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Efficacy of Entomopathogenic Nematodes for the Control of the Western Flower Thrips *Frankliniella occidentalis*

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Dedicated to my late grandmother

Dawiti Bedhaso

Abstract

Efficacy of Entomopathogenic Nematodes for Control of the Western Flower Thrips *Frankliniella occidentalis*

Lemma Ebssa

Since its accidental introduction from California into Europe in the early 1980s, the western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) has become an important cosmopolitan pest of vegetables and ornamentals in greenhouses. Due to its cryptic feeding behaviour and life strategy, control of WFT is extremely difficult. Entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) are known to infect the soil-dwelling development stages of WFT. However, high concentrations of EPNs are required to assure high control levels of WFT. The general objectives of this study were (i) to assess factors that might be responsible for the high EPN concentrations needed for WFT control and (ii) to combine EPNs with other biocontrol agents that target the foliar-feeding development stages of WFT, with the overall aim of improving the biological control of *F. occidentalis*. The experiments were mainly carried out in a growth chamber. For experiments with plants, green beans *Phaseolus vulgaris* L were used as a model plant and Fruhstorfer Erde as a commercially available growing substrate. Depending on the nature of the respective experiments EPN concentrations of 50, 100, 200, 400, or 1000 infective juveniles (IJs) cm^{-2} were used.

In a screening experiment involving 16 EPN species/strains, variability among nematodes in their pathogenicity to WFT was confirmed. In general *Heterorhabditis* spp. were more pathogenic to WFT than *Steinernema* spp. When selected EPN species were further tested at different concentrations, temperatures, and host densities, superiority of *H. indica* Poinar (strain LN2) to other EPN species/strains could be shown, and up to 80% WFT corrected mortality was obtained at 400 IJs cm^{-2}. In general, increasing EPN concentrations resulted in an increase in thrips mortality. EPN strains originated from warmer and cooler climate performed better at higher and lower temperatures, respectively. Independent of the geographic origin of the EPN species/strains, highest thrips mortality was attained at 25°C. Host densities affected efficacy of EPNs differently depending on the foraging behaviour of the nematodes, i.e., ambushers that follow a 'sit and wait' strategy, and cruisers that

actively search for hosts. Unlike in the ambusher *S. bicornutu* Tallosi, Peters, & Ehlers, WFT mortality caused by the cruiser *H. indica* increased with increasing host densities but depending on concentrations.

When tested at different substrate moisture levels and different amounts of post-application irrigation levels, differences in WFT mortality due to varying EPN concentrations depended on nematode species/strain. The lower EPN concentration was sufficient and resulted in similar WFT control compared to a four-fold increase in concentration only when moisture level was kept at >78% relative moisture content for the cruiser *H. indica*. However, to obtain a higher thrips mortality by the ambusher *S. bicornutum*, always higher moisture levels were required. Furthermore, it was only at an appropriate amount of post-application irrigation (that then resulted in a relative moisture content of 88%) or a sufficient volume of EPN application (that caused a moisture content closer to the saturation point of the substrate) that the lower EPN concentration resulted in a similar WFT mortality compared to the higher concentration.

When tested at varying depths of thrips pupation, a higher concentration of the cruiser *H. indica* was required for WFT that pupated deeper than 1.0 cm. At such a pupation depths, increasing concentrations of the ambusher *S. bicornutum* did not result in a higher WFT control. At high thrips densities and/or EPN concentrations, a greater proportion of the thrips tended to avoid pupating deep.

The cruiser *H. bacteriophora* Poinar (strain HK3) persisted longer at a higher than a lower concentration. The ambusher *S. carpocapsae* (Weiser) could persist relatively long even at a lower concentration. In a separate experiment early and repeated applications of *H. bacteriophora* at 200 IJs cm^{-2} resulted in a better WFT control than one-time application of the same nematode at 400 IJs cm^{-2} irrespective of the time of application.

When assessing the single and combined effects of EPNs and releases of the predatory mite *Amblyseius cucumeris* (Oudemans) for WFT control in a controlled environment experiment, control levels of up to 83% were achieved by combined applications of the two natural enemies. Thrips control in the combined treatment was significantly better than in both individual applications of the biocontrol agents. In general, the extent of WFT control depended on the density and concentrations of mites and nematodes, respectively. Results in a similar greenhouse experiment were less straightforward, with no differences

between individual and combined applications of the same biocontrol agents. Most likely the compatibility of EPNs and mites highly depend on the climatic conditions in greenhouses, with extreme temperatures and low humidity generally being unfavourable for the biological control of WFT.

