungi, the double (EPF and EPN) and the triple (EPF, NeemAzal-T and EPF) combinations reduced survivals to 3%. As the triple combination did not give an extraordinary performance, taking costs into account, the use of EPN and EPF would be preferred.

With respect to nematode-fungi interaction, the special mutualistic association of the EPN with bacteria of the genus *Xenorhabdus* for *Steinernematidae*, should be considered. The symbionts inhibit host immune reactions and kill the host rapidly (Lacey *et al*., 2001). Successful EPF infection needs a sequence of steps: attachment

to the host cuticle, germination, penetration, growth and reproduction of the fungus. It kills the host by depletion of nutrients, digestion of tissue or release of toxins. Thrips already infested by the quick-acting nematodes are less mobile, and because of the mentioned symbiotic activity, more susceptible to the fungi and likewise, thrips infected by the fungi should be a weak and easy target for the nematodes thus explaining synergism.

Regarding the dose-response experiments first, a clear relation between dose and efficacy was seen for the EPF; hence, increasing the conidia concentration of EPF in the soil would be one option for optimization of efficacy. Moreover, it was obvious that at every dose, the combination with EPN consistently performed better than its counterpart with neem. For instance, a more noticeable significant difference was recorded between the highest EPF concentration (10^8 conidia) + EPN and the lowest (10^6 conidia) + EPN (p<0.001) than its counterpart 10^8 conidia + NeemAzal-T and 10^6 conidia + EPN (p<0.025) and 10^6 conidia + NeemAzal-T (p<0.005). While many authors have reported positive interaction between the two entomopathogens (Georgis, 1997; Ansari *et al*., 2008b; Williams *et al*., *et al*., 2013). Mohan *et al*., (2007) reported that neem oil caused general delay in conidial growth of *B*. *bassiana*. In addition, in neem-sensitive isolates, the growth of *B*. *bassiana* was decreased but not totaly inhibited resulting in an antagonistic effect. The highest nymph mortality of *B*. *tabaci* was recorded when *B*. *bassiana* was topically applied with drenching application of neem (Islam *et al*., 2011). Also Niassy *et al*., (2012c) reported that AZA in high dosage could have negative effect on sporulation and vegetative growth of *M*. *anisopliae* ICIPE-69. However, this could also be due to overestimation of toxicity because pesticides are delivered on rather inert material than on plant substrate. Plants enzymes can detoxify the pesticide or sequestor it in its waxy leaf cuticle making it less available to natural enemies (Desneux *et al*., 2005; 2006).

Our findings provide evidence of interaction between AZA-based insecticides and the entomopathogens, which allows use of NeemAzal-T with reduced rates, hence also improving the economic trade-off. However, we must admit that a detailed economic valuation was not the aim of this study. Reduction of the operational dose of NeemAzal-T to 0.25% or 0.5% with EPN may be equally effective or even better than a full recommended dose. Surprisingly the dose response for EPN unlike in NeemAzal-T

and EPF was not clear. The simultaneous application of the EPN and NeemAzal-T might have had an effect on the nematodes such that an increase in concentration did not cause a corresponding decrease in adult emergence. Although reports about compatibility between neem formulations and EPNs have been recorded, Meyer *et al*., (2012) reported subtle effects of NeemAzal-U on the plant parasitic nematode *M*. *incognita*. Also, a soap surfactant used for application of neem oil caused 23-25% mortality of *S*. *feltiae* (Krishnayya and Grewal, 2002).

Conversely, combining very low concentrations of EPN (100 IJ/cm^2) with NeemAzal-T resulted in satisfactory control compared to the operational dose of EPN. Practically, EPNs are used against soil-dwelling stages of WFT, and the dose rates presently needed for sufficient control are not economical (Premachandra *et al*., 2003a). Therefore, this study provides further validation that low doses of EPN can be used in combination with other biocontrol agents to offer more satisfactory results.

2.5 Conclusions

The combined use of the soil-applied AZA formulations NeemAzal-T and Neem pellets with the entomopathogens such as the EPN *S. carpocapsae* (commercial product Nemastar®), and EPF (non-commercial isolate of *M*. *anisopliae* ICIPE-69) might be a strategy to improve the efficacy of controlling the soil-dwelling stages of WFT in comparison with the use of the individual components. In all the EPF-based combinations, survivals ranged between 14% and 3% when considering secondary mortality due to mycosis of the emerged adults. However, our results were obtained only in microcosm in a controlled environment with artificially synchronized thrips cohorts. These data need to be confirmed under glasshouse or field conditions with naturally established thrips populations consisting of individuals of different developmental stages and propagating over longer time periods. Moreover, the influence of other factors such as varying abiotic conditions or the influence of competition among the guild of natural enemies should be considered. If additive or even synergistic effects of neem and entomopathogens can be confirmed in more realistic crop culture situations such as field studies, this combination may offer a more powerful and reliable tool for thrips control compared to single biocontrol agent treatments. In addition, sequential treatments and split application of the entomopathogens may offer an interesting option as well. In case thrips are detected

early, neem may provide initial population reduction followed by split application of the entomopathogens which can offer a long-term control of the pupal stage of WFT. More research is needed to establish the effect of neem on the virulence of entomopathogens as different compatibility studies show some conflicting results. Also, research into the molecular background of innate defense responses in WFT following the combined application of neem and entomopathogens would be interesting.

3.0 Additive and synergistic interaction amongst *Orius laevigatus* (Heteroptera: Anthocoridae), entomopathogens and azadirachtin for controlling western flower thrips (Thysanoptera: Thripidae)

Abstract

This study evaluated the efficacy of the foliage-dwelling predator *Orius laevigatus* (Fiber), soil-applied entomopathogens and azadirachtin, both as single treatments and in various combinations, as well as their interaction effects in controlling western flower thrips *Frankliniella occidentalis* (Pergande). Evaluated products were Nemastar® *Steinernema carpocapsae* (Wesier) (Rhabditida: Steinernematidae), *Orius laevigatus* (Re-natur), *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycota: Hyphomycetes) isolate ICIPE-69, and NeemAzal-T (Trifolio). The predator *O. laevigatus* was introduced at different densities and also targeted different larval stages of western flower thrips. Efficacy against western flower thrips was significantly improved by combined treatments, achieving 62-97% mean reduction in western flower thrips emergence from the soil, compared to 45-74% in single treatments. Among the ten treatment combinations that were tested, two synergistic and eight additive interactions were found. Significant differences were observed between efficacy of *Orius* and *M*. *anisopliae* as well as among combinations with and without *Orius*. *Metarhizium*-based treatments reduced western flower thrips survival by 93-99.6% when late mortality due to mycosis was considered. Halving the number of released predators did not significantly reduce efficacy (86-96% versus 76-88% thrips reduction), and when *Orius* was introduced to target the first larval stage (L1) of western flower thrips, 96-98% reduction was achieved, compared to only 71-89% when targeting larval stage 2 (L2). In conclusion, early release of *O*. *laevigatus,* either alone or preferably in combination with soil-applied NeemAzal-T and/or entomopathogens, can be a successful and reliable biocontrol strategy for western flower thrips.

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3.1 Introduction

Western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is an important pest of ornamentals and vegetables that causes extensive economic losses in greenhouse and open-field plant production (Reitz *et al*., 2011). Western flower thrips have an affinity to tight/cryptic feeding sites within buds or on rapidly growing tissues such as young leaves and flowers, and this cryptic behaviour is a major challenge because it makes thorough coverage difficult when using insecticides. Moreover, pupation occurs in the soil and a certain part of the population is escaping from plant delivered compounds. In addition, resistance to conventional insecticides has been recorded worldwide (Jensen, 2000). Even populations resistant to insecticides that are currently in wide use, such as imidacloprid and fipronil (Herron and James, 2005) or spinosad (Bielza *et al*., 2007), have been detected. In addition, there is a global tendency towards reducing the use of synthetic pesticides because of associated problems of resistance, environmental contamination, adverse effects on non-target organisms and demand for pesticide-free foods (Gao *et al*., 2012b). Biocontrol could therefore be a convenient alternative option providing economic and eco-toxicological benefits (Bonsignore and Vacante, 2012).

In its life cycle, western flower thrips occurs as highly mobile adult, immobile but protected egg in plant tissue as well as first and second larval stage in the crop canopy where it is relatively vulnerable to predatory mites or bugs preferring the same habitat. In contrast, the descending late second larval stage escapes such antagonists to pupate in the soil or leaf litter (Steiner *et al*., 2011), but tends to be more vulnerable to soil dwelling predators and pathogens (Holmes *et al*., 2012). Therefore, an ideal biocontrol strategy would target these soil-dwelling stages as well as the foliage-inhabiting adult and larval stages (Rahman *et al*., 2011).

The *Orius spp*. (Hemiptera: Anthocoridae) were shown to be effective predators for thrips in both field and greenhouse crops throughout the world as they are the only predators that attack thrips even in cryptic habitats such as flower buds (Blaeser *et al*., 2004, Silveira *et al*., 2004, Islam *et al*., 2010). They are also strong flyers and agile hunters, with wider operational radius for prey location compared to other biocontrol agents (Nderitu *et al*., 2010). They can prey on all thrips stages as well as other pests like aphids, spider mites, whiteflies and moth eggs. They also feed on pollen, which

enables them to build a population in pollen-bearing crops when thrips are not available (Sanchez *et al*., 2000).

However, there are conflicting reports concerning the success of *Orius* in controlling western flower thrips. While significant efficacy has been reported in several studies (Silveira *et al*., 2004; Blaeser *et al*., 2004; Xu *et al*., 2006; Bosco *et al*., 2008; Weintraub *et al*., 2011) other authors observed only low control of thrips by *Orius* as compared to other biocontrol agents (Medina *et al*., 2003; Shipp and Wang, 2003; Pozzebon *et al*., 2015). Therefore, it might be worthwhile to test the release of *Orius spp*. in combination with additional measures compatible with natural enemies such as botanicals or entomopathogens for efficient and persistent pest suppression. The following are interesting candidates:

Biorational products containing active ingredients such as azadirachtin derived from the neem tree *Azadirachta indica* and often termed simply neem products are general-purpose botanical pesticides today widely used in different crops, in particular in organic production systems (Islam *et al*., 2010). They have several advantages: first, multiple mechanisms of action such as repellence, moulting and oviposition disruption, growth reduction, and increased mortality of immature stages (Mitchell *et al*., 2004) and hence low risk for selection of resistant pest biotypes. Second, extremely low toxicity to humans and often with relatively high selectivity concerning natural enemies if direct contamination of sensitive stages is avoided. Third, they can be applied either alone (Kumar and Poehling, 2006; Thoeming *et al*., 2006) or in combinations with entomopathogens that are “compatible” (causing mortality < 20%) and their joint efficacy can potentially benefit from synergistic interaction (Mohan *et al*., 2007; Islam *et al*., 2010). However, the active ingredient azadirachtin is susceptible to photo-degradation by UV radiation, and hence soil application is advantageous since it minimizes exposure to sunlight. For soil treatments in particular water based formulations of azadirachtin have been proven to be most efficient in controlling pests (Thoeming *et al*., 2006; Karanja *et al*., 2015).

Entomopathogenic fungi (EPF) such as *Metarhizium aniosopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitacea) has also been described as an efficient biocontrol agent against western flower thrips (Ansari *et al*., 2007; 2008a). The relative

safety and specificity of these fungal pathogens may enhance their acceptance by growers for use in pest management programs (Gao *et al*., 2012b) however their need for very specific conditions regarding relative humidity (RH) and temperature may limit their use in many cropping systems if applied to the crop canopy (Ekesi *et al*., 2000). According to Mfuti *et al*., (2016), EPF conidia applied on foliage have short persistence due to environmental factors such as UV light, temperature and rain. This short persistence requires frequent application at short intervals. On the other hand, the high RH inside flowers where adult western flower thrips feed and in the soil where thrips mostly pupate are convenient microclimatic environments for fungal infection and therefore better performance (Cloyd, 2009). Susceptibility of thrips to soil treatment with *Metarhizium anisopliae* has already been shown (Brownbridge, 2006; Skinner *et al*., 2012; Otieno *et al*., 2016).

Another option is to use entomopathogenic nematodes (EPN) such as *Steinernema carpocapsae* (Rhabditida: Steinernematidae). In above-ground application the efficacy of EPN is limited due to their sensitivity to desiccation and UV radiation (Shapiro-Ilan *et al*., 2010), however, they were found to be suited for infecting the cryptic pupal stages of western flower thrips in the soil, and efficiently reducing thrips emergence when drenched into the soil at high rates to compensate for limited dispersal capacity (Premachandra *et al*., 2003a, 2003b; Buitenhuis and Shipp, 2005; Ebssa *et al*., 2006; Otieno *et al*., 2016). The EPN applied to the soil can be combined without any detrimental interaction with other biocontrol agents, in particular foliage-dwelling predators of thrips. A detailed study of the interaction among various combinations of entomopathogens and azadirachtin targeting the soil stages of western flower thrips in a microcosm experiment was reported by Otieno *et al*., (2016). In addition to that, the present study includes *O*. *laevigatus* as a control agent, with the aim of assessing the efficacy of biocontrol agents that possibly could act in combination above and below ground for integrated thrips management. We evaluated basically the effect of releasing *O*. *laevigatus*, combined with soil application of NeemAzal-T, *S. carpocapsae* and *M*. *anisopliae,* against western flower thrips. A detailed economic valuation is however beyond the scope of this study.

3.2 Materials and methods

3.2.1 Host plant and western flower thrips rearing

Two-week old bean seedlings (*Phaseolus vulgaris* L.) var. 'Speedy' (Fabaceae) seedlings were used as host plants. Individual seedlings were transplanted into 16 cm diameter plastic pots filled with 600 g of commercial substrate (CS) Fruhstorfer Erde, type P (Archut GmbH, Lauterbach-Wallenrod, Germany); 50% peat, 35% clay, 15% humus; pH 5.7-6.3; 124-185 mg N, 120-179 mg P_2O_5 ,190-284 mg K_2O and 0.8-1.4 g salt content per L. The seedlings were kept under greenhouse conditions of 22 ± 2°C, 65 - 75% RH and a 16:8 h light: dark photoperiod. To obtain thrips of the same age, synchronized rearing of western flower thrips was established on pods of French beans in 0.75 litre glass jars (Leifheit, Nassau, Germany) according to a protocol by Berndt *et al*., (2004).

3.2.2 Predatory bug

Orius laevigatus were provided by Re-natur (Stolpe, Germany) in bottles containing 500 individuals dispersed in vermiculite, and were released within one day after receipt.

3.2.3 Entomopathogenic fungi (EPF)

Metarhizium anisopliae isolate ICIPE-69 commercialized strain by International Centre of Insect Physiology and Ecology (Nairobi, Kenya) with identification characteristics stated in Niassy *et al*., (2012b) was used in this study. *M. anisopliae* was obtained as dry conidia and stored at 4°C. Before use, an infection cycle was run on western flower thrips adults to ensure virulence to that target. Conidia from infected western flower thrips cadavers were isolated onto potato dextrose agar medium (PDA), supplemented with peptone and yeast extract. Two week old fungal cultures maintained at 25°C and grown on artificial medium less than three times after isolation from western flower thrips were used for the experiments. An aqueous conidial suspension was then prepared by scraping off dried conidia from the PDA plates into 15 ml test tubes using a sterile spatula. To determine the number of conidia per gram of dry powder, a 0.1 g sample was aseptically mixed with 100 ml of sterile 0.05% Tween 80. The solution was vortexed for five minutes to break chains or aggregates of conidia to achieve a homogenous suspension. The conidial suspension was then adjusted to 10^7 conidia per 100 ml (Islam *et al*., 2010) using a Fuchs-Rosenthal haemocytometer (Marienfeld GmbH & Co. KG, Lauda Königshofen, Germany) and a compound microscope (x400

magnification) (Goettel and Inglis, 1997). Viability of conidia was assessed before the experiments to adjust the desired concentration of viable conidia per ml for every bioassay. Briefly, five aliquots of conidia solution diluted to 10^6 conidia/ml were added to a Petri dish filled with agar, which was incubated at 25°C for 24 hours in darkness. Lacto-phenol cotton blue was used to stop conidial growth and to stain the fungal conidia. Germinated and non-germinated conidia were counted by placing agar cubes under a compound microscope (x400 magnification) and evaluating 100 conidia. A conidium was considered germinated if the germ tube was twice the length of the conidia.

3.2.4 Entomopathogenic nematodes (EPN)

Nemastar® based on *S. carpocapsae* was obtained as a clay formulation from e-nema GmbH (Raisdorf, Germany) and stored at 4°C until use. Before exposure to western flower thrips, nematodes were allowed to acclimatize at ambient room temperature for four hours. Thereafter, 1 g of the product was dissolved in 50 ml of de-ionized water, and 1 ml of the suspension was mounted on a microscope slide to assess EPN density and mobility. A final concentration of about 80,500 infective juveniles (IJ) in 25 ml of de-ionized water was prepared by quantification and dilution giving a concentration of 400 IJ per cm^2 soil per experimental pot (Premachandra *et al*., 2003b).

3.2.5 NeemAzal-T

NeemAzal-T solution (1% active ingredient of azadirachtin dissolved in water) was obtained from Trifolio-M GmbH (Lahnau, Germany). A concentration of 1 ml/kg of substrate was used. For each pot (600 mg of the substrate Fruhstorfer Erde), 0.6 ml of the basic solution was diluted in 100 ml de-ionized water. The suspension was shaken for 30 minutes in a mechanical shaker before application.

3.2.6 General experimental procedure

Male and female *Orius* were isolated in a plastic bottle prior to the experiments. Only females were used since they are generally larger and feed more for reproduction (Xu *et al*., 2006). Each experimental unit consisted of a potted bean plant in a plastic pot (16 cm diameter) enclosed by a plexiglass cylinder (diameter 15 cm, height 40 cm) serving as tightly fitting microcosm. Proper ventilation was ensured by the open top and four additional holes (diameter 3 cm) in the cylinder wall, which were covered with thrips-proof nylon gauze (pore size 64 µm).

Ten one-week old adult western flower thrips females and two males were selected from a synchronized rearing unit and transferred into a clean Eppendorf tube (2 ml) using a fine hairbrush. The Eppendorf tube was fixed on the petiole of the bean plant and afterwards opened to allow the adults to crawl/fly out of the tube and infest the plant. The edge between cylinder and pot was sealed with parafilm to prevent thrips from escaping. Thrips were allowed to feed and to lay eggs on the plants, and on the fifth day when L1 had hatched, two female *Orius* were introduced using the same delivery technique as with thrips. On the eighth day when L2 should start descending to the soil for pupation (Berndt *et al*., 2004), the microcosm was cautiously opened to avoid any escape of thrips, and the soil treatments were quickly applied such that the L2 stage targeted here didn't escape. For NeemAzal-T application 0.6 ml of the basic solution diluted in 100 ml de-ionized water was drenched to the soil. The EPN suspension of about 80,500 infective juveniles (IJ) in 25 ml of de-ionized water was also drenched using a Pasteur pipette and five minutes later the soil surface was further irrigated with another 25 ml of distilled water for better percolation of the nematodes to lower soil layers where the western flower thrips pupate. For EPF, the whole conidia suspension (100 ml) was applied to each pot, which ensured a total wetting to field capacity of the upper soil layer that should be passed by the descending thrips larvae.

A blank treatment with only de-ionized water was set up as a control since pre-tests showed no significant differences between the blanks of the compounds used and the water control. Twelve days after the introduction of the western flower thrips adults, the cylinders were removed and the bean plants were cut off. *Orius* were found to be still active on the canopy but barely any thrips adults. To assess efficacy of the treatments, the number of emerging western flower thrips was recorded for seven days using an emergence trap as described in Otieno *et al*., (2016). In brief, the emergence traps consisted of an inverted pot of the same size as the one used for the substrate in the microcosm and tightly fitting on the substrate. The positively phototactic emerging thrips were attracted to a light exposed hole on top of this elector unit and guided into an adjusted Eppendorf tube (trap). All experiments were conducted in a climate chamber (Johnson Controls GmbH, Mannheim, Germany). The conditions were 23 ± 2°C, 50-60% RH and 16:8 L: D photoperiod.

3.2.7 Basic treatments and combinations

The full list of treatments and dosages used is shown in Table 3.1. A completely randomized design with fifteen replicates per treatment was used in all experiments. Additionally, in EPF treatments a test for late mycosis was performed. Adult thrips emerging from treatments with EPF were incubated at 25˚C in 55 mm diameter Petri dishes filled with a 5 mm plaster of Paris: charcoal (9:1) layer overlaid by a Whatman filter paper of the same diameter. 1.5 ml of sterile water was added to maintain high humidity. Most (> 90%) of the incubated adults died after two days, and the cadavers of these specimens were examined under a binocular microscope for spores and hyphae on their surface for identification of the suspended EPF and to confirm mycosis as the cause of death.

3.2.8 Two densities of *Orius* (1 or 2 adults)

To assess density dependent variation in *Orius* efficacy, one vs. two adult *Orius* were released in different combinations with EPF, EPN and azadirachtin using the experimental procedure as described above, and the soil treatments were applied as in the previous experiment.

3.2.9 Efficacy of *Orius* targeting different larval stages of WFT

In an additional set-up, *Orius* was released on the fifth and eighth day after introduction of western flower thrips adults to target the L1 and L2 stages of western flower thrips, respectively. The soil treatments and combinations with EPF, EPN and Neem were applied as in experiment 1.

3.2.10 Statistical analysis

In all experiments, the number of emerging thrips per pot was modelled as Poisson counts in a generalized linear model (GLM) (McCullagh and Nelder, 1989) with a logarithmic link function and over-dispersion (“quasi-Poisson”). Treatment means were compared using all pair-wise contrasts (Tukey test) of the generalized linear model (GLM) parameters at a family-wise type I error rate of 5%. Interactions of treatments applied as combinations were evaluated as described for a very similar experimental setup in Otieno *et al*., (2016). Null hypotheses of additivity were formulated as linear combinations of GLM parameters in a way that they reflected the mixing ratios shown in Table 3.1. Combinations that deviated significantly ($p < 0.05$) from additivity were judged as synergistic if fewer thrips emerged from soil than expected under additivity,

or otherwise as antagonistic. All data were analyzed using R version 3.1.3 (R Core Team, 2015).

Table 3.1: Single and combined treatments identified by *Orius laevigatus* released in the canopy and soil treatments given as amount of active compounds/pot (600g of soil) and percentages (relative to the single treatment doses).

	Treatment (s)	**Canopy treatments**	**Soil treatments**		
		***O. laevigatus* (*Orius*)**	***M. anisopliae*-ICIPE (EPF)**	**NeemAzal-T (Neem)**	***S. carpocapsae* (EPN)**
A	**Single treatments**				
	O. laevigatus	2 adults	-	-	-
	M. anisopliae (EPF)	-	10^7 conidia	-	-
	NeemAzal-T (Neem)	-	-	6 mg AZA**	-
	S. carpocapsae (EPN)	-	-	-	80435 IJ* (400IJ/ cm^2)
B	**Double combinations – (Soil treatments as % of single treatment used - see A)**				
	EPF + EPN	-	(67%)		(33%)
	EPF + Neem	-	(40%)	(60%)	-
	Neem + EPN	-		(75%)	(25%)
	Orius + EPF	2 adults	(100%)		
	Orius + EPN	2 adults	-	(100%)	-
	Orius + Neem	2 adults	-	-	(100%)
C	**Triple combinations- (Soil treatments as % of single treatment used - see A**				
	EPF + Neem + EPN	-	(33%)	(50%)	(17%)
	Orius+EPF+EPN	2 adults	(67%)	-	(33%)
	Orius+EPF+Neem	2 adults	(40%)	(60%)	-
	Orius+EPN+Neem	2 adults		(75%)	(25%)

*IJ = Infective juveniles; **AZA = Azadirachtin

3.3 Results

3.3.1 Single treatments

All treatments were significantly superior to the control according to the Tukey test of GLM parameters at a family-wise 5% level ($p<0.001$). Emergence of adult western flower thrips was reduced on average by 45-74% compared to the control group, with *Orius* being the most efficient single treatment (Fig. 3.1), although there were no significant differences except between *Orius* and *M. anisopliae* ICIPE-69 ($p = 0.002$, Tukey test). The daily trend of emergence also showed that *Orius* outperformed other treatments having the least number of daily emergences of western flower thrips adults (Fig. 3.2).

3.3.2 Double combinations

All *Orius*-based double combinations revealed significantly higher efficacies than other double combinations without *Orius* ($p<0.001$ for every pair-wise comparison, Tukey test) (Fig. 3.1). This clear difference was also highlighted by the daily trend (Fig. 3.2). The *Orius* based combinations caused 89-97% reduction in emergence while those that did not include *Orius* ranged between 62 and 69% reduction in emergence. The most efficient among the double combinations was *Orius* + Neem with 97% reduction.

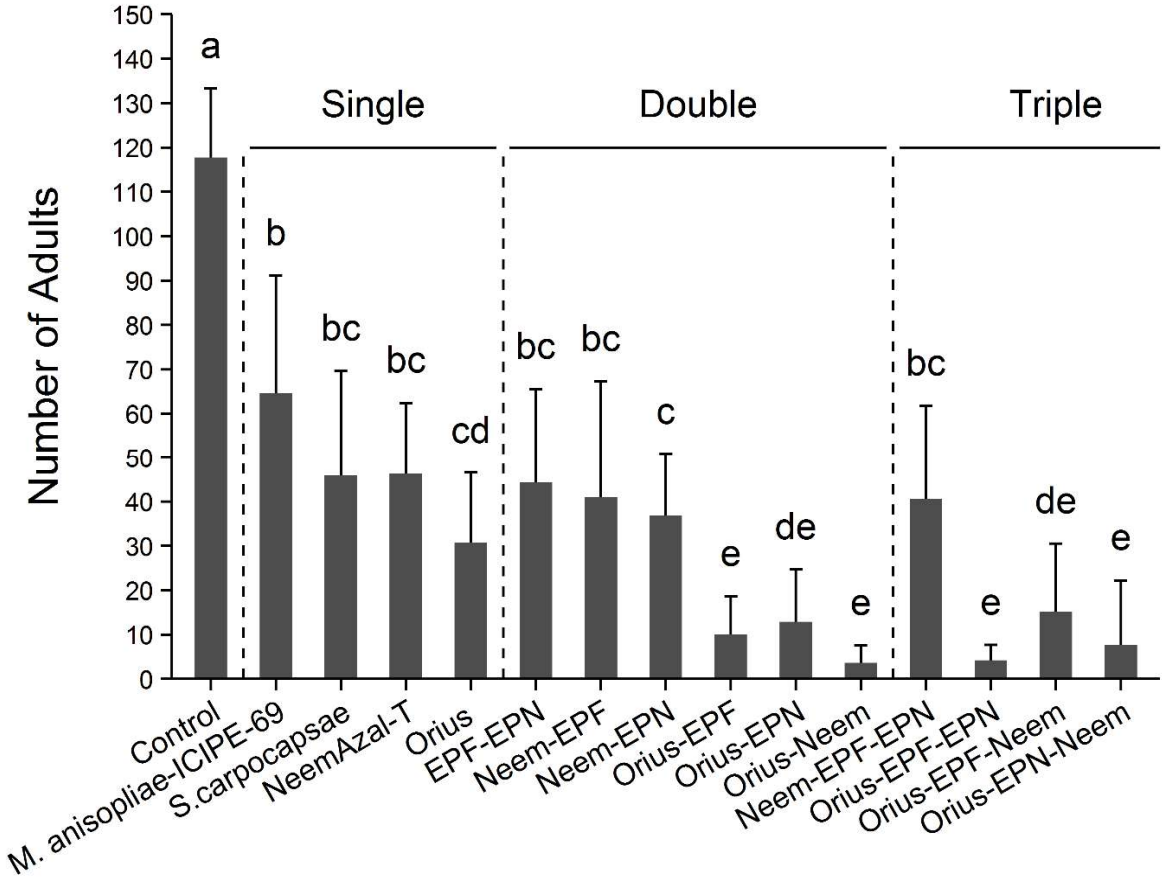

Fig. 3.1 Average number of emerging adults of western flower thrips after seven days (mean + SD) in single and combined treatments using *Orius laevigatus*, NeemAzal-T solution (1% azadirachtin AZA), *Metarhizium anisopliae* ICIPE-69, Entomopathogenic fungi-EPF) and *Steinernema carpocapsae* (Entomopathogenic nematode-EPN), grouped into single, double and triple treatments. Treatments sharing no common letters are significantly different at a multiple type I error level of 5% (quasi-Poisson analysis and Tukey test).

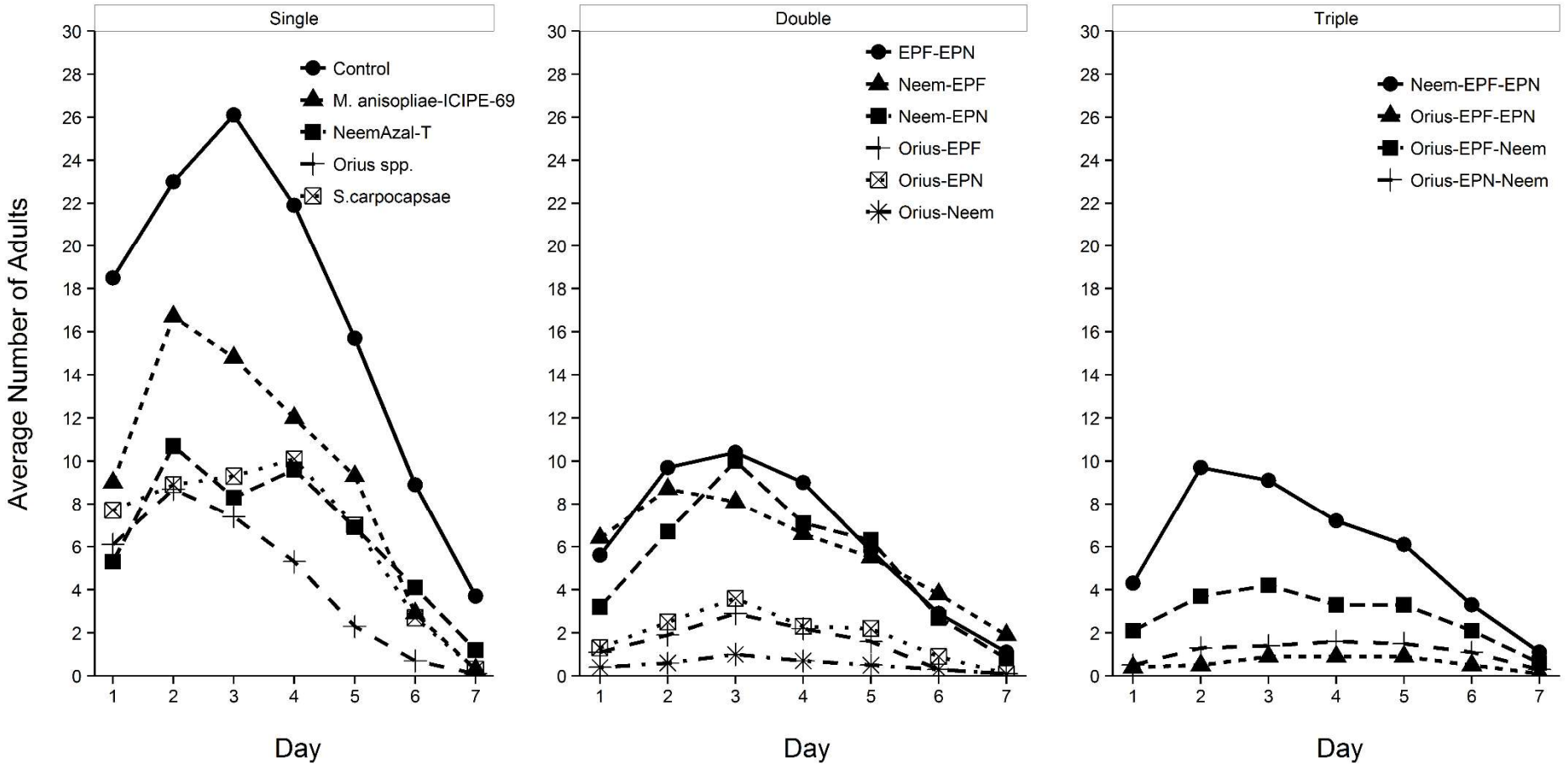

Fig. 3.2 Trend lines showing average number of emerging adults of western flower thrips within seven-day period in single and combined treatments using *Orius laevigatus*, NeemAzal-T solution (1% azadirachtin A), *Metarhizium anisopliae* ICIPE-69 (EPF) and *Steinernema carpocapsae* (EPN), grouped into single, double and triple treatments.

3.3.3 Triple combinations

Triple combinations showed consistently high performance of *Orius*-based combinations, the reduction in thrips emergence ranged between 84 and 96%. The Tukey test showed highly significant differences between *Orius*-based triple combinations and Neem + EPF + EPN ($p<0.001$). The latter only resulted in 65% reduction in adult emergence.