Results of this study clearly indicate that environmental conditions such as host density, temperature, pupation depth, substrate moisture content, and post-application irrigation are important factors that are partly responsible for the requirement of high EPN concentrations for WFT control. Thus, identifying efficient EPN species/strains against the soil-dwelling life stages of WFT and testing the nematodes under such environmental conditions can lead to higher levels of WFT control at lower EPN concentrations. Moreover, the appropriate time and frequency of EPN applications and their potential to be applied along with other natural enemies of WFT will contribute to improving the biological control of WFT.

Keywords: biological control, entomopathogenic nematodes, *Frankliniella occidentalis*, nematode concentration, western flower thrips

Zusammenfassung

Effizienz entomopathogener Nematoden zur Bekämpfung des Kalifornischen Blütenthrips *Frankliniella occidentalis*

Lemma Ebssa

Seit der Einschleppung des aus Kalifornien stammenden Kalifornischen Blütenthrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) nach Europa Anfang der 80er Jahre, hat sich der Thrips zu einem der bedeutensten Schädlinge im Unterglasbereich an Gemüse- und Zierpflanzen entwickelt. Auf Grund seiner kryptischen Lebensweise und Nahrungsaufnahme ist die Bekämpfung von *F. occidentalis* sehr schwierig. Entomopathogene Nematoden (EPN) (Rhabditida: Steinernematidae und Heterorhabditidae) können die bodenbewohnende Entwicklungsstadien von *F. occidentalis* infizieren. Allerdings müssen derzeit vergleichsweise hohe Konzentrationen von EPN verwandt werden um einen befriedigenden Bekämpfungserfolg zu gewährleisten. Die Ziele der vorliegenden Arbeit sind (i) Faktoren zu identifizieren, die für die hohen EPN Konzentrationen zur Bekämpfung von *F. occidentalis* verantwortlich sind und (ii) Applikationen von EPN mit anderen biologischen Bekämpfungsmitteln zu kombinieren, die auf die oberirdische vorkommenden Entwicklungsstadien von *F. occidentalis* wirken. Das Hauptziel dieser Arbeit ist eine Verbesserung der derzeitigen biologischen Bekämpfung von *F. occidentalis*. Die meisten Versuche wurden in Klimakammern durchgeführt, und in allen Experimenten die Pflanzen involvierten wurden Gartenbohnen *Phaseolus vulgaris* L. und Fruhstorfer Erde als Substrat verwandt. In den Versuchen wurden EPN Konzentrationen von 50, 100, 200, 400, oder 1000 Dauerlarven (DL) cm^{-2} eingesetzt.

Zunächst wurden in einem Screening-Versuch die Pathogenität von 16 EPN Arten oder Stämme gegenüber *F. occidentalis* untersucht. Es zeigte sich allgemein, dass mit einer Behandlung mit *Heterorhabditis* spp. ein höherer Bekämpfungserfolg erzielt werden kann als mit *Steinernema* spp. Insbesondere *H. indica* Poinar (Stamm LN2) erwieß sich gegenüber den anderen EPN Arten/Stämmen konnte bei unterschiedlichen Konzentrationen, Temperaturen und Wirtsdichten zumeist als überlegen. Dieser Stamm verursachte bei einer Konzentration von 400 DL cm^{-2} eine korrigierte Mortalität von bis zu

80%. Allgemein bewirkten steigende EPN Konzentrationen eine erhöhte Thripsmortalität. Es zeigte sich des weiteren, dass EPN Stämme aus warmen bzw. kalten Klimaten eine bessere Leistung bei hohen bzw. tiefen Temperaturen aufwiesen. Unabhängig von der geographischen Herkunft der EPN Arten/ Stämme wurden die höchsten Thripsmortalitäten bei 25 °C beobachtet. Die Effizienz der EPN wurde in Abhängigkeit von ihrer Suchstrategie ("foraging behaviour") unterschiedlich durch variierende Wirtsdichten beeinflusst. Beispielsweise verfolgen „ambusher“ eine sogenannte "sit-and-wait" Strategie, während "cruisers" aktiv geeignete Wirte suchen. Im Gegensatz zum "ambusher" *S. bicornutu* Tallosi, Peters & Ehlers stieg die durch den "cruiser" *H. indica* verursachte Mortalität von *F. occidentalis* mit steigender Wirtsdichte in Abhängigkeit der EPN-Konzentrationen an.