3.3.4 Mycosis

The total fungal efficacy was realized when adult thrips that succumbed after emergence from the delayed effect of mycosis were taken into consideration as well. There were no significant differences in the number of cadavers which were not infested by the fungi between all EPF based treatments ($p\geq0.066$). The results ranged from EPF (applied singly) where 93% total mortality was recorded, to *Orius* + EPF + EPN where more than 99% kill was achieved when late mycosis was taken into consideration (Fig. 3.3).

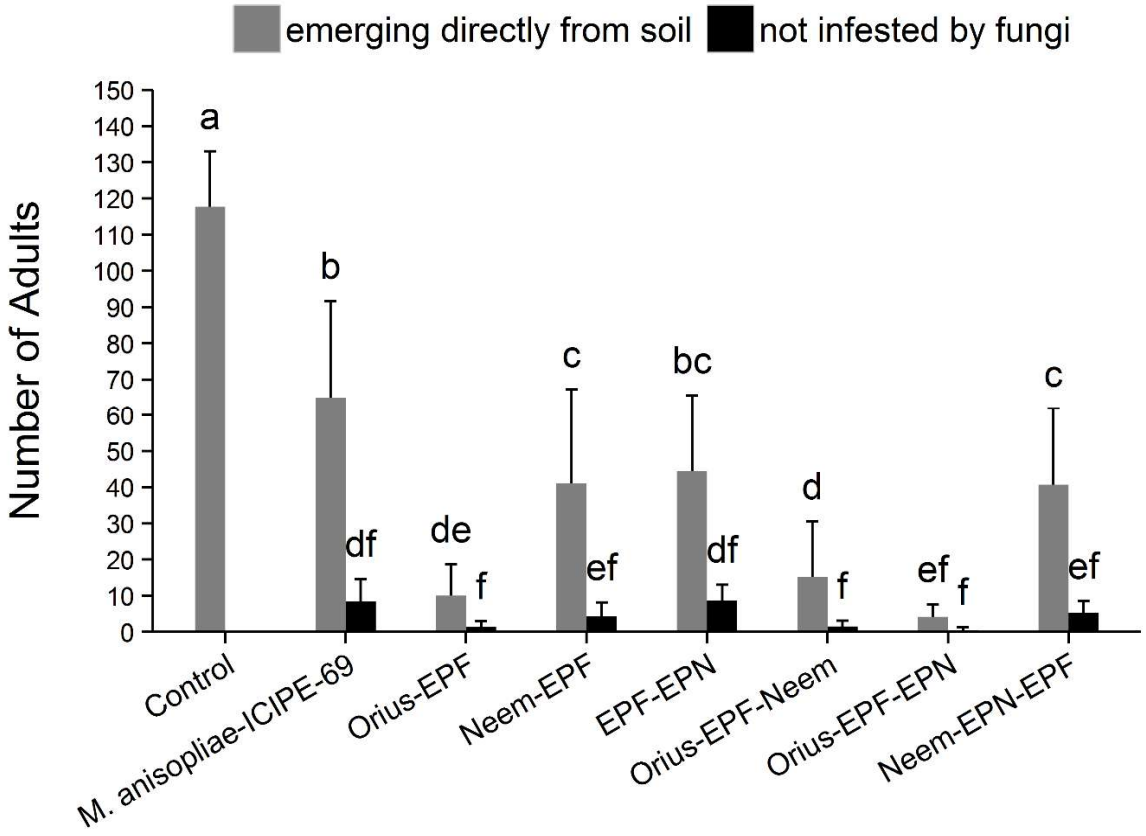

Fig. 3.3 Average number of emerging adults of western flower thrips after seven days (light grey columns) and surviving healthy adults not infested by mycosis in *Metarhizium anisopliae* ICIPE-69-based treatment combinations (dark grey columns). Treatments sharing no common letters are significantly different at a multiple type I error level of 5% (quasi-Poisson analysis and Tukey test).

3.3.5 Interaction effects

Most combined treatments resulted in additive effects (Table 3.2). However, the double combination *Orius* + Neem and the triple combination *Orius* + EPF + EPN allowed significantly fewer emergences than would have been expected under the assumption of additivity ($p=0.013$ and $p=0.004$, respectively, hence synergistic effects can be assumed (Table 3.2).

Table 3.2: Evaluation of treatment combinations: estimated percent deviations from additivity with 95% confidence intervals, p-values, and conclusions about interactions based on quasi-Poisson GLM analysis.

Treatment combination	**Estimated % deviation from additivity**	**95% confidence interval**	**p-value**	**Interaction**
EPF + EPN	+30.3	-2.6;+74.4	0.075	Additive
Neem + EPF	+29.2	-4.6;+75.1	0.098	Additive
Neem + EPN	+25.5	-9.7;+74.5	0.177	Additive
Orius + EPF	+68.4	-11.6;+220.8	0.113	Additive
Orius + EPN	-6.8	-49.5;+71.9	0.821	Additive
Orius + Neem	+229.4	+27.9;+748.2	0.014	Synergistic
Neem + EPF + EPN	+27.2	-5.1;+70.6	0.108	Additive
Orius + EPF + EPN	+259.6	+49.6;+764.4	0.004	Synergistic
Orius+EPF + Neem	-9.0	-48.0;+59.1	0.741	Additive
Orius+EPN + Neem	+57.2	-22.1;+217.4	0.207	Additive

3.3.6 Two densities of *Orius* (1 or 2 adults)

All treatments showed additional reduction in average western flower thrips emergence when two predators were introduced instead of one to the microcosm, although the differences were not always significant. Combinations with a single predator caused on average between 76 and 88% reduction while those with two predators resulted in 86-96% reduction (Fig. 3.4). However, when *Orius* was introduced alone a significant difference between one *Orius* (76% reduction) and two *Orius* (96% reduction) ($p=0.039$, Tukey test) could be observed. In combined treatments there was no significant difference between introducing one or two adult *Orius* ($p \geq 0.57$).

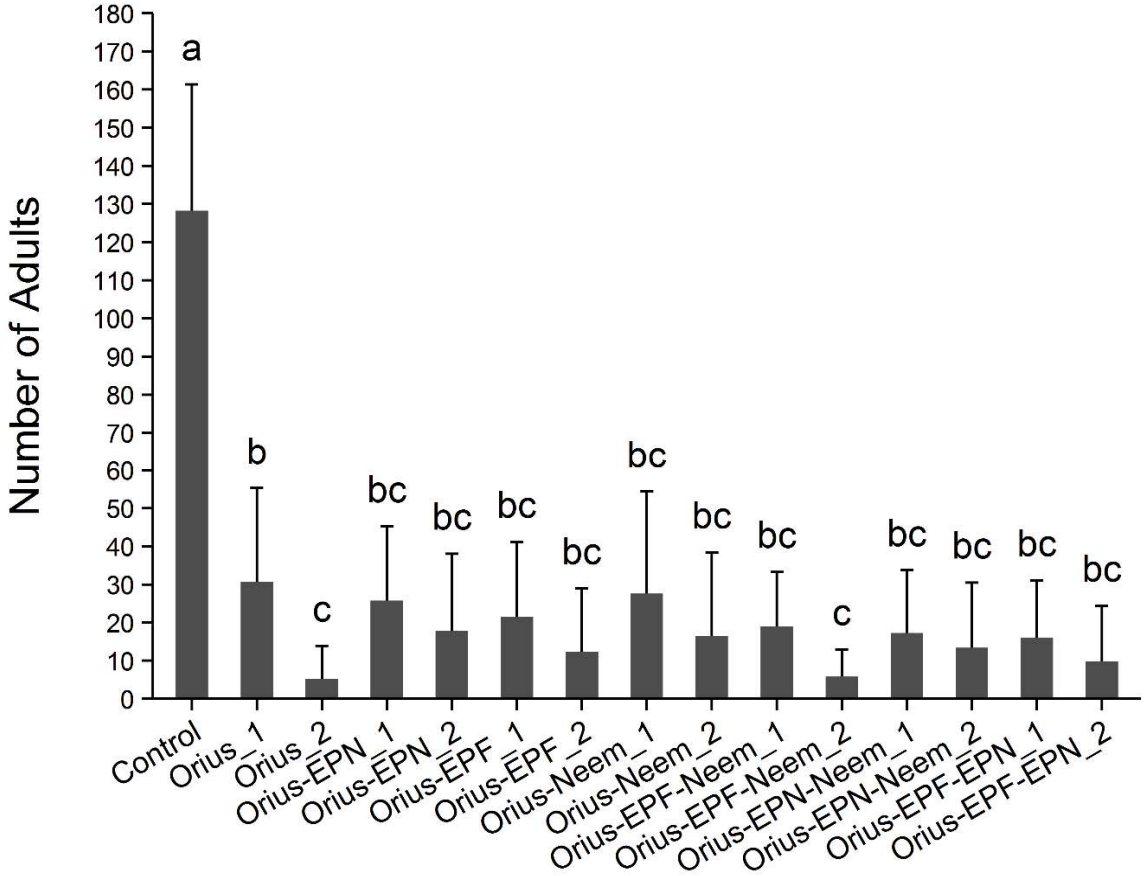

Fig. 3.4 Average number of emerging adults of western flower thrips after seven days (mean + SD) when one (Orius_1) or two (Orius_2) adults of *Orius laevigatus* were introduced on the plants in combination with NeemAzal-T solution (1% azadirachtin AZA), *Metarhizium anisopliae* ICIPE-69 (EPF) and *Steinernema carpocapsae* (EPN) applied to the soil. Treatments sharing no common letters are significantly different at a multiple type I error level of 5% (quasi-Poisson analysis and Tukey test).

3.3.7 Efficacy of *Orius* targeting different larval stages of WFT

Targeting the L1 stage of western flower thrips was always significantly more successful than targeting the L2 for every treatment. For example, *Orius* alone resulted in 98% reduction when targeting the L1 and only 78% when targeting the L2 stage of western flower thrips ($p<0.001$, Tukey test). Generally, combinations with *Orius* targeting the L1 stage of western flower thrips resulted in 96-98% average reduction in emergence with no significant differences amongst them ($p\geq0.69$) (Fig. 3.5). On the other hand, combinations with *Orius* targeting the L2 stage of western flower thrips caused average reduction of 71-89%. Significant differences were only recorded between *Orius* + EPF_L2 (the lowest performer) and *Orius* + EPN_L2 ($p<0.001$, Tukey test) and *Orius* + EPN + Neem_L2 ($p<0.001$, Tukey test).

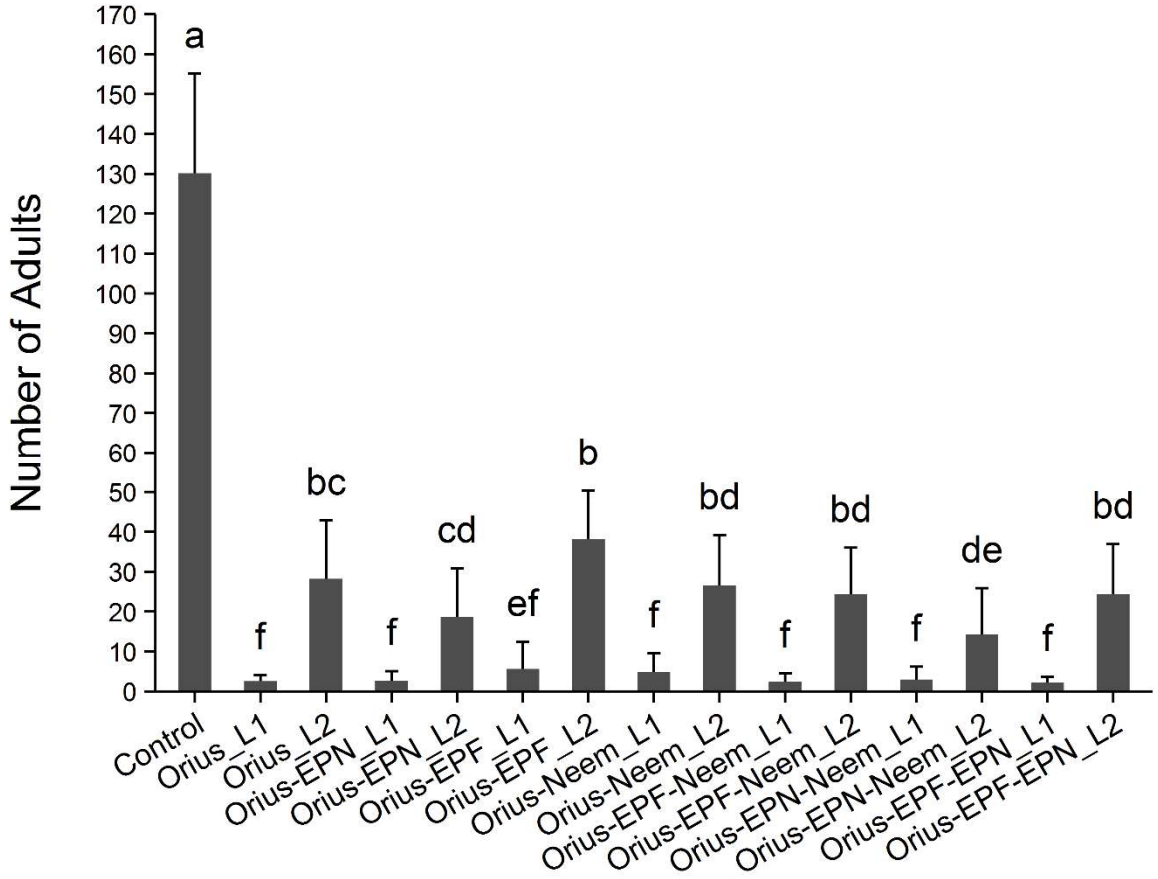

Fig. 3.5 Average number of emerging adults of western flower thrips after seven-days (mean + SD) when *Orius laevigatus* were introduced on the plants to target larval stages 1 (L1) and 2 (L2) in combinations with soil treatments by NeemAzal-T solution (1% azadirachtin AZA), *Metarhizium anisopliae* ICIPE-69 (EPF) and *Steinernema carpocapsae* (EPN). Treatments sharing no common letters are significantly different at a multiple type I error level of 5% (quasi-Poisson analysis and Tukey test).

3.4 Discussion

Regarding the effect of single treatments on western flower thrips in experiment 1, *Orius* was the most efficient. The results of soil application of *M. anisopliae* ICIPE-69, NeemAzal-T and *S. carpocapsae* as single treatments were consistent with earlier findings by Otieno *et al*., (2016). The slightly better performance of EPN compared to EPF could be explained by the fact that infective juveniles have the ability to find their host actively and are therefore better suited to locate the cryptic pupal stages of western flower thrips than conidia of EPF whose success depends on contact by chance when thrips move in the soil or by passive distribution with water in the soil (Williams *et al*., 2013). Initially, the EPN showed higher efficacy than the EPF, although

the overall effect of EPF was substantially increased when thrips mortality due to late mycosis was taken into consideration. The larvae migrating in to the soil might have acquired sub-lethal infection (relatively few conidia) but as mortality is dose related, infection took longer to develop; therefore, more individuals survived through to adult stages but succumbed thereafter (Ansari *et al*., 2008a). Nearly complete control of western flower thrips could be expected from the EPF-based treatments.

Combined application of control agents may enhance the efficacies of single products. This phenomenon was observed in the first experiment, efficacy against western flower thrips was significantly improved in combined treatments compared to single treatments. In particular, *Orius*-based double and triple combinations performed significantly better than their non-*Orius*-based counterparts. Comparable results were obtained by Rahman *et al*., (2011), in other studies, Down *et al*., (2009) used *O. laevigatus* to disseminate conidia of EPFs to control aphids, whiteflies and thrips, indicating that *Orius* is not affected by this pathogen. Studies with other sucking pests for instance the application of *Beauveria bassiana* and neem together increased the mortality of *B. tabaci* (Islam *et al*., 2010; Islam and Omar, 2012). In contrast, combined application of *M. anisopliae* and *Neoseiulus cucumeris* (Oudemans) releases did not further reduce the density of adult western flower thrips compared with sole application of *M. anisopliae* or *N. cucumeris* (Nyasani *et al*., 2015).

An important aspect of combined treatments is the direction and intensity of the resulting joint efficacy. Combining different control agents may result in either positive or negative interactions depending on the type of control agent and target pest. These control agents may act independently against different targets in a given host; hence, their effect would be simply additive. However, they may also complementarily augment/increase the sensitivity of the target organism and ideally interact synergistically. On the other hand, control agents could impair each other, resulting in reduced total efficacies. In these studies, only positive interaction was found within all treatment combinations, most of them with additive properties, but (*Orius* + Neem) and (*Orius* + EPF + EPN) combinations showed clear synergistic responses.