Bei Versuchen mit unterschiedlichen Substratfeuchten und unterschiedlichen Bewässerungsmengen nach einer EPN Anwendung hing die Mortalität von *F. occidentalis* bei wechselnden EPN-Dichten von der jeweiligen Nematodenart oder –stamm ab. Niedrigere EPN-Konzentrationen erwiesen sich als vergleichbar erfolgreich wie vierfach höhere wenn z.B. bei dem "cruiser" *H. indica* die Substratfeuchte bei >78% relativer Feuchte gehalten wurde. Jedoch benötigt der "ambusher" *S. bicornutum* immer höhere Substratfeuchtegehalte um ähnlich hohe Thripsmortaltitäten zu verursachen. Außerdem ergaben sich nur bei ausreichender post-applikation Bewässerung (die in einem relativen Substratfeuchtegehalt von 88% resultierte) oder bei einer EPN-Anwendung, die zu einem Feuchtegehalt nahe des Sättigungspunkts des Substrat führte, vergleichbar hohe Thripsmortalitäten bei geringen und hohen EPN-Konzentration.

Wenn sich *F. occidentalis* tiefer als 1 cm verpuppte wurden hohe Konzentrationen des "cruiser" *H. indica* benötigt. Bei solchen Verpuppungstiefen führten steigenden Konzentrationen des „ambusher“ *S. bicornutum* nicht zu einem verbesserten Bekämpfungserfolg bei *F. occidentalis*. Bei hohen Thripsdichten und/oder EPN Konzentrationen verpuppte sich der mehrzahl der Thripse in den oberen Bodenschichten (bis 96 %).

Die Persistenz des "cruisers" *H. bacteriophora* Poinar (Stamm HK3) war länger bei höheren als bei niedrigen Konzentrationen, während der "ambusher" *S. carpocapsae* (Weiser) auch bei geringen Konzentrationen eine relativ hohe Persistenz aufwies. Bei gesplitteten Behandlungen bewirkten frühe und wiederholte Anwendungen von 200 DL

cm^{-2} von *H. bacteriophora* einen höheren Bekämpfungserfolg als eine einmalige Anwendung derselben Nematodenart mit doppelter Konzentration (400 DL cm^{-2}).

Bei kombinierten Applikationen von EPN und der Raubmilbe *Amblyseius cucumeris* (Oudemans) konnten in einem Experiment unter kontrollierten Bedingungen ein bis zu 83%iger Bekämpfungserfolg von *F. occidentalis* erzielt werden. Die Thripsbekämpfung war in der kombinierten Variante signifikant besser als in den Einzelanwendungen der beiden natürlichen Gegenspieler. Allgemein hing das Ausmaß des Bekämpfungserfolges von der Dichte bzw. Konzentration der Milben und Nematoden ab. In einem nachfolgenden Gewächshausversuch konnten diese Ergebnisse aber nicht bestätigt werden, da hier keine Unterschiede im Bekämpfungserfolg von *F. occidentalis* zwischen der kombinierten und der einzelnen Anwendung der natürlichen Gegenspieler erzielt wurden. Ursache hierfür waren höchtswahrscheinlich die phasenweise sehr hohen Temperaturen und niedrigen Luftfeuchten in dem Gewächshaus. Wahrscheinlich ist die Kompatibilität von EPN und Raubmilben stark von den klimatischen Verhältnissen im Gewächshaus abhängig.

Zusammenfassend zeigen die Ergebnisse dieser Studie, dass biotische Faktoren wie z.B. Wirtsdichte, Temperatur, Verpuppungstiefe, Feuchtegehalt des Substrates und das Ausmaß der post-applikation Bewässerung die entscheidenden Faktoren sind, die für die hohen EPN-Konzentrationen, die z.Z. für eine erfolgreiche *F. occidentalis*-Bekämpfung benötig werden, verantworlich sind. Ein Testen von potentiell vielversprechenden EPN Arten/ Stämmen zur Bekämpfung der bodenbürtigen Entwicklungsstadien von *F. occidentalis* unter solchen Bedingungen in zukünftigen Screening-Versuchen kann zu einer verbesserten Thripsbekämpfung bei niedrigeren EPN-Konzentartionen führen. Darüber hinaus kann der geeignete Behandlungszeitpunkt sowie gesplittete Behandlungen, aber auch der kombinierte Einsatz von EPN und anderer natürlicher Gegenspieler zu einer verbesserten biologischen Bekämpfung von *F. occidentalis* beitragen.