Throughout the experiments, combined application of azadirachtin with EPN or EPF resulted in increased efficacy against western flower thrips. Ebssa *et al*., (2006)

reported that the use of EPN together with the foliar-dwelling *N*. *cucumeris* provided better control (83% mortality) as compared to individual releases of natural enemies. The underlying mechanisms of these synergistic interactions are unclear, but it is postulated that one agent may stress or alter the behaviour like feeding or movement of the target pest making it more susceptible to the other control agents. For example, *M. anisopliae* infected insects may be less mobile which gives EPN more time to penetrate the host (Ansari *et al*., 2004). Alternatively, plant-dwelling predators can evoke escape behaviour of the prey, making the prey available for soil-foraging antagonists or biopesticides. Ebssa *et al*., (2006) and Rahman, *et al*., (2011) noted that foliar-dwelling mites increased the likelihood of thrips falling off the plant onto nematode-treated soil where they were liable to be infected. Azadirachtin weakened the western flower thrips by being a physiological stressor or behavioural modifier, thereby predisposing them to the microbes or reducing the defense response of the thrips, making them an easier target for nematode penetration and more susceptible to fungi. The nematode-associated symbionts release toxins that inhibit host immune reactions (Lacey *et al*., 2001). Such synergies can be exploited to enhance control of pests, while concomitantly reducing the cost of control for growers since each biocontrol agent can be used at a lower dose.

There are contrasting reports concerning the compatibility of azadirachtin and *O*. *laevigatus*. Biondi *et al*., (2012) reported that azadirachtin mainly acts as a moulting disruptor and hence cannot affect adult insects and is therefore harmless to the adult predator, and according to Angeli *et al*., (2005) azadirachtin has no effect on *O*. *laevigatus* exposed via direct contact or by ingestion of infected eggs of *Ephestia kuehniella* (Zeller). Contrastingly, Bonsignore and Vacante, (2012) reported that rotenone, NeemAzal-TS (oil formulation of azadirachtin) and *S*. *feltiae* reduced numbers of *O*. *laevigatus* and Tedeschi *et al*., (2001) showed high direct toxicity of neem (azadirachtin oil formulation) to the mirid *Macrolophus caliginosus* (Wagner). But it should be recognized that in our studies the azadirachtin treatment (soil) and *Orius* (plant canopy) were spatially divided, thus enabling selectivity even in case of possible contact toxicity.

The number of released predators could be critical for an optimal balance between efficacy and expenditure on the control agent. Therefore, in an additional experiment

we checked if the density of the predator could be reduced without impairing its effectiveness. In the single *Orius* treatment, predation of thrips was significantly higher when two predators were released per microcosm compared to only one. However, the drawback of introducing only one predator was largely compensated for by combined treatments with other biocontrol agents, showing clearly a stabilizing effect of combinations. This improved reliability of combined treatments is of practical importance. Although the predators were of uniform age and sizes, variation in efficacy between experiments still existed. Such incidences can greatly affect the success of a single component of the biocontrol program and thus in addition justifies the use of an integrated approach. Two control agents with different mechanisms and different intrinsic variability can act complementarily for a given target site of a host, resulting in an additive or synergistic effect. This may overcome the inefficiency or problems with inconsistency when using each agent alone.

Another important issue is the timing of application of biocontrol agents. In case of the soil-delivered EPN and EPF as well as drenching of neem, application just before the late L2 descends to the soil ensures the best performance (Premachandra *et al*., 2003b). In case of predator release, timing is more crucial since target susceptibility and hence predatory success rate can vary greatly with progress in development of the prey. Most often, early release of predators is key for successful biocontrol (Cloyd, 2009). This work corroborated the need for early releases by showing that introducing *Orius* to target the L1 was significantly more successful than targeting the L2 stage of western flower thrips. Apart from being able to feed on more juveniles at this stage, it also allowed time for better establishment of the predator, interaction between prey and predator, and therefore good control of thrips (Cloyd, 2009). Further studies should include long term effect of the EPF and neem on population development of the predator and also, using other *Orius spp*. with other biocontrol agents to determine if our observation with *Orius laevigatus* is consistent.

3.5 Conclusions

In summary, this study indicates that *Orius laevigatus* is effective in controlling thrips and also suitable to be released in combinations with soil applied azadirachtin and entomopathogens (EPN and EPF). These combinations resulted in additive and synergistic effects, covering gaps of efficacy and improving the reliability of single agent treatments. Therefore, selection of biocontrol agents should not only depend on their individual efficacies, but also on the potential for complementary action against the target pest. Timing of release is key to successful thrips control when using the predator *Orius*. The best results were observed when targeting the first larval stage of thrips. Moreover, using *Orius* in combination with other soil applied biocontrol agents can help to manage thrips populations in the crop canopy efficiently with very low predator densities. However, further greenhouse experiments should be conducted to validate these results.

4.0 Efficacy of LED enhanced blue sticky traps combinbed with the synthetic Lurem-TR for trapping of western flower thrips

Abstract

Pest monitoring is an important tool for a successful integrated pest management program. Blue sticky traps are commercially used for monitoring western flower thrips (WFT), *Frankliniella occidentalis* in greenhouses. However, efficacy of such blue sticky traps depends on reflection of a broad wavelengths pattern (colour) not adapted to maximum sensitivity of WFT photoreceptors. The related behavioural response (attraction) is also limited by the reflected intensity depending on the ambient light conditions. This study explored the differential attractiveness (measured by trap capture) of a spectrum of narrow-bandwidth light emitting diodes (LEDs) to WFT. Subsequently, the trapping efficacy/attractiveness of the most efficient blue LED in combination with a blue sticky trap (LED-blue sticky trap) and the addition of an attractive lure (Lurem-TR) was evaluated in flight cages under greenhouse conditions. First, using release-recapture studies we established that LEDs with peak emission of 445 nm were clearly more effective than conventional blue sticky traps. In choice experiments up to 2.7 fold and in no-choice experiment up to 2.1 fold more thrips were recaptured by the LED-blue sticky traps compared to conventional reflecting blue sticky traps. Lurem-TR improved performance of blue sticky traps and LED-blue sticky traps by a 2.3 and 2.0-fold increase of trapping efficacy, respectively as compared to those without Lurem-TR. The use of LEDs and an attractant lure is a promising approach to increase the attractiveness and specificity of visual traps, however these results should be further validated under more complex field conditions.

*Submitted to *Journal of Pest Science* as: **Otieno J.A.,** Stukenberg, N., Weller, J. and Poehling H.-M. (2016). Development and efficacy of LED enhanced blue sticky traps combined with the synthetic lure Lurem-TR for trapping of western flower thrips (*Frankliniella occidentalis*)

4.1 Introduction

Production of high-value crops is oftenly done under protected cultivation where pests and diseases play a major role in determining their performance (Raviv and Antignus, 2004). Western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is an important horticultural pest worldwide (Kirk and Terry, 2003. It affects many economically important plant species, directly by reducing yield and market quality, through feeding, oviposition damage or indirectly by transmission of virus pathogens, most notably tospoviruses (Morse and Hoddle, 2006; *Natwick et al*., 2007). Their small size and cryptic behaviour enable them to remain undetected through quarantine control measures, and their spread was mainly facilitated by increase in international plant movement (Vierbergen, 1995; Kiritani, 2001). Chemical control is not only handicapped by the rapid development of resistance to conventional chemical pesticides (Bielza *et al*., 2007; Bielza, 2008) but also by the complexity of cryptic life cycle with eggs inserted in plants epidermis, larvae hidden at the bottom of flower buds. While pupation occurs either in the soil (Berndt *et al*., 2004) or concealed flowers sites (Buitenhuis and Shipp, 2008) depending on the host plant and the prevailing environmental conditions (Steiner *et al*., 2011) resulting often in unsatisfactory efficacy of foliar treatments (Harbi *et al*., 2013). Alternative biocontrol options are most desirable and progressively being developed (Otieno *et al*., 2016) but they need to be targeted in time and space. Hence, in chemical as well as biocontrol measures timely, accurate and reliable monitoring of WFT is essential (Affandi and Emilda, 2009; Bout *et al*., 2010). Monitoring of thrips is based on visual (colours, shape, and size) or olfactory (host odour) signals which are primary cues used for host finding. As a flower feeder, the canopy dwelling WFT use colour and scent cues for detection and orientation to their host plants (de Kogel and Koschier, 2002). According to electrophysiological studies by Matteson *et al*., (1992), WFT have a di-chromatic visual system with peak sensitivity around 540 nm (green-yellow) and 365 nm (UV range). On the other hand, Natwick *et al*., (2007) reported sensitivity to blue by *F. occidentalis* and *T. tabaci* in onions when using coloured traps (reflective mode), and according to Liu and Chu, (2004) blue and white were more attractive to WFT as compared to other colours. More detailed studies on colour vision of different thrips species corroborated the high attractiveness of blue wavelength in thrips trapping (Chen *et al*., 2004; Chu *et al*., 2006; Natwick *et al*., 2007). However, Kirk, (2002) noted that blue sensitivity in WFT is based on a very specific wavelength and that most commercial blue traps do

not exploit maximum response from thrips because their relatively broad wavelength reflection is not exactly fitting with the sensitivity peak. A more precise target of WFT visual sensitivity independent of the illuminating, reflection stimulating light should be possible with LEDs as recently shown in a pilot study with whiteflies (Stukenberg *et al*., 2015). LEDs are solid state, semiconductor light sources and, when used in traps for agricultural pests, offer some advantages such as small size, low weight, high mechanical stability, low temperature sensitivity, high reliability/efficacy, long operating life time and low cost (Chen *et al*., 2004). LEDs emit narrow-bandwidth light and light intensity can be regulated and most attractive wavelengths specifically selected (Kim and Lee, 2012; Stukenberg *et al*., 2015). Because LEDs emit low thermal radiation and resulting heat is transferred from the LED chip to the heat sink on its backside, LEDs can be installed close to plant canopy (Massa *et al*., 2008). Because the LED light devices take up little space, they have long lifetime and low power consumption and they may enable pest monitoring in places where conventional light sources are impractical. Additionally, the operation with low voltage makes the technique safe for the application in greenhouses (Yeh and Chung, 2009). In case of thrips, it was shown that blue LEDs (465 nm peak emission) in combination with blue traps can enhance catches of WFT (Chen *et al*., 2004; Chu *et al*., 2006) but a detailed study for characterization of the most attractive wavelength with LED light sources has not been carried out.

With respect to research on semiochemical lures (pheromones and allelochemicals), Hamilton *et al*., (2005) showed that WFT male aggregation pheromones can increase sensitivity of traps at low level of infestation or in crops with low damage threshold. Plant derived compounds used as attractive lures (kairomones) could enable earlier and more accurate detection of WFT (Davidson *et al*., 2007). Furthermore, traps (reflective blue colour cards) have been used together with different odour lures in different crops to improve their effectiveness (Gómez *et al*., 2006; van Tol *et al*., 2007; Niassy *et al*., 2012; Harbi *et al*., 2013; Sampson and Kirk, 2013; Teulon *et al*., 2014; Muvea *et al*., 2014). A first generation commercial WFT lure is Lurem-TR, an interspecific kairomone based on methyl isonicotinate, a pyridine compound (host plant derived attractant) which has been tested for improved thrips monitoring and management (Teulon *et al*., 2007b; Nielsen *et al*., 2010). Methyl isonicotinate has been reported to stimulate walking and take-off behaviour in WFT (van Tol *et al*., 2012).

The aim of this study was to find an optimal combination of all three trap components, LED, reflective colour trap and lure. However, a major drawback was the missing detailed information on WFT spectral sensitivity, hence we first explored the most attractive wavelength by examining WFT response to a spectrum of different narrow-bandwidth LED light as compared to the conventional blue reflective traps. Then we ascertained how WFT responds to blue sticky traps and blue traps equipped with specific LEDs in combination with Lurem-TR attractant alone or in the presence of a host plant.

4.2 Material and methods

4.2.1 Host plant and thrips rearing

Western flower thrips are mostly flight active as newly hatched adults searching for feeding sites. This stage was achieved by infestation of two-week old potted bean seedlings (*Phaseolus vulgaris* L. var. 'Speedy') separately enclosed in acrylic glass tubes/microcosms (diameter 15 cm, height 40 cm) set up with WFT adults from a synchronized thrips culture. Two weeks later, the plants were cut off from the pot and as a result of positive phototaxis, emerging adults from the pupation site in the substrate were trapped using a photoeclector (see Otieno *et al*., 2016). The eclector consisted of an inverted pot (upside down) with same dimensions as those used for culturing the seedlings, provided exact fitting when placed together. A hole was drilled in the base of the upper inverted eclector pot and a 10 ml Pipette tip inserted ensuring a tight fit. A 2 ml Eppendorf tube was placed on top of the pipette tip to capture the emerging adults. For ventilation, four holes, each 2 cm in diameter were drilled on the sides of the pot and covered with thrips proof nylon screen (64 µm pore size). Adults collected from the Eppendorf tubes were used in the experiments immediately.

4.2.2 Blue sticky traps and semiochemical

Blue sticky traps (Horiver-TR® 10 x 25 cm) and a commercial semiochemical (kairomone) for thrips (Lurem-TR) were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands). The reflection spectrum of the blue sticky trap was measured with the spectrometer Lambda 900 UV/VIS/NIR (Perkin Elmer, Rodgau, Germany) containing a 30 cm integrating sphere and a tungsten-halogen and deuterium lamp. In our experiments, Lurem-TR dispensers were respectively placed on or close by a blue sticky trap, as recommended by the manufacturer. We changed

the dispensers after each experimental trial (max. 6 days) although the manufacturer recommends replacement in 42-day interval.

4.2.3 Light emitting diodes (LEDs) and trap screens

Initially, the most effective LEDs had to be selected from arange of different LED colours to study the visual attraction of *F. occidentalis*. LEDs were obtained from Roithner Laser Technik GmbH (Vienna, Austria) and Osram GmbH (Munich, Germany). Spectra of selected LEDs were measured with the spectrometer Avaspec 2048-2 (Avantes, Apeldoorn, Netherlands). Spectra and specifications are given in Table 4.1 and Fig. 4.1. The spectral range from blue to orange was investigated using standard LEDs because of their availability in various wavelength ranges, especially the green-yellow range, where high-power LEDs are missing. Standard LEDs generate only low radiant fluxes and especially for yellow a high number had to be used to obtain a sufficient intensity. The spectral range from violet to blue was investigated using high-power (HP) LEDs because they are available in the desired wavelengths and only single LEDs were used to generate even higher intensities of the respective colours, as compared to the standard LEDs. LED-panels were constructed by mounting several standard LEDs on circuit boards or single HP-LED on aluminium plates respectively. They were optionally inserted on the backside of manufactured cubic frames (10.5 x 13 x 10.8 cm) made of grey PVC (4 mm). To generate evenly LED illuminated surfaces, specific acrylic glass with light scattering properties for LED backlighting (PLEXIGLAS® LED 0M200 SC, Evonik Industries AG, Essen, Germany) was used to close the front side of the frames. The inner side of the hereby generated boxes was covered with mirror foil (PEARL GmbH, Buggingen, Germany) to achieve an even light distribution on the acrylic glass screen. The LEDs were operated with a switchable power supply (MW7H50GS, MEAN WELL, New Taipei City, Taiwan) in combination with LED drivers and PWM dimmers (Standard LEDs: Miniboost, Nano-Dim V2; HP-LEDs: LED Slave V4 PWM; PCB Components, Hildesheim, Germany). The setup allowed adjustment of individual LED intensities via rotary potentiometers. Drivers and potentiometers for each LED type (standard, HP) were placed in aluminium casings respectively and LED panels and power supplies were connected to it. To measure, adjust and equalize photon fluxes (unit µmol m^{-2} s^{-1}) of the LED screens, the LI 250 Light Meter and the LI 190 Quantum Sensor (LI-COR Biosciences GmbH, Bad Homburg, Germany) were used in darkness.

4.2.4 LED equipped blue sticky traps

For constructing LED equipped sticky traps the backside of a blue sticky trap was covered with black plastic film and a small hole (1 x 1 cm) was cut into the middle of the trap. Aluminium panels like those described above, with one blue LED respectively, were slightly curved and attached on the backside with adhesive tape so that the LED (Osram Oslon deep blue, Table 4.1) fits in the hole on the sticky trap. Up to eight LED equipped traps were used simultaneously. The traps operated with a plug in LED power supply (ELP10X1PS, Sunrise Power Transformers GmbH, Hamburg, Germany).

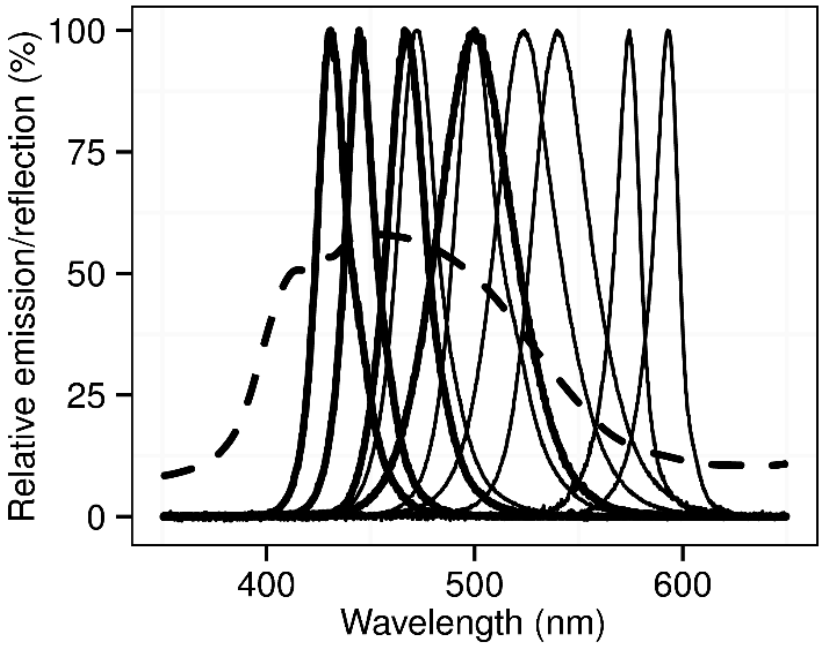

Fig. 4.1 Spectra of LEDs (solid lines: Thin = standard LEDs; Thick = high-power LEDs) and the blue trap (dashed line) used in the experiments.

Table 4.1 Specifications of used LEDs

Colour	Design	Manufacturer	Type	Peak wavelength, measured (nm)	No. of LEDs/ Panel
Violet	High-Power LEDs (5mm)	Roithner	H2A1-H435	431	1
Deep blue		Osram	Oslon SSL 80 LD CQ7P	445	1
Blue		Osram	Oslon SSL 80 LB CP7P	466	1
Cyan		Roithner	H2A1-H490	500	1
Blue	Standard LEDs (5mm)	Roithner	RLS 5B475-S	474	8
Cyan		Roithner	B5-433-B505	498	8
Green 1		Roithner	B5-433-B525	517	8
Green 2		Roithner	LED 545-01	537	16
Yellow		Roithner	LED 565-O3U	576	60
Orange		Roithner	CY5111A-WY	594	9

4.2.5 Experiments

All experiments were conducted in the central greenhouse compartment of the Institute of Horticultural Production Systems, Department Phytomedicine (Leibniz University Hannover).

4.2.5.1 Recapture choice experiments without host plants

Initially, recapture choice experiments without host plants were performed to investigate the basic attractiveness of LED colours, blue sticky traps, Lurem-TR and LED equipped blue sticky traps and the resulting preferences for *F. occidentalis*. These experiments were conducted in a cage (1 x 1 x 0.8 m, Fig. 4.2a) with thrips proof gauze on the sides and transparent foil on top.

Experiment 1

The preference for the six LED colours blue, cyan, green 1, green 2, yellow and orange was evaluated using LED trap screens equipped with standard LEDs (Table 4.1) and

equalized photon fluxes (0.38 µmol m^{-2} s^{-1} in 70 cm distance) as already described. The screens were covered with transparent plastic film which was coated with insect glue (Temmen GmbH, Hattersheim, Germany). Thrips were released in one corner of the cage and the traps were arranged 70 cm distant on the periphery of a quarter circle and at a distance of 5 cm to each other (Fig. 4.2a). LED trap screens were placed on small wooden blocks 2 cm high to ensure that thrips have to fly or to hop on the trap. The order (colours) of the traps in the arrangement was randomized in each experimental trial. In each trial 100 thrips were released and left in the cage one hour for orientation towards the trap screens. The number of thrips recaptured on the traps was counted thereafter. The experiment was replicated 14 times. Depending on the availability of thrips, 2–4 trials were conducted per day between 11:00 and 15:00.

Experiment 2

In the second experiment the attractiveness of the four LED colours violet, deep blue, blue and cyan generated by high-power LEDs (Table 4.1) was investigated with 13 replicates and the same set up and procedure. The intensities were equalized to 0.55 µmol m^{-2} s^{-1} in 70 cm distance which corresponds to the highest intensity of the cyan LED while the intensity of violet and blue LEDs had to be reduced.

Experiment 3

Here the deep blue high-power LED trap screen (Osram Oslon deep blue, 445 nm peak wavelength, Table 4.1) found to be most atrractive from the previous experiment was compared with a blue sticky trap (10 x 10 cm) attached to a non-illuminated screen device exactly fitting in size to the front panel. The traps were arranged in a distance of 30 cm to each other and the position was changed randomly over 14 replicates. The procedure was otherwise the same like in the previous experiments.

Experiment 4

In the fourth experiment four deep blue high-power LEDs with different intensities were compared with the blue sticky trap. The highest intensity corresponds to the one from the previous two experiments while the others were reduced in 25% tiers (100/75/50/25% = 0.55/0.41/0.28/0.14 µmol m^{-2} s^{-1} in 70 cm distance). The order was also changed randomly over 13 replicates.

Experiment 5

This experiment was designed to compare the effect of Lurem-TR on thrips attraction in presence and absence of a visual LED stimulus. Two trap screens with deep blue LEDs were arranged at a distance of 70 cm to each other. A dispenser of Lurem-TR was fixed on one of the traps and the position was changed randomly. The experiment consisted of two treatments. In the first treatment the LEDs were off and the only signal for orientation was the Lurem-TR. In the second treatment the LEDs were both set to their maximum intensity (0.83 µmol m^{-2} s^{-1} in 70 cm distance) for combining the visual and olfactory cue. Each treatment was replicated 12 times.

Experiment 6

The sixth experiment evaluated the preference for a deep blue LED trap screen compared to an LED equipped blue sticky trap (described above). The set up was similar to experiment 3 but traps were positioned at 70 cm distance to each other. The positions of traps were changed randomly over 10 replicates.

4.2.5.2 Choice experiment with host plant

Subsequently a choice experiment with host plants and blue sticky traps equipped with or without LED (LED-blue sticky trap, as already described) and Lurem-TR was conducted in a long tunnel-like cage measuring 2 x 0.5 x 0.5 m (Fig. 4.2b) placed on a greenhouse table. The sides were covered with thrips proof gauze and the bottom was covered with black plastic film (Phormisol R; Bonar TF, Lokeren, Belgium) to reduce light reflection. A sodium vapour lamp positioned centrally above the cage was switched on for supplementary light. A pot with three two-week old bean seedlings was placed in the middle of the cage as thrips host plants. About 100 freshly hatched adult thrips from synchronized rearing were released on the plants by hanging the Eppendorf tube on it and left to settle for one day. On the second day, both a blue sticky trap and a LED-blue sticky trap were placed at both ends of the cage (i.e. 4 traps in total) at a distance of 1 m to the host plants respectively and a distance of about 20 cm to each other (Fig. 4.2b). On one side only a Lurem-TR-dispenser was placed in between the two traps. Each experiment lasted a total of 6 days when the number of thrips on the traps was counted. Lurem-TR dispensers were changed after every experiment. The experiment was replicated 16 times. The Lurem-TR was placed on alternative sides in every experiment and the position of the traps changed respectively.

4.2.5.3 No-choice experiment with host plant

Finally, the attractiveness of the above described trap types in presence of host plants were examined in a series of no-choice experiments. The first sequence compared the blue sticky trap with and without Lurem-TR, the second one the blue sticky trap and LED-blue sticky trap and the third one LED-blue sticky trap with and without Lurem-TR. Eight thrips proof cages (1 x 0.6 x 0.6 m, Fig.4.2c) which were placed in two rows on the ground of the greenhouse were used. The supplementary light from sodium vapour lamps was on. In each cage a pot with bean seedlings was placed at one end of the box and about 60 adult thrips were released on it and left to settle for one day. A trap was positioned on the other end of the cage at a distance of 90 cm the next day. Each experiment lasted a total of 6 days when the number of thrips on the traps was counted. New Lurem-TR dispensers were used for each experiment. Because every sequence compared two trap variants and eight cages were available, only four replicates could run simultaneously. To achieve a total of 16 replicates the experiment was replicated four times, for each sequence respectively. The experiments were block randomized based on the row of cages and Lurem-TR treatments to avoid contamination by diffusion of the semiochemical.

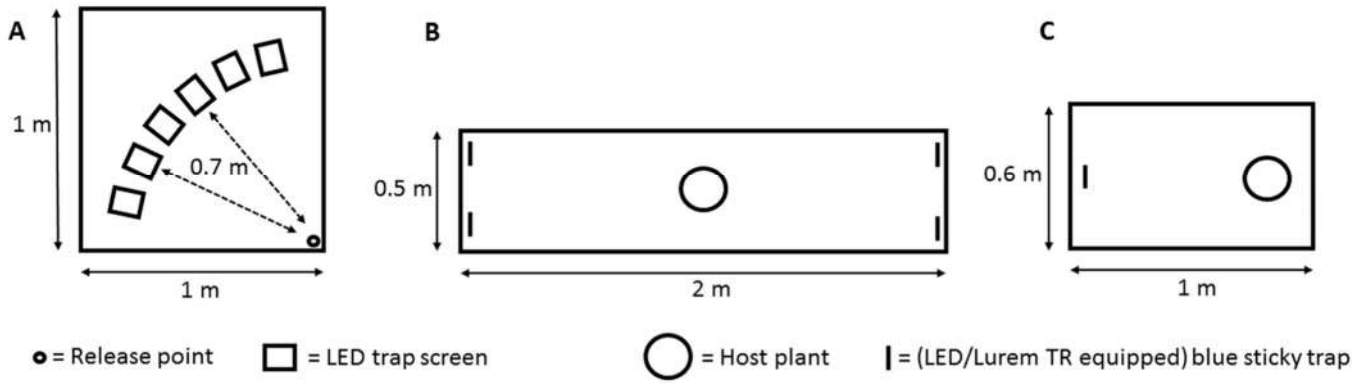

Fig. 4.2 Scheme of experimental cage setups used for the experiments. (A) recapture choice experiments; (B) choice experiment with host plant; (C) no-choice experiments with host plants in eight cages simultaneously.

4.2.6 Statistical analysis

All data were analyzed in accordance with a completely randomized and blocked experimental design using generalized linear models (GLM) with the assumption of a quasi-poisson distribution. The experimental trials were included in statistical analysis as block factors, whereas the numbers of trapped thrips were included as randomized treatment effects**.** Multiple comparisons (R-package "multcomp", Tukey or user defined contrasts) were then undertaken to examine differences between numbers of thrips trapped by each trap in an experiment (McCullagh and Nelder, 1989; Hothorn *et al*., 2008; R Core Team, 2016).

4.3 Results

In experiment 1, the blue LEDs (474 nm peak wavelength) had the highest recapture rate (60 to 75%) among standard LED equipped trap screens significantly different to the other LED colours ($p < 0.0001$). There were no significant differences between green 1 (517 nm) and green 2 (537 nm) as well as between cyan (498 nm) and yellow (576 nm). However, trap rates differed significantly between orange (594 nm) and the two green traps ($p = 0.017$ / 0.0048, Fig. 4.3). In experiment 2 investigating a narrower range of spectra, using HP-LEDs violet (431 nm), deep blue (445 nm) and blue (466 nm) we recorded similar recaptures rates between 25 to 35%. In contrast, cyan (500 nm) traps showed significantly lower recapture rates (5 to 10%, $p < 0.0001$, Fig. 4.4). In experiment 3 comparing the conventional blue sticky trap and the deep blue LED trap significant differences were recorded in recapture rates of 10 to 15% and 85 to 90%, respectively ($p < 0.0001$, Fig. 4.5). When offering an intensity (brightness) gradient with blue LEDs (experiment 4), trapping rates increased in relation to increasing intensity but only when intensity was reduced to 25%, a significant difference to the highest intensities (75% and 100%, $p = 0.0007$ / 0.0003) occurred. At this intensity the trapping rate was equal to the blue sticky trap (Fig. 4.6).

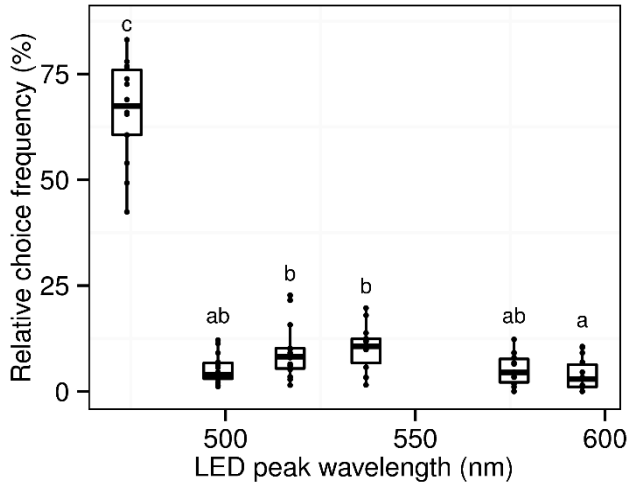

Fig. 4.3 Data points with box plot (median, inter, upper; lower quartiles) showing relative frequencies of western flower thrips catches in multiple choice experiment with various LED colours (standard LEDs): blue (474 nm), Cyan (498 nm), green 1 (517 nm), green 2 (537 nm), yellow (576 nm) and orange (594 nm). Different letters indicate significant differences (GLM, Tukey test, α = 5%).

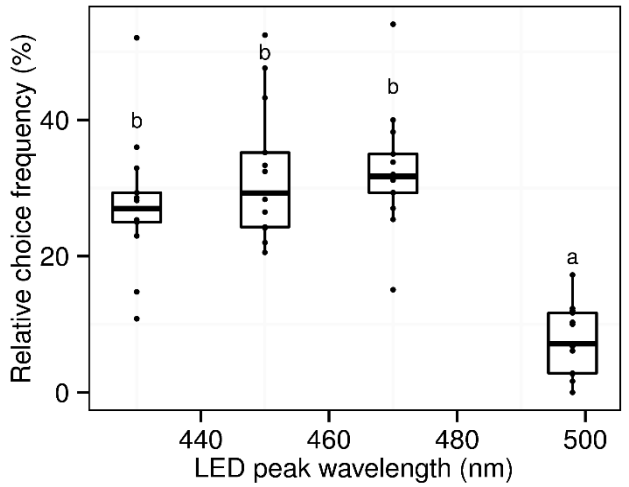

Fig. 4.4 Data points with box plot (median, inter, upper; lower quartiles) showing relative frequencies of western flower thrips catches in multiple choice experiment with different high-power LEDs in the violet-blue range: violet (431 nm), deep blue (445 nm), blue (466 nm), cyan (500 nm). Different letters indicate significant differences (GLM, Tukey test, α = 5%).

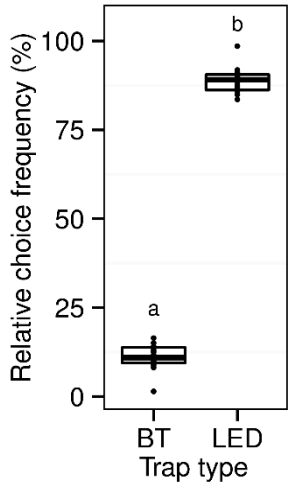

Fig. 4.5 Data points with box plot (median, inter, upper; lower quartiles) showing relative frequencies of western flower thrips catches in choice experiment comparing blue sticky trap (BT) and blue LED (deep blue 445 nm). Different letters indicate significant differences (GLM, Tukey test, α = 5%).

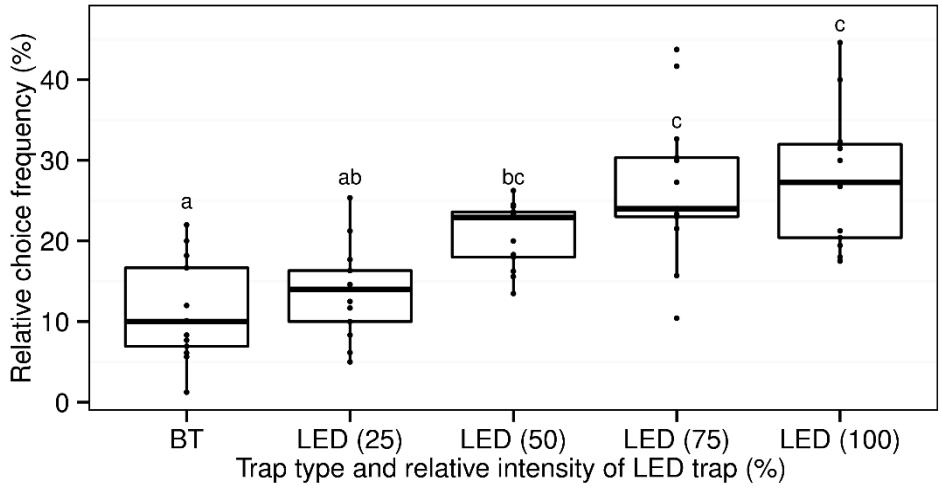

Fig. 4.6 Data points with box plot (median, inter, upper; lower quartiles) showing relative frequencies of western flower thrips catches in choice experiment comparing blue sticky trap (BT) and blue LED (deep blue 445 nm) at different relative intensities (25%, 50%, 75% and 100%). Different letters indicate significant differences (GLM, Tukey test, α = 5%).