Schlagwörter: Biologische Bekämpfung, Entomopathogene Nematoden, *Frankliniella occidentalis*, Kalifornischer Blütenthrips, Konzentration der Nematoden

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Abbreviations

AMC	Actual moisture content
ANOVA	Analysis of variance
CM	Corrected mortality
DANA	Days after nematode application
df	Degree freedom
EPN(s)	Entomopathogenic nematode(s)
F	Statistical *F*-value
Fig.	Figure
GLM	General linear model
IJ(s)	Infective juvenile(s)
L:D	Relation of light to darkness
L1	First instar larvae
L2	Second instar larvae
LSD	Least significant difference
MC	Moisture content
ns	Non-significant
P	*P*-value (statistical significance level)
r	Correlation coefficient
R^2	Coefficient of determination in regression
rh	Relative humidity
RMC	Relative moisture content
SAS	Statistical analyses system
SE	Standard error of the mean
t	Statistical *t*-value
w/w	Weight of water to weight of the substrate with the water
WFT	Western flower thrips
WHC	Water holding capacity

1 General Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is the most worldwide-distributed species of the genus *Frankliniella*, which includes about 180 species, causing a significant impact on crop plant (Mound, 1997; Mound and Kibbi, 1998). Pergande (1895) reported *F. occidentalis* for the first time from a specimen collected in California, USA. The international spread of this pest from its origin was started in the late 1970s most likely due to the intense use of insecticide in horticulture that left insecticide resistant strains. It continued to distribute mainly through the movement of horticultural materials and becomes one of the major worldwide crop pests (Frey, 1993b; Kirk, 2002; Kirk and Terry, 2003).

Adult western flower thrips are very small in size having a length of 0.9–1.4 mm from the tip of antenna to the tip of the abdomen (van Lenteren et al., 1995). The life history of WFT consists of an egg, two actively feeding larval stages (L1 and L2), two non-feeding stages (prepupa and pupa) followed by an adult. Adults deposit eggs in parenchymatous tissues of plants (leaves, buds, flowers, or young fruits). Depending on environmental conditions and nutrient levels female WFT may live for 20–45 days and lay 150–300 eggs per female (Gaum et al., 1994; Hulshof et al., 2003). The first and second larvae resemble a mature version of the adult apart from the absence of wings and genital appendages, and having reddish eyes and less segmented antennae. Upon maturity, the majority of the second instar larvae descend to the soil for pupation at a depth of 1.5–2.0 cm (van Lenteren et al., 1995). The larvae develop to prepupal and then to pupal stages both of which are immobile unless disturbed. Fully matured pupae change to adult and emerge from the soil to re-colonise plants. The reproduction lifecycle of WFT depends on several factors among which temperature is the most important. At 25–30 °C, WFT requires less than 10 days to complete its lifecycle. In the greenhouse, *F. occidentalis* reproduces continually, producing more than 10 generations per year (Bene et al., 1998).

WFT is one of the most important pests of greenhouse-grown vegetable and ornamental crops worldwide (Giliomee, 1989; Katayama et al., 1997; Malipatil et al., 1993; Oda et al., 1997; Shipp et al., 1991; van Lenteren et al., 1995). The pest attacks also several outdoor plants being a highly polyphagous species with at least 250 plant species from 65 families

(Anonymous, 1989; Yudin et al., 1986). It causes direct damages by feeding on or ovipositing in young fruits and ornamentals resulting in silvery scarring or even malformation. Flower abortion may result in total loss of fruits. The dark faecal droplets and the scars by WFT on leaves, flowers, and fruits significantly reduce the market values of the products (Rosenheim et al., 1990; van Lenteren et al., 1995). In addition to the reduction in aesthetic qualities of crops, Shipp et al. (1998b) reported that more than 1200 larval-days per sweet pepper plant has negative impacts on plant physiology and yield. WFT causes also indirect damages by vectoring tospoviruses (e.g. *Tomato spotted wilt virus* (TSWV), impatiens necrotic spot virus (INSV), *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV), and *Chrysanthemum stem necrosis virus* (CSNV)). Out of more than nine thrips species that can transmit tospoviruses, *F. occidentalis* is the major vector (Mound, 2002; Nagata et al., 2002; Wijkamp et al., 1995). Among the tospoviruses, INSV and TSWV, which are transmitted mainly by WFT, are serious diseases of many economically important crops (Daughtrey et al., 1997; Nagata et al., 2002). TSWV attacks 174 plant species from 35 plant families including dicots and monocots (Goldbach and Peters, 1994; Parrella et al., 2003; Peters et al, 1996) having a host range of ornamentals, vegetables, and field crops, which is a unique host range among plant-infecting viruses. INSV is also a common viral disease on ornamental plants of more than 300 species from 50 plant families (Windham et al., 1998). The polphagous nature of WFT attacking several plants including high value crops under protected cultivation, high vectoring efficiency of important plant viruses of high value crops, and its worldwide distribution are some of the factors that may categorize WFT among extremely important plant pests of high priority requiring urgent control.