When the volatile attractant was added, Lurem-TR increased the number of thrips recaptured on the trap screens when the lights were off (experiment 5), although there were no statistical differences (p = 0.361). The combined effect of the visual LED stimulus (both LED lights on) and Lurem-TR resulted into a significant increase in the

number of thrips recaptured on the blue LED trap combined with the attractant (p < 0.0001). The total number of recaptured thrips was also significantly increased when the LEDs were on (p < 0.0001, Fig. 4.7). The blue sticky traps equipped with a deep blue LED recorded no significant difference to the blue LED trap screen (experiment 6), with slightly more thrips recaptured in the former (p = 0.167, Fig. 4.8). Therefore, consecutive experiments only used LED equipped blue sticky traps.

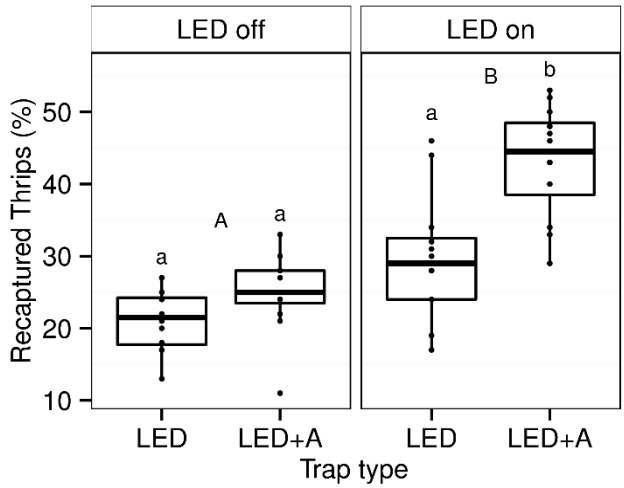

Fig. 4.7 Data points with box plot (median, inter, upper; lower quartiles) showing recaptured western flower thrips in choice experiment with blue LED (deep blue 445 nm) with (LED+A) or without Lurem-TR (LED) when the LED lights were switched either on or off. Different letters indicate significant differences within each panel (small letters) and between the panels (capital letters) (GLM, user defined comparisons, α = 5%).

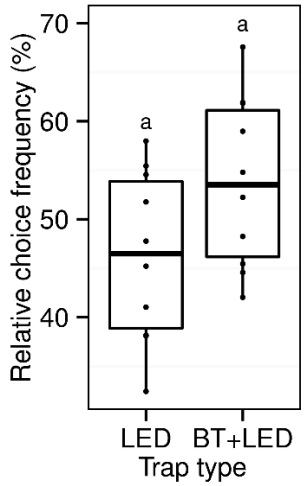

Fig. 4.8 Data points with box plot (median, inter, upper; lower quartiles) showing relative frequencies of western flower thrips catches in blue LED trap screen as compared to blue sticky traps equipped with LED (BT+LED). Different letters indicate significant differences (GLM, Tukey test, α = 5%).

In the six-day choice experiment with host plants, significantly more thrips (2.5-fold) were trapped on the side of the flight cage with Lurem-TR than the side without the attractant ($p < 0.0001$). On both sides, the LED-blue sticky traps recaptured more thrips than the blue sticky traps, 2.7 and 2.04 fold in both Lurem-TR minus and plus, respectively ($p < 0.0001$). However, there was no significant difference between thrips caught on blue sticky traps plus attractant and LED-blue sticky traps ($p = 0.908$). The recapture rate was 5 and 12%; 13 and 27% on blue sticky trap and LED-blue sticky trap for Lurem-TR minus and plus respectively (Fig. 4.9).

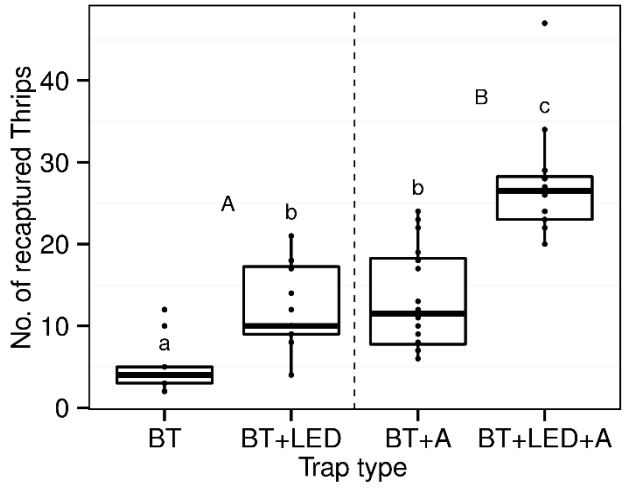

Fig. 4.9 Data points with box plot (median, inter, upper; lower quartiles) showing number of recaptured western flower thrips in choice-experiment with host plants. One side composed of blue sticky traps (BT) and LED-blue sticky trap (BT+LED). The other side was equipped with Lurem-TR and the same trap types (BT+A; BT+LED+A). The two sides are separated by a dotted line. Different letters indicate significant differences overall (small letters), and between the two sides (capital letters) (GLM, user defined comparisons, α = 5%).

Lurem-TR and LED consistently enhanced the efficacy of the blue sticky traps in the no-choice experiments with the host plant. First, when comparing blue sticky traps with and without addition of Lurem-TR, the performance of traps was significantly increased (2.3-fold) by the lure with recapture rates of 11% and 25% in blue sticky trap alone and with attractant respectively ($p < 0.0001$, Fig. 4.10a). Moreover, when blue sticky traps were equipped with an LED, 2.07-fold more thrips were caught on the LED-blue sticky trap compared to blue sticky trap alone ($p < 0.0001$). The recapture rate was 17.8% and 36.9% on the blue sticky trap and LED-blue sticky trap respectively (Fig. 4.10b). Finally LED-blue sticky traps plus Lurem-TR further increased recapture rates up to 75% as compared to LED-blue sticky trap without Lurem-TR with 37.5%, recaptures ($p < 0.0001$, Fig. 4.10c).

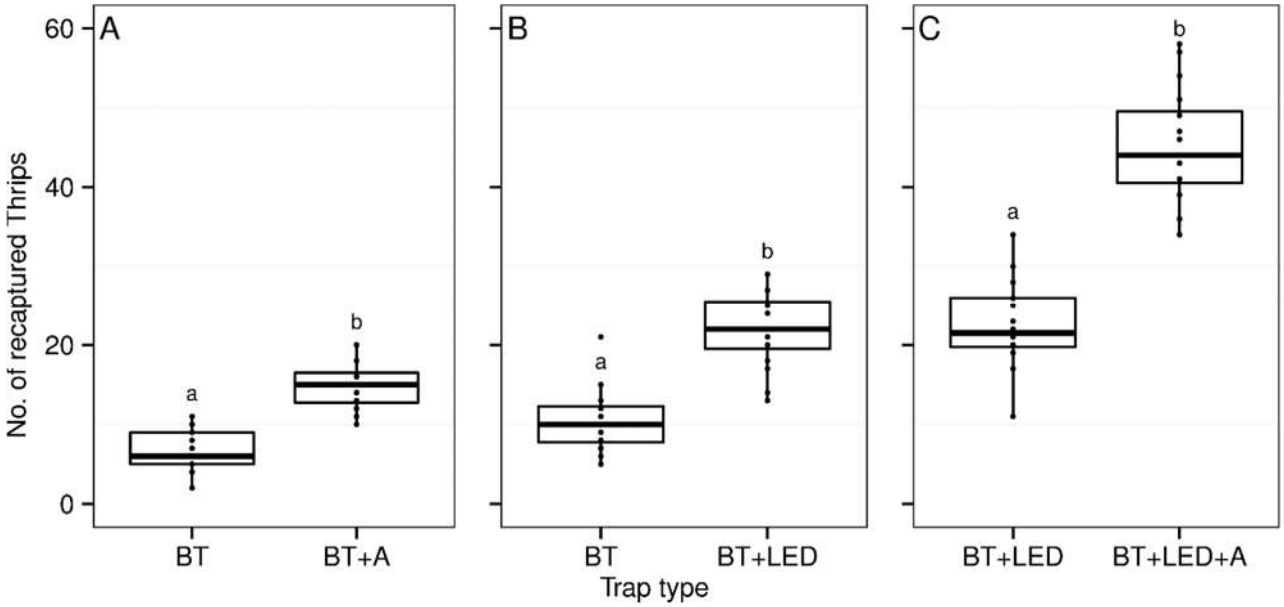

Fig. 4.10 Data points with box plot (median, inter, upper; lower quartiles) showing number of recaptured western flower thrips catches in no-choice experiments with host plants. The first (A) sequence compared the blue sticky trap alone (BT) and blue sticky traps with Lurem-TR (BT+A), the second (B) blue sticky trap (BT) and LED-blue sticky trap (BT+LED) and the third (C) LED-blue trap alone (BT+LED) and LED-blue trap with Lurem-TR (BT+LED+A). Different letters indicate significant differences (GLM, Tukey test, α = 5%).

4.4 Discussion

In experiment 1, more thrips were attracted to LED blue (474 nm peak wavelength) as compared to other LED colours (Fig. 4.3). This confirms findings by Chen *et al*., (2004), Chu *et al*., (2006) and Natwick *et al*., (2007) that thrips are attracted to blue wavelengths. However, when investigating LED lights from a narrower spectrum the LED deep blue (445 nm) was slightly more attractive but not significantly different from LED violet (431 nm) and the longer wavelength LED blue (466 nm) (Fig.4.4). Hence, the attraction to the blue wavelength is not very specific in terms of bandwidth range. It can be concluded that any blue LED between 430 and 480 nm wavelengths would give comparative performance for LED based traps. However, regarding the blue range, other studies reported that blue/violet wavelengths (380-450 nm) could be less attractive to WFT as compared to green-yellow. But these studies were of completely different design with flowers as coloured targets, reflecting rather broad spectra of wavelength (Blumthal *et al*., 2005)**.** We observed only low response to green-yellow. But we can't ascertain that green-yellow is totally unattractive due to the multiple choice set up, which only proved that blue was relatively more attractive. Moreover, there is an indication that the green-yellow range represents a second attractive domain, inferior to the blue one (Fig. 4.3). Our findings correspond with the notion that blue sticky traps have been proven as a rapid and cost effective tool for monitoring of thrips population (Liu and Chu, 2004; Chu *et al*., 2006) with specific examples in broccoli (Chen *et al*., 2004), head lettuce and onions (Natwick *et al*., 2007).

Attraction of WFT to the blue LEDs was clearly related to the brightness (intensity) of the LED (Fig. 4.6). In general light intensity dependent reactions from herbivore insects are described by Döring and Chittka, (2007) ; Johansen *et al*., (2011). Liang *et al*., (2010) reported that flight activity of thrips is regulated by both temperature and light intensity. Matteson and Terry, (1992) also asserted that WFT attraction to blue sticky traps was correlated with light intensity.

The relation between wavelength and brightness is a key factor for determining colour sensitivity of herbivorous insects. Responses to different wavelength and interactions of wavelength and brightness can be explained with the underlying physiological response of the thrips' photoreceptors. A photoreceptor acts as

a photon counter and the wavelength dependence is based only on its sensitivity (Döring and Chittka, 2007). At an equal wavelength, as in our experiment, higher number of photons received in the same sensitivity range of the receptor will increase the physiological response. However, when considering different wavelength or broad wavelength spectra, a bright light with high number of photons at a wavelength far from the sensitivity peak may even cause the same physiological response in the photoreceptor cells as a dim light at the peak sensitivity. Accordingly, if two light sources (reflected photons from target or emitted from light source) have the same wavelength but different intensities, the brighter target elicits a higher behavioural response and is therefore preferred (Döring and Chittka, 2007). In particular, the intensity and wavelength of reflective targets depends also on the quality of illuminating light which can lead to variable observations. For example, fluorescent lamps which provide more irradiance in green-yellow wavelengths (500-600 nm) than violet-blue wavelength (350-450 nm) could make green-yellow appear much brighter than the shorter wavelength pattern (Blumthal *et al*., 2005). Regarding the relatively broad sensitivity range of a speculative “blue” receptor of WFT and the broad reflective wavelength range of “yellow” appearing targets of high brightness can result in higher attractiveness than low brightness blue to WFT. This has led to the erroneous conclusion that other colours apart from blue are more attractive to thrips (Kirk, 2002).

In experiment 5, when LED lights were switched off in LED vs LED plus Lurem-TR, thrips (though comparatively low numbers) were still recaptured on the whitish light scattering trap screen surface. Other researchers have reported the comparative attractiveness of white and yellow surfaces to WFT (Liu and Chu, 2004; Johansen *et al*., 2011; Li *et al*., 2011) and other thrips species (Roditakis *et al*., 2001). However, these studies did not provide spectral qualities of the coloured surfaces and it can be assumed that the explanation for that phenomenon equals the above given discussion of receptor sensitivity broadness and sensitivity vs brightness relation.

What is the most efficient trap? Regarding the visual orientation of WFT our results clearly show that a blue sticky trap equipped with a high-power (HP) LED with appropriate wavelength (445 nm peak wavelength) and a sufficient intensity is the most reasonable solution for application in thrips monitoring. Similar findings were reported by Chu *et al*., (2005), where blue traps equipped with a single standard LED were more

attractive to thrips as compared to blue LEDs. Because we used high-power LEDs which provide a much higher intensity than standard LEDs, it is very likely that these high power LED traps could perform much better under conditions in greenhouse crops, based on our observations on light intensity and trapping efficacy (Fig. 4.6). Additional LEDs may need to be added under conditions with competitively high global radiation for instance on sunny days.

Adding Lurem-TR resulted in more than two-fold-increase in WFT catches both on the blue sticky trap and LED-blue sticky trap. The improvement of trapping efficacy by this specific lure has been described from several other studies: Broughton and Harrison, (2012) observed a threefold increase in capture of WFT with Lurem-TR in western Australia. Harbi *et al*., (2013) in Tunisia caught more than twice as many WFT in pepper using blue sticky traps with Lurem-TR. Other researchers reported improved catches in sticky traps using Lurem-TR (Teulon *et al*., 2007b; Till *et al*., 2009; Niassy *et al*., 2012a; Muvea *et al*., 2014; Teulon *et al*., 2014). Using blue sticky traps with Lurem-TR or LED-blue sticky traps alone could also be a viable and improved alternative for conventional blue sticky traps. This was clearly demonstrated by the results (Fig. 4.9) and inference could also be made to Fig. 4.10, though they were not directly compared.

However, we observed the most distinct improvement with Lurem-TR amendment when using newly hatched thrips adults and in the experimental design without host plants. The method of rearing thrips in this study, enhanced their motivation to fly/disperse. Liang *et al*., (2010) reported that thrips flight activity is mainly regulated by their physiological circadian rhythm such as photoperiod reaction and starvation time. External factors like light intensity, relative humidity and temperature helps to provoke the flight motivation (Liang *et al*., 2010). The adults used here emerged from the soil and were collected immediately but after starving for at most 24 hours. According to Davidson *et al*., (2006) and Liang *et al*., (2010) hungry thrips (starved between 4h and 24h) responded more to odours and showed increased flight activity than non-starved thrips. As a consequence, lures may be more effective against invading thrips than those resident within the crop (Davidson *et al*., 2006; Davidson and Butler, 2009). Indeed, we found that comparatively lower numbers of thrips dispersed when the host plant was introduced in to the experiments. Usually, when

thrips are released from a point source their first reaction is to feed (Rhainds and Shipp, 2004) and since they are thigmotactic and cryptic, the addition of host plants could reduce dispersal motivation, given that dispersal activity of thrips is normally slow as compared to other insects (Rhainds and Shipp, 2004). The impact of the attractant was reduced in experiments that included the host plant and only 2.3 and 2.0-fold increase (no choice) and 2.7 and 2.1 fold increases (choice) both with blue sticky trap and blue sticky trap-LED could be achieved. Thrips lure is known to be more effective when used for trapping thrips in areas with non-host as compared to those with host plants (Davidson *et al*., 2009). In at least one other insect host plant attractive cues like colour and other volatiles may affect the effectiveness of the lure (Blackmer and Cañas, 2005). Final host discrimination is obviously a complex reaction to colours and olfactory cues, which may also explain why polyphagous thrips like the western flower thrips show varying colour preferences in different environments (Hoddle *et al*., 2002).