Managing and efficient control of WFT is difficult because they: are very small and easy to overlook; spend one-third of their life cycle in the soil; like to feed and hide in flowers, flower buds, and leaf buds making them hard to spot and reach with pesticides; live on a wide variety of host plants; are opportunistic by feeding on other insects and mites; reproduce rapidly in warm greenhouses; hide in plant materials and are transported worldwide; transmit tospoviruses to a wide range of plant species; and are resistant to a number of insecticides. As low as 5 adults per trap per week was reported a damage threshold level of some ornamental plants (Frey, 1993a). On the other hand, a loss in commercial value of crops as a consequence of the development of dark spots caused by TSWV results in almost zero economic threshold (Frey, 1993a; Maymo et al., 2002; Sadof

and Raupp, 1997; Windham, 1998). To combat the high reproduction rate and low economic threshold levels of WFT in several high value crops, several groups of insecticides have been repeatedly applied. However, this practice has lead to develop resistant strains of WFT to commonly used insecticides aggravating the problem of WFT (Immaraju et al., 1992; Zhao et al., 1995). There are limited ranges of natural enemies, including anthocorid bugs of *Orius* spp. (Heteroptera: Anthocoridae) and phytoseiids mites of *Amblyseius* spp. (Acarina: Phytoseiidae) (Shipp and Wang, 2003; van Lenteren, et al., 1995). However, these predators attack only the foliage-feeding life stages of WFT and hence, the soil-dwelling late L2, prepupae and pupae are not within the reach of these biocontrol agents. Moreover, due to the low economic threshold levels of WFT in many crops (e.g., Shipp et al., 1998a,b), releases of *Orius* spp. and *Amblyseius* spp. have resulted in insufficient control levels of WFT (e.g., Jarosik and Pliva, 1995). Therefore, research on different directions of WFT biological control strategies have been initiated. In these new approaches, the soil-dwelling life stages of WFT are targeted by soil-dwelling biological control agents, that include entomopathogenic nematodes (EPNs) (e.g. Chyzik et al., 1996; Ebssa et al., 20001a,b) and soil-dwelling predatory mites (Berndt et al., 2004). Premachandra et al. (2003b) also indicated that the prospects of combined releases of EPNs and *Hypoaspis aculeifer* Canestrini (Acarina: Laelapidae) against WFT. Moreover, several attempts were made to use other control options (Antignus et al., 1996; CAB International, 1999; Lindquist and Casey, 1990) including entomopathogenic fungi (Azaizeh et al., 2002; Vestergaard et al., 1995) and parasitic nematodes (Arthurs and Heinz, 2003). However, each control strategy individually applied does not seem to provide a sufficient control of WFT populations mainly because of the low economic threshold levels. Hence, WFT has to be targeted from several possible directions, meaning that only a combination of several and compatible control strategies will assure that the pest will be kept below the economic threshold level(s).

Entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) are important biological control agents of a great variety of insect pests (Poinar, 1986). EPNs have a simple life cycle that includes the egg, four juvenile stages, and the adult. The third juvenile stage, called infective juveniles (IJs), carries symbiotic bacteria in its gut. *Xenrohabdus* and *Photorhabdus* are the two bacterial genera mutualistically associated with EPNs from genera *Steinernema* and *Heterorhabditis*, respectively (Boemare, 2002). After entering the haemocoel of insect host via natural

openings or spiracles the IJs release the associate bacteria, which then proliferate and produce a wide range of toxins and hydrolytic exoenzymes that are responsible for the death of insect host within 24–48 h and bioconversion of the insect cadaver into a nutrient soup that is ideal for growth and reproduction of the nematodes (Frost and Clarke, 2002; Smart, 1995).

As EPNs are found naturally in the soil, they are particularly suited for control of soil-inhabiting insects (Ehlers, 1996). Researches already reported the successful use of EPNs against many insects under field conditions (e.g., Boselli et al., 1997; Shapiro-Ilan et al., 2004b; Sulistyanto et al., 1996). EPNs possess certain attributes that make them potentially attractive biocontrol agents. EPNs can be mass-produced (Ehlers et al., 1998; Surrey and Davies, 1996) and hence are available commercially, have a rather broad host range (Poinar, 1986) but only limited effects on non-target organisms (Bathon, 1996), are fast-acting (Smart, 1995), are widespread (Hominick et al., 1996) thus can be obtained from every ecology, are amenable for genetic improvement (Burnell and Dowds, 1996), and are easily compatible with other control measures like insecticides and other biocontrol agents (Koppenhöfer and Kaya, 1997; Premachandra et al., 2003b; Rovesti and Deseo, 1991; Shapiro-Ilan et al., 2004a).

However, the IJs emerging from the insect cadaver or applied as biological control agents and searching for hosts may encounter many potential hazards, for instance extremes in temperature, soil texture, soil moisture, UV light, toxic root exudates, secondary plant metabolites, inter- and intra-specific competition in EPNs, and natural enemies of EPNs (Brown and Gaugler, 1997; Kaya and Koppenhöfer, 1996). Generally, factors operating at the time of application and over the following few hours are most critical for the establishment and efficacy of EPNs in the soil. Foraging behaviours of EPN affect, directly or indirectly, the extent of tolerance of the nematodes to some of these factors (Glaser, 2002; Lewis, 2002). Based on their capability to attach to mobile versus sedentary hosts, which is expressed through their ability of movement during foraging and their response to host cues, EPN species are classified as ambusher, cruiser, or intermediate (Lewis, 2002). The ambushers have a sit-and-wait strategy by remaining near or at the soil surface and nictating to attach to mobile insects whereas cruisers have an active searching strategy by responding to chemical cues from host or plant exudates and attacking even sedentary hosts (Lewis, 2002).

In the control of WFT so far only few species/strains of EPN were tested. In these studies, though up to 80–90 and 60% WFT mortality were obtained under laboratory and microcosm conditions, respectively, high nematode concentrations were required (Ebssa et al., 2001a,b). These have necessitated screening greater numbers of EPN species/strains, and testing several factors that may improve the efficacy of the applied nematode. Furthermore, the compatibility of efficient EPN strains against WFT with other biocontrol agents was envisaged. Thus, the general objectives of this study were to enlarge the screening for well-suited EPNs species/strains in their efficacy, assess biotic and abiotic constraints for efficient use of EPN, and investigate compatibility of EPN with other biocontrol agents of WFT.

2 Effectiveness of Different Species/Strains of Entomopathogenic Nematodes for Control of Western Flower Thrips *Frankliniella occidentalis* at Various Concentrations, Host Densities, and Temperatures

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Abstract

Entomopathogenic nematode (EPN) species/strains (Rhabditida: Steinernematidae and Heterorhabditidae) were tested at a concentration of 200 infective juveniles (IJs) cm^{-2} against mixed soil-dwelling life stages (i.e., second instar larvae, prepupae, and pupae) of the western flower thrips (WFT) *Frankliniella occidentalis* (Thysanoptera: Thripidae), in a plant growing substrate under laboratory conditions. The different EPN species/strains resulted in WFT corrected mortality (CM) values ranging between 2.6 and 60%. In general, *Heterorhabditis* spp. were more virulent than *Steinernema* spp. Increasing concentrations of selected EPN species/strains led to an increase in thrips CM, with significant differences among species/strains. *Heterorhabditis indica* (strain LN2) caused higher CM than the other strains, with 30 and 90% as highest CM at the lowest and highest concentration tested, i.e. 100 and 1,000 IJs cm^{-2}, respectively. In an experiment with different WFT densities, CM caused by *S. bicornutum* negatively correlated with host density. However, with *H. indica*, CM increased, though not always significantly, with increasing host densities. Generally, the effect of host density on efficacy of EPNs depended on concentrations. When tested over a range of temperatures, 25 °C was the optimal temperature for both *H. indica* LN2 and *S. bicornutum*. *Heterorhabditis indica* LN2, tropical in origin, and *S. bicornutum*, isolated from a more temperate environment, performed better at higher and lower temperatures, respectively.