An important issue in this small scale experiments, was the possibility of interference of traps with and without luring because of the volatility of the lure. Odour-baited traps may increase the efficacy of nearby unbaited-traps. This interference is known to affect even outdoor traps placed 10 m apart according to Teulon *et al*., (2007a; 2014). This effect is expected to be worse in the greenhouses like in this case where the enclosed structure with relatively limited air circulation would inhibit dissipation of odour into the wider environment (Teulon *et al*., 2014). Therefore, optimal lure spacing is important to maximise thrips management.

Nevertheless, the highest recaptured rate (75%) with host plants was recorded when both LED-blue sticky trap and Lurem-TR were used together. Visual cues are stable, omni-directional and are not influenced by wind. They are more important in long distance host finding, while olfactory cues are important at close range (Klowden, 2007). The use of the attractant was the key to achieve high thrips recapture rates considering spatial conditions. This was demonstrated in choice experiment with host plants, where the thrips used the attractant as the key determinant to decide on which side of the flight tunnel to move, before being attracted to the light traps with 2.5 fold more thrips to the side with attractant. Chu *et al*., (2005) reported that WFT response to light occurs over short distance although this could change with bigger visual traps,

Natwick *et al*., (2007) noted that the larger the visual trap the smaller the effect of odour.

The special “take off motivation” by the lure might especially become important in real crop stands where thrips live in cryptic areas. Once they get into the dispersal mode the long distant visual attraction to (LED) traps comes into play. This increased activity could also be utilized to improve efficacy of insecticides or biopesticides by exposing a greater number of thrips to spray application (van Tol *et al*., 2012).

Use of flight chambers to study insect behaviour has limitations. The insects are not exposed to natural environment with various light signals and intensities besides other olfactory and climatic factors. It also ignores food reward with host plants which may arrest thrips despite the presence of an attractant (van Tol *et al*., 2012). In this case, more reliable results with LEDs were recorded in autumn than during summer. Most likely the relatively low ambient light conditions in autumn and winter enhances the effect from the LEDs better as compared to bright conditions in summer when LEDs might be barely noticeable for thrips.

4.5 Conclusions

The LED light sources have proved to be superior to the conventional blue sticky traps and are therefore well suited as optical traps for monitoring WFT. Similar findings for whiteflies were reported by Stukenberg *et al*., (2015). Adding narrow-bandwidth LED light to the blue sticky traps greatly enhanced their performance for trapping thrips. These LED based sticky traps have been used with trap plants to increase the efficacy of thrips control (Shipp *et al*., 2011). However, the attractiveness of blue LEDs is intensity dependent, at low intensities the number of thrips recaptured were comparable to blue traps.

Based on these results, Lurem-TR improved trapping efficiency of WFT. This confirmed findings by Abdullah *et al*., (2015) that adding an attractant to sticky cards can increase their attractiveness and sensitivity leading to a more efficient monitoring and evaluation of the efficacy of control strategies. Lurem-TR is also known to be volatile, chemically stable and a relatively low toxicity to natural enemies. The attractant can enhance other control methods of WFT such as lure and kill, mass trapping and push and pull (Niassy *et al*., 2012a; Sampson and Kirk, 2013). The results obtained here should be further validated under field condition with ambient abiotic conditions, because the performance of LED-blue sticky trap can be affected in particular by natural sunlight properties. In addition, the use of Lurem-TR in the field would reduce the possible contamination effect to the control treatments which could not be avoided in the greenhouse set up with limited air circulation. Although the present study has demonstrated that WFT has wavelength-specific behavioural responses to blue, this does not clearly prove that it has capabilities to discriminate colours, which would include opponent processing at a neural stage. More reliable behavioural or physiological evidence is therefore needed to draw a comprehensive picture of the spectral sensitivity and related behavioural responses in WFT.

5.0 General discussion

Western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most important pests of ornamentals and vegetables that causes extensive economic losses in greenhouse and open-field plant production (Reitz *et al*., 2011). It's control is difficult because it is a polyphagous pest with a wide host range (Morse and Hoddle, 2006). The WFT are small and have an affinity to cryptic feeding sites like flower buds, high reproduction rate leading to overlapping generations in a crop and ability to pupate in the soil (Berndt *et al*., 2004). It is also the key vector of several destructive plant viruses from the genus Tospovirus (Bunyaviridae) (Webster *et al*., 2011). The difficulties in using conventional insecticides to control WFT (Reitz et al., 2011) has led to growing interest in novel and effective integrated pest management alternatives (Funderburk, 2009). The success of biorational insecticides does not depend only on efficacy against the target pest, but also on low virulence against non-target organisms especially the beneficials. This search for environmentally friendly and sustainable control methods has given more attention to biocontrol in the recent past.

The adoption of biocontrol agents as a pest control strategy is handicapped by the rudimentary knowledge on precise timing and the appropriate numbers of biocontrol agents to be released for effective pest control (Hoodle *et al*., 2004). This can only be achieved by strict-regular monitoring and evaluation of the target pest population. In addition, the cryptic nature of thrips, with eggs inserted in epidermal cells, larvae hidden in flower buds and pupae in the ground implies that they are consequently unaffected by foliar treatments (Harbi *et al*., 2013). Hence timely, accurate and reliable monitoring of WFT is an essential component of IPM to prevent economic damage (Bout *et al*., 2010). Most control strategies have targeted the canopy life stages (adult and larvae), while the soil-dwelling pupal stages have been ignored yet they act as reservoirs for re-infestation (Ansari *et al*., 2008a). Moreover, given the amount of time spent in the soil, these stages make ideal targets for soil-dwelling antagonists or entomopathogens, preferably when they are used as part of an IPM strategy. Most high valued crops in horticulture also have low damage thresholds and there is no single biocontrol method that can effectively control WFT. Therefore, the use of a suite of natural enemies, entomopathogens as well as selective application of a low-risk pesticide seems to offer a sustainable control. Interactions resulting from combined

use of biocontrol agents could range from antagonism to additivity or synergy depending on the specific combination of control agents, target pest, rates and timing of application. Positive interaction (additivity or synergy) may improve the efficacy and reliability of a single control strategy. This thesis reported two studies aimed at improving thrips control using the biorationals (predators, entomopathogens and botanical), and an approach evaluating the suitability of LED-blue sticky traps baited with Lurem-TR for improving thrips monitoring which is necessary for the success of an IPM program.

The initial study aimed at the combined effect of soil-applied azadirachtin with entomopathogens for integrated management of the soil-dwelling stages of WFT. NeemAzal-T was the most efficient in controlling WFT, however, this was surpassed when secondary mortality due to mycosis (EPF) was considered. Eight combinations resulted into positive interactions (synergism and additive) and one resulted into antagonism. The dose response study proved that combining low concentration of EPN with NeemAzal-T or vice versa could be equally effective or even better than the full recommended dose.

Soil application allowed optimal performance for the neem formulations since foliar application has the drawback of rapid degradation by UV radiation besides possible side effect to the canopy dwelling non-target organisms (Kumar and Poehling, 2006). Despite having the same active ingredient and concentration, NeemAzal-T solution outperformed the Neem pellet formulation. Possibly, the water-based (NeemAzal-T solution) could release high concentration of AZA in the soil for an acute and effective control of WFT unlike the slow release formulation (Neem pellet). In a different study set up, the systemic effect of these two formulations were assessed in controlling two whiteflies species by Karanja *et al*., (2015). Neem pellet was shown to be more persistent in the soil. It could provide a long-term and more efficient plant protection property than the NeemAzal-T solution. While, NeemAzal-T provided high concentration of AZA initially producing higher efficacy but because it is prone to leaching effect, this reduced the availability of AZA for plant uptake. This direct effect against the soil dwelling thrips stages provided by NeemAzal-T was more relevant in our study. May be the use of more irrigation water could increase the amount of AZA

released by the pellet formulation into the soil for an acute/fast control of WFT population.

The EPNs are known to actively search for their hosts and kill them, and after infection thousands of nematodes can be produced from a single infected insect host. They have both high reproduction potential as well as self-sustaining system after the first introduction (Kaya and Gaugler, 1993). In this study the use of EPN did not result into satisfactory control. The species *S*. *carpocapsae* is an ambush forager and can successfully control fast moving surface adapted pests while cruisers like *Heterorhabditis spp*. can infect less mobile or even sedentary hosts living deep in the soil and the intermediate foragers like S. *feltiae* combines both ambush and cruiser foraging ability to find their hosts. May be any other species with cruising foraging ability could have given a better performance than *S*. *carpocapsae*, for example, according to Premachandra *et al*., (2003b), *S*. *feltiae* proved more virulent than *S*. *carpocapsae*.

In addition, the nematodes are also not effective in controlling the foliage-dwelling stages unless specifically formulated for this purpose, this is due to their sensitivity to desiccation and UV radiation and therefore soil application targeting the soil stages of WFT was the best option. Because the majority of the thrips are on the foliage, effective control of thrips populations with these nematodes alone is rarely achievable, the high dose rates of EPNs needed for sufficient control (more than 80%) of the soil-dwelling stages of WFT are not economical (Premachandra *et al*., 2003a). Though some success against certain above-ground targets have been achieved, currently research is targeting improved formulation of the EPN (Shapiro-Ilan *et al*., 2010). For example, Mureithi, (unpublished results) used Chitosan to enhance and stabilize the efficacy of *S*. *carpocapsae* in low moisture and high temperature conditions. More emphasis is also laid on combined use of EPNs with other control agents since they are compatible and may be tank mixed with most chemical herbicides, fungicides and insecticides including bacterial and fungal products (Koppenhöfer and Grawal, 2005).

The EPN has the free-living infective juveniles (IJs) which actively pursue their host and once they gain entry through the natural openings, they kill their hosts with the aid of the symbiotic bacteria residing in their gut, killing the host within 24-48 hours. The

conidia from the EPF infect the host via the cuticle as the host moves over the treated soil. The conidia stick to the cuticle, germinate, penetrate into the body wall of the insect, multiply and cause the host to stop feeding and eventually dies within a few days after infection. While azadirachtin has multiple effects on the pest, it is an ecdysone disruptor/chitin synthesis inhibitor in addition to alteration of juvenile hormone titre. This could prolong moulting or alternatively it could act as physiological stressor (Schmutterer, 1997). Using these control agents in combinations resulted in higher efficacies in controlling WFT. Possibly, azadirachtin reduced thrips defence potentially making them an easier target to the nematodes or alternatively, gave more time for the fungal conidia to penetrate through the insect cuticle or both mechanisms could be responsible for these results (Akbar *et al*., 2005). On the other hand, thrips already infested by the quick-acting nematodes are less mobile because of the activity of the symbiotic bacteria and therefore more susceptible to the fungi and likewise, thrips infected by the fungi should be a weak and easy target for the nematodes thus explaining synergism.

More thrips cadavers showed mycosis in the EPF-based combinations which had lower conidia concentrations than single EPF treatment. Possibly, the migrating larvae might have acquired relatively few conidia but since mortality is dose dependent, infection took long to develop, allowing many to survive to adult stage but later succumbed (Ansari *et al*., 2008a). This showed that secondary late-mortality by mycosis is a major contributing factor to the success of EPF-based treatments and full efficacy of the EPF should be inclusive of mortalities due to mycosis. However, growers may not agree with such a slow kind of reaction since mostly a fast action on an already increasing pest population is expected. Indeed there remains a certain risk that the pests could still cause some economic damage to the crop between the time of infection by the EPF and when it finally sucumb to death due to mycosis, even though the EPF have sub-lethal effects on the pest that reduces its impact on the crop (Ansari *et al*., 2010).

This study was carried out in controlled climate rooms and closed microcosm with suitable optimal conditions for the performance of the various biocontrol components, this is difficult to attain in a natural set-up. For example, the EPF needs high relative humidity to germinate, shading from UV radiation which affects their viability and

optimal temperature of 23-28°C (*B*. *bassiana*) (Zimmermann, 2007a) and 15-35°C (*M*. *anisopliae*) (Zimmermann, 2007b). While *S*. *carpocapsae* are also sensitive to desiccation and UV radiation, optimal temperature (22-28°C) besides other soil factors like pH (4-8) and soil texture (sandy soil) are favourable. There is need for further validation of these results in a more practical setting before fully implementing the system.

There was need for economic valuation of the biocontrol components used. However, this was not possible because some products were still on trial for example, the NeemAzal-T solution and Pellets, also the *M*. *anisopliae* IPP-2539. While *M*. *anisopliae* ICIPE-69 is marketed as Campaign® by Real-IPM-Kenya, the samples used here were obtained directly from the cultures at the ICIPE's Arthropod Germplasm Centre. It was not possible to get their market prices. Combined use of biocontrol agents may not be a common practice with growers. Their use may be influenced by the type of crop (vegetable or flowers), economic threshold, susceptibility to tospoviral infection and the target market for the products. Growers producing vegetables like cucumber, beans, tomatoes for local markets which only look at yield may find this practice unreasonable. Though thrips damage will lower the quality of any produce, hence low returns for the producers (see Fig. 1.4). However, both fresh vegetable export growers and the wider ornamental growers with flowers with very low damage thresholds and strict market prohibitions on WFT transfer with produces to countries where it is a quarantine pest may find this the only option. This is necessitated by the demand for pesticide-free and sustainable production of these crops by the EU market. Additionally, growers with crops which are highly susceptible to viral infection will use integrated approach to secure their crops against diseases. This high efficacy obtained by these combinations would be a viable option.

Generally, the use of biocontrol combinations could be expensive at the face value but in the long run, it has proved to be more cost-effective and sustainable than their chemical counter parts. This is because once they are established, the system is self-sustaining making it cheap in the long-run. Because they are safe to the users and environmentally friendly, biocontrol products are widely acceptable and highly priced by the consumers through fair trade labels. The nematodes reproduce in large numbers and are therefore self-regulating after the initial introduction. The fungal conidia can also remain viable for a long time in the soil. *Metarhizium anisopliae* was

reported to be more persistent in the soil (up to 3.5 years) than *B. bassiana* (survived for less than 1 year) (Vänninen *et al*., 2000).

Comparatively, it's expensive to develop new pesticides due to fast resistance development because of intensive management in greenhouses which results from high selection pressure. Cost/benefit analysis also showed that biocontrol is the most cost effective method in the long run, (biocontrol 1:30 and chemical control 1:5). Though biocontrol researchers deal with a more complex ecological variability than chemical researchers. Cost of development of a natural enemy was estimated at 2 million US dollars and chemical pesticides at 50 US million dollars (van Lenteren, 1993). Another important fact is that, pesticides control large number of pests, have large markets and therefore justifies spending on research, development and registration because it can be paid by the potential income. Since most biological control agents suffer from the very specificity that makes it attractive. As a consequence, they have narrow spectrum of application that better fits the need for environmental protection unfortunately, this has smaller market and is less able to support research, development and registration cost (Fallis, 2013). In addition, for the microorganisms/biorationals there is lack of clear guidelines on data requirements for registration between countries. There is need for regulatory urgencies for different countries to publish special guidelines for registration in their countries and a further move to harmonize these standards to help in faster registration of these products within different continents. Otherwise unlike their chemical counter parts, biorationals deliver value in a way that is consistent with the evolving consumer preference, environmental stewardship and short and long term public policy (Fallis, 2013).

The use of entomopathogens for crop protection is still not widely adopted because it is expensive to produce them in large quantities for commercial use. Again a purely biological control system lacks the power of immediate intervention that saves the crop. They also have short shelf life, and the pests must be present before the pathogens can be usefully applied thus making preventative treatment difficult. In the recent past, cheaper and cost effective methods of producing entomopathogens has been developed. Current research is focused on improving their quality-formulation and performance to make them more cost-competitive with chemical pesticides (Lacey *et al*., 2001). Today's formulations are stable and easily mixed for spay application using conventional spay equipment. Replacing the use of harmful pesticides with "soft"

botanicals like azadirachtin (water based formulation) will ensure their optimal performance. Soil application of the entomopathogens will also reduce their risk of being killed by the pesticides as compared to when they are applied in the canopy.