2.1. Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important polyphagous greenhouse and field pest in many parts of the world (van Lenteren et al., 1995). Vegetables and ornamental crops are among the most important host plants of WFT. It causes direct damage on the plants and indirect damage as a vector of important plant viral diseases, e.g. tomato spotted wilt virus (Marchoux et al., 1991).

WFT post-embryonic development involves two larval instars (i.e., L1 and L2) as well as prepupal and pupal stages. Upon maturity, the late L2 moves from the plant and enters the soil (Berndt, 2002). At a depth of 1.5–2.0 cm, the larvae develop into prepupae and subsequently pupae. Both prepupae and pupae are immobile unless disturbed and do not feed. Shortly after emergence from soil, adult WFT feed on leaves and flowers of the host plant. The adult starts laying eggs in parenchymatous tissues of their host plant within 72 h after emergence (Moritz, 1997; van Lenteren et al., 1995).

Entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) have a simple life cycle that includes the egg, four juvenile stages, and the adult (Boemare et al., 1996). The third-stage juvenile, often termed dauer larva or infective juvenile (IJ), is the infective life stage of EPNs. IJs live in symbiosis with bacteria that they release after host penetration. The bacteria then cause the septicemic death of the insect within 48 h (Bedding and Molyneux, 1982). EPNs are important biological control agents of a great variety of insect pests (Poinar, 1986). However, EPN species/strains vary in their virulence (expressed in terms of pathogenicity) against different host insects (e.g., Mason and Wright, 1997). In addition, efficacy of EPN species/strains is affected, among others, by concentration, host density, and temperature (Zervos et al., 1991).

Previous studies have shown that soil-dwelling stages of WFT are susceptible to EPNs (Chyzik et al., 1996; Ebssa et al., 2001a; Premachandra et al., 2003a). In these studies six strains of *Heterorhabditis* spp. and 11 strains of *Steinernema* spp. were tested. However, high concentrations were required to effectively control WFT, rendering this control approach presently not economical. Thus, we hypothesized that selection of a more virulent EPN species/strain and an improvement of environmental factors lead to an enhanced control of WFT. Therefore, the objectives of the present study were to assess the

efficacy of a large number of EPN species/strains and to evaluate the most effective nematodes at different concentrations, host densities, and temperatures.

2.2. Materials and Methods

Nematode and thrips cultures

All EPN species/strains used in this study were obtained from the Institute of Phytopathology, Christian-Albrechts University Kiel, Germany. Except for *S. feltiae* (Nemaplus® [E-Nema GmbH, Raisdorf, Germany]), the different EPN species/strains were reared in the laboratory at 23 ± 2 ºC in greater wax moth larvae *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) by a modified production system described by Kaya and Stock (1997). All nematodes were stored at 4 °C until used, except for the *H. indica* strains that were kept at 15 °C. To obtain uniform-aged insects for the experiments, WFT was reared on pods of green beans *Phaseolus vulgaris* L. (Fabaceae) in an incubator (23 ± 2 ºC, 50 ± 60% relative humidity (rh), and L16:D8 h photoperiod) following the modified protocol described by Ullman et al. (1992).

Assay arena

Six g of Fruhstorfer Erde, a commercially available growing substrate (Archut GmbH, Lauterbach-Wallenrod, Germany), was added into a plastic pot (diameters of 8 and 5 cm at the top and bottom, respectively, and a height of 6 cm), leading to a substrate depth of 1.5 cm and top area of 23.75 cm^2. The substrate is composed of humus, clay and peat in a proportion of 15:35:50, respectively, and has a high water holding capacity (i.e., 480%, weight of water to weight of oven-dry substrate) due to the high proportion of peat. The moisture content of the substrate at the time of WFT introduction into the pots was ca. 50–55% w/w (i.e., weight of water to weight of the substrate with the water). Twenty late L2 WFT (8 to 9 days old after the emergence of the neonate) were transferred to the top of the substrate in the pots. The L2 descended into the substrate immediately after the transfer. A Petri dish (diameter 10 cm) was used as a cover for the pot. A small hole (diameter 20 mm) was drilled in the center of the Petri dish, onto which nylon tissue (64 µm pore size) was glued to allow ventilation but preventing thrips from escaping. The inner part of the Petri dish, except the hole, was painted with insect glue (Temmen GmbH, Hattersheim, Germany) (and hereafter referred to as ‘sticky trap’) to trap emerging adult thrips. Two

days later, thereby enabling some of the L2 to moult into prepupae and pupae (Premachandra et al., 2003a) and thus providing a population mixture of different developmental stages in the substrate, different suspensions of EPNs were pipetted on the top of the substrate. The nematodes were not more than 1 month old after being harvested from the *G. mellonella* larvae and were acclimatized for at least 6 h at room temperature before use. To prepare a new concentration from nematodes in a stock culture, the quantification method described in Kaya and Stock (1997) was used.