The second study focused on the effect of combined release of the predator *Orius laevigatus* and the "soil package" (soil-applied entomopathogens and NeemAzal-T) for controlling western flower thrips. The first study only targeted the soil-stages of WFT, meaning the population of the foliage stages developed unchecked and could be a constant source of pest re-infestation in an open field. This could be made worse by possible survivors from the soil treatments and immigrating thrips. There was need to introduce the foliage-dwelling predator in addition. Although, *O. laevigatus* alone was also handicapped because it fails to forage in the soil hence combining both "soil package" above and foliage-dwelling predator was the most appropriate approach. Efficacy against WFT was greatly improved in the combinations as compared to single treatments. *Orius*-based combinations performed better than their non *Orius*-based counter parts. All the ten treatment combinations resulted in complementary interactions (additive and synergism) thus covering gaps of efficacy and improving the reliability of single agent treatments. Generally, the above discussed successful combination of two or more entomopathogens to suppress pest populations was further enhanced by combined use of a foliar predator which attacked the foliar stages of the pest for a holistic integrated biocontrol of WFT. This multiple approach increased pressure on the pest population given that they have different modes of action hence lower chances of competition.

Neem formulations have been used in combination with EPNs (Krishnayya and Grewal, 2002; Meyer *et al*., 2012). This combined efficacy benefited from synergistic interaction with *B. bassiana* (Mohan *et al*. 2007, Islam *et al*., 2010) and *S. carpocapsae (*Mahmoud, 2007). Neem has also been used with *A. cucumeris* and *H. aculeifer* (Thoeming, 2005; Thoeming and Poehling, 2006), *E. warrae* (Kumar *et al*., 2010). However, there are contrasting reports concerning the compatibility of neem and *O. laevigatus*. Angeli *et al*., (2005) reported that neem had no effect on
O. laevigatus exposed via direct contact or by ingestion of infected eggs of *Ephestia kuehniella* (Zeller) additionally, Biondi *et al*., (2012) stated that neem is a moulting disruptor and hence has no effect on adult predators. In contrast Bonsignore and Vacante, (2012) and Tedeschi *et al*., (2001) reported negative effects of neem on *O.*

laevigatus and *Macrolophus caliginosus* respectively. It should be noted that the formulations used in these two studies were oil formulations of azadirachtin (NeemAzal-TS). In our study, water-based azadirachtin formulation was used and there was spatial separation between Neem (soil-applied) and *O*. *laevigatus* (foliage-applied). Therefore, a detailed study to ascertain residual toxicity of different formulations of azadirachtin, their effect on fitness or oviposition of *Orius* should be carried out.

Release density is critical in optimizing efficacy and balancing cost of production. As single treatments, two *Orius* performed better than introducing one predator. However, when combined with other biocontrol agents, reducing the density of *Orius* did not impair its efficacy. The use of combinations allowed reduced application rates even for the soil treatments (azadirachtin and the entomopathogens). For up-scaling of these results it can be concluded that in addition to the possible reduction of predator mass release (economic aspect) fluctuations in predator: prey relations which will normally naturally occur across crop stand will be “buffered” by the additional treatments.

More crucial was timing to attack the most susceptible stage of the target pest. The optimal timing for the soil treatments was before L2 descends to pupate (Premachandra *et al*., 2003b). Although *Orius* can feed on all life stages of thrips, but it consumes higher numbers of the younger stages (eggs and L1) than on the later stages (L2 and adults) (Elimem and Chermiti, 2012), introducing *Orius* to target L1 was more successful than targeting L2. However, in a natural crop stand such an exact timing is difficult to achieve, in particular when the thrips population is already established and the pests occur with all different life stages at the same time. This confirms that early release of predators as soon as possible after immigration (see monitoring below) plays an important role in the success of biocontrol (Cloyd, 2009).
In addition, experiments with variable thrips densities were not performed. It could be speculated that may be *Orius* killing capacity was not reached in our microcosm experiments. Although, these results cannot be ignored given that thrips hatching from the control treatment had an average of up to 130 adults, this number was sufficiently high for only two *Orius*. In another study, bi-weekly introduction of 10 adults of *O. insidiosus* per plant with 92-103 thrips failed to reduce thrips on tomatoes to economically acceptable levels (Ship and Wang, 2003).

The generalist omnivore *Orius* can survive and reproduce in crop habitats in absence of thrips by feeding on other pests like whiteflies or aphids besides feeding on alternative foods or exhibit prey switching, this enables it to establish well ahead of the cropping season and prevent subsequent rapid build-up of the pest population (Coll and Guershon, 2002). This is a desirable trait for biocontrol early in the growing season. On the other hand, this phenomenon can hinder optimal performance of the predator on the target pest, diverting its attention to other sources of food in a more natural/complex greenhouse set up. However, this could not be evaluated in our set-up where only thrips were provided hence a high efficacy was expected. However, Arno *et al*., (2008) reported that WFT was clearly preferred by *O. laevigatus and O. majusculus* when simultaneously presented with whiteflies and *O. insidiosus* preferred WFT over *Tetranychus urticae* (Koch) (Xu *et al*., 2006).

Lastly, integrated approach of pest control has challenges in ensuring that optimal conditions for each component are met to allow for maximum performance of each treatment. The climate chamber provided the optimal conditions of temperature (23 ± 2˚C), high relative humidity (50-60% RH) and long days (16:8 h light: dark photoperiod). These were the best conditions for *Orius* to forage. In the natural set up, meeting an optimal temperature, relative humidity, light duration for nematodes, fungi and the predator may be challenging. Either the conditions will enhance the performance of one component but be less favourable for the optimal performance of other components. For example, shorter day-light conditions are known to initiate diapause in some species of *Orius*.

The last study was about the efficacy of Light Emitting Diodes (LED)-based blue sticky traps combined with the synthetic lure Lurem-TR for monitoring of *F. occidentalis*. Monitoring is the backbone of successful integrated pest management especially the use of biocontrol tools. The success of augmentative release of biocontrol agents is based on timely and adequate release of the natural enemies (Hoodle *et al*., 2004). Hence prompt and accurate monitoring will guide growers on correct timing and adoption of a more comprehensive and enhanced control methods to finally come up with the most efficient suppression methods for the pest populations. In this study, the LED light based traps were clearly preferred over the conventional blue sticky traps.

This was used to construct LED-blue sticky traps which greatly improved the performance of the blue sticky traps. The performance of this set up was further enhanced by over two-fold increase in the number of WFT recaptured when the synthetic Lurem-TR lure was included.

Although blue sticky traps are more effective for catching thrips, many growers use yellow traps because they can be used to scout for other important pests such as winged aphids, adult whiteflies, leaf miners, fungus gnats, and shore flies. In addition, thrips are generally easier to see on yellow sticky cards than on blue ones. Although, in this study, the most attractive was the high-power (HP) LED-blue sticky trap with Lurem-TR, growers can also opt for either LED-blue sticky traps or blue sticky traps baited with Lurem-TR since there were no significant differences between these two treatments. Similar findings were reported by Chu *et al*., (2005). In addition, LED light devices take up little space, they have long lifetime and low power consumption and they may enable pest monitoring in places where conventional light sources are impractical. Besides, their operation with low voltage makes the technique safe for the application in greenhouses (Yeh and Chung, 2009). Massa *et al*., (2008) reported that LEDs emit low thermal radiation since the resulting heat is transferred from the LED chip to the heat sink on its backside hence they can be installed close to plant canopy as inter-lights. Unlike most conventional traps which depend on reflection, LEDs traps work on radiation and not on reflection mode hence they can be used even in low global radiations. In our study, better results were obtained during autumn and winter than in summer. These periods of low light conditions might have enhanced the effect from LEDs as compared to the bright conditions in summer. This makes the use of LEDs more applicable amongst crop stands in the greenhouse.

Even though the potential of host plants odours to mask the lure odour has not been studied into details, there are indications that the impact of the attractant was reduced in experiments that included the host plants. Host plant attractive cues like colour and other volatiles may affect the effectiveness of the lure (Blackmer and Cañas, 2005). Results from studies using live plants have been variable primarily due to the plant species tested. Thrips lures are usually known to be more effective when used with non-host plants. Pheromone lures are not a control device, and issues associated with longevity of the scent within the greenhouse during certain times of the year and how effective the pheromone lure is when many plant types are flowering is still rudimentary

understood (Cloyd, 2009). As quality of plant resources changes, thrips which is polyphagous may resort to host switching depending on resources availability, they may utilize weeds and other flowers to ensure an all year round population of thrips. Lurem-TR lure could dissipate to influence trap catches in unbaited-traps, this effect could be experienced up to 10 m away according to Teulon *et al*., (2007a; 2014). This phenomenon could be utilized to maximise thrips management in a wider environment by optimal lure spacing. This study was in the greenhouse with limited air circulation and the potential of the lure was possibly under-utilized.

Methyl isonicotinate is known to initiate “take off” behaviour in thrips. This increased activity could improve efficacy of insecticides or biopesticides by exposing a greater number of thrips to spray application (van Tol *et al*., 2012). The attractant can also enhance other control methods of WFT such as lure & kill, mass trapping and push & pull (Niassy *et al*., 2012a; Sampson and Kirk, 2013). However, the use of semiochemicals for mass trapping is still not established, it needs a stronger lure as well as integration with other mortality factors like biocontrol agents for better performance. Although this integration can be hindered by some negative effect of the lure especially on the persistence of fungal conidia. For example, methyl isonicotinate was reported to have antifungal properties (Teulon *et al*., 2011). This was confirmed by Niassy *et al*., (2012a) where there was complete inhibition of conidial germination 2 days after fungal exposure to the semiochemical therefore decreased thrips mortality two days post inoculation. Other semiochemicals that are compatible with entomopathogens needs to be screened for use. Methyl isonicotinate known to have low toxicity to mammals was also repellent to *O*. *insidious* and *Ceranisus menes* (Walker) (Waite, 2013). Contrastingly, Lurem-TR increased trap catches of brown lacewings (Broughton and Harrison, 2012). Being and interspecific kairomone, more research should be done to establish its attractiveness to a wide range of natural enemies or else another attractant which is more selective at detecting and monitoring WFT should be incorporated.

A critical view should be given to the experimental arenas used here. Use of flight chambers to study insect behaviour has limitations. The insects are not exposed to natural environment and it also ignores food reward with host plants which may arrest thrips despite the presence of an attractant (Raviv and Antignus, 2004). More realistic

conditions which include polychromatic radiation sources are needed for further evaluation. Alternatively, these results should be validated in a field condition where insects are exposed to ambient abiotic conditions (light signals and intensities, olfactory and climatic factors). Here a critical point is that the performance of LED-blue sticky trap can be affected by natural sunlight properties. This study used high-power LEDs which provide a much higher intensity than standard LEDs, it is very likely that these traps could perform much better under realistic conditions in greenhouse crops and may be the most reasonable solution for application in thrips monitoring.
A recent study aimed at improving monitoring found the even surface and fixed position of the sticky traps well suited for an automated identification and counting of insects using image processing software (Böckmann, 2015). The concept of area specific decision support system developed by Böckmann, (2015) for optimal use of beneficial is a reliable and cost efficient monitoring system which provides growers with area specific information on pests and beneficial density. This system gives the much desired correlation between the trap catches and the pest population on the crops which directly translates into the observed crop damage. Lures may be useful in increasing the attractiveness and selectivity of the sticky traps to catch more target pests and preserve their natural enemies. This monitoring driven introduction of natural enemies in this system could be more economical as compared to introduction of beneficials at predefined intervals.

Generally, the use of biocontrol requires considerable knowledge, but it has clear benefits in terms of reliable pest control, lack of phytotoxicity to young plants, residues in marketed products, re-entry and pre-harvest interval and better crop quality. Stringent and local legislatives that govern registration of biocontrol agents in various countries has hindered their fast adoption. However, the great emphasis placed by European union on IPM as part of the agricultural policy may lead to innovations in the way biopesticides are regulated and adopted (Chandler *et al*., 2011). Previously, biocontrol was only used when chemical control was insufficient, impossible or undesirable. But currently, crop protectionists consider biocontrol a powerful option when used in an IPM program. However, it is relatively “easy” to develop an IPM program-with emphasis on the use of integrated biological control for a single pest. The implementation of this program becomes complicated by the fact that most crops are affected by multiple pest species and growers have to contend with each of the pests effectively (Funderburk, 2009). This calls for stringent and fact-based decision

making to ensure proper crop management and improved productivity. To accommodate this complex management decision making, there is critical need for a management program that integrates control tactics for most economically important pests within a cropping system. This decision should be based on economic threshold using a reliable monitoring system with emphasis on timely application of efficient biological control methods. This concern highlights the importance of a need based integrated biological control approach for WFT using an omnivorous predator like *O. laevigatus* and/or soil application of azadirachtin and entomopathogens for a holistic management of both foliage dwelling and soil inhabiting life stages. There is need to review and reinforce improved pests monitoring systems and integrated biological control as major components of the overall IPM program and not view each as an independent goal in itself.

The interaction between multiple agents is classified as synergistic, antagonistic or simply additive depending on how much the observed combination responses differs from the expected responses under the null hypothesis. These assumptions are difficult to validate a priori due to lack of knowledge of the mechanisms of actions once the control agents are used together (Tang *et al*., 2015). Therefore, a befitting study to evaluate these interactions and confirm their efficacy is important. This study recorded mostly, the desired additive and synergistic interactions. Although, it should be noted that synergy is a measure of the degree of interaction, while efficacy is a measure of phenotypic response to treatment combinations. There is a possibility of having a treatment combination that exhibits strong responses yet does not necessarily show a synergistic response and this may be insufficient to reach the satisfactory efficacy (Tang *et al*., 2015). For instance, in combined use of azadirachtin products and entomopathogens, the active ingredient may produce the response while an oil surfactant used in its formulations may show adverse effect on the entomopathogens thus lowering the overall efficacy of the combination. Alternatively, the resulting efficacy may be statistically below the expected null hypothesis (additivity) and the interaction will be termed as antagonistic. Therefore, it is important to carefully select the treatments, use an appropriate experimental design for combination treatments and a statistical evaluation of the interaction effect which takes into consideration the contributions of individual components of the treatments and their overall effect in efficacy of pest control.

In conclusion, the success of these biocontrol agents could only be assured if there is a prompt and reliable monitoring system. This work proved that blue sticky traps equipped with narrow-bandwidth high-power (HP) Light Emitting Diodes (LEDs) and Lurem-TR lure is a promising approach to increase the attractiveness and specificity of visual traps leading to a more efficient monitoring system. Secondly, for an integrated biological control approach, improvement of thrips control was possible by intelligent combination of soil applied NeemAzal-T with S. *carpocapsae* and/or *M. anisopliae* ICIPE-69 to control the soil dwelling stages of WFT. Nearly complete control of western flower thrips could be expected from the EPF-based treatments but only after including mortality resulting from the late mycosis. The predator *O. laevigatus* could effectively control the foliage dwelling adult and larval stages of WFT when facing a tradable thrips population (density). Most of these combinations showed complementary interactions (synergistic or additive). This could improve reliability and inefficiency of single antagonist. Using the combinations, it was possible to use low concentrations of particularly NeemAzal-T, EPN and reduced application rate of *O. laevigatus* did not affect its efficacy. The best results were obtained when *O. laevigatus* was applied to target L1 stage of WFT. This approach which combines the use of a more efficient/sensitive monitoring technique will ensure timely application of the biocontrol approaches. Alternatively, should the pest population be shown to be approaching economic threshold based on monitoring data, growers can use NeemAzal-T for first knock-down effect then follow it with the predator and entomopathogens for a long lasting control of the most economically important greenhouse pest. However, for evaluation of performance under practical cultural conditions more extensive studies on integration of both monitoring and control strategies fitting into IPM programs have to be performed. This should focus on interaction effects and possible side effects to the biocontrol agents used in the program.

6.0 References

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- BASIS Crop protection certificate of competency by BASIS (UK) (2011)
- Women's leadership and management course, a member of African Women in Agricultural Research and Development (AWARD) fellowship CGIAR Gender and Diversity program (2010)
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- A member of the African Association of Insect Scientists (IAAS) and Entomological Society of Ghana (2005)
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Publications and Research

Otieno, J.A., Pallman, P., and Poehling, H.-M. (2016). The combined effect of soil-applied azadiractin with entomopathogens for integrated management of western flower thrips. J. Appl. Entomol. 140:174-186.

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Erklärung

Hiermit erkläre ich, Jacinter Atieno Otieno dass ich die vorliegende Dissertation mit dem Titel “Integration of soil-applied azadirachtin with predators, entomopathogens and optical/chemical traps for the management of Western flower thrips, Frankliniella occidentalis Pergande (Thysanoptera: Thripidae)“ selbstständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ferner erkläre ich, dass die Dissertation weder im Ganzen noch in Teilen bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Declaration

I Jacinter Atieno Otieno hereby declare that this thesis, entitled “Integration of soil-applied azadirachtin with predators, entomopathogens and optical/chemical traps for the management of Western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae)” with the exception of references to other people’s work which I have duly acknowledged all the experimental work described in this thesis was carried out by me and this thesis, either in whole or in part, has not been presented elsewhere for another degree.