In all experiments, a control treatment was pipetted with distilled water instead of nematode suspension. The experiments were carried out in a growth chamber at 23 ± 2 °C, ca 70% rh, and L16:D8 photoperiod unless indicated otherwise. All experiments were repeated twice over time, with two and three replicates, respectively, giving a total of five replications per treatment. In all repetitions, an assay arena was randomly assigned to a treatment depending on the types of experimental designs used in the respective experiment (for details see below). Starting from the second day after EPN application, emerged WFT adults on the sticky traps and the top of the substrate in the pots were daily counted under a binocular for one week until no more adult thrips were observed in the assay arena. EPN efficacies in the different experiments were assessed using data on WFT adult emergence.

Efficacy of EPN strains (experiment I)

EPN strains listed in Table 2.1 were tested against a mixture of different soil-dwelling life stages of WFT, using the assay arena described above. All strains were applied at 200 IJs cm^{-2} using a completely randomized design.

EPN Concentration studies (experiment II)

The most pathogenic EPN strains from the EPN efficacy experiment were further tested at 100, 150, 200, 400 and 1,000 IJs cm^{-2} in the assay arena. The experiment was conducted in a split plot design, using concentrations as a sub-factor and EPN species/strains as a main factor.

Efficacy of EPN as affected by WFT densities (experiment III)

Three population densities of soil-dwelling life stages of WFT were established in the assay arena by introducing 10, 20, or 50 late L2 into the assay arena. Two days later, *H. indica* LN2 or *S. bicornutum* were pipetted to the arena at concentrations of 100, 200, or 400 IJs cm^{-2}. The numbers of L2 and concentrations were combined factorially for a given EPN strain.

Effect of temperature on the efficacy of EPNs against WFT (experiment IV)

Suspensions of *H. indica* LN2 and *S. bicornutum* at concentrations of 100 and 400 IJs cm^{-2} were applied to a host density of 20 WFT per arena. After application of the EPNs, the assay arenas were transferred to different climate controlled growing chambers at 20, 25, 30, or 35 °C (± 2 °C in all cases), and the efficacy of the EPNs was recorded as previously described. A split-split-plot design with EPN species as main factor, concentration as sub-factor, and temperature as sub-sub-factor was used. The four temperature regimes were chosen as representative ranges of temperatures in greenhouse plant production.

Statistical analyses

WFT mortality data were corrected for control mortality using Abbott's corrected mortality (CM) formula (Abbott, 1925). The efficacy of EPN strains was evaluated using the CM data. The CM data were arcsine transformed before subjected to statistical analyses. Data of experiments repeated over time were checked for homogeneity of variance using the HOVTEST = LEVENE option of SAS version 8 (SAS Institute, 1999) and pooled only when variance homogeneity could be assumed. The data were analyzed using the PROC GLM procedure in SAS to determine single or interaction effects of factors (SAS Institute, 1999). For correlation analyses Pearson's correlation coefficient (r) was used with the PROC CORR procedure of SAS; furthermore, regression analyses were performed using the PROC REG procedure in SAS. Lack-of-fit was used to determine the appropriate model in the regression analyses. Whenever significant interactions were observed between factors, the level of one factor was compared at each level of the other factor. CM means caused by EPN applications under different conditions were compared to zero (the CM of the control treatment) using Dunnett's two-sided test. When significant factor effects were detected by means of ANOVA, CM means at different levels of the respective

factor were compared using Tukey's multiple means comparison procedure unless mentioned otherwise. A significance level of $\alpha = 0.05$ was used in all analyses. Data are presented as means ± SE.

2.3. Results

Efficacy of EPN (experiment I)

The EPN species/strains varied greatly in terms of efficacy against WFT, with CM values ranging between 2.6 and 60.2% (Table 2.1). Mean (+ SE) CM values <50% were recorded for 67 and 43% of the tested *Steinernema* and *Heterorhabditis* species/strains, respectively. The commercial product Nemaplus® and the hybrid *Heterorhabditis bacteriophora* strain PS8 were among the least effective strains.

Except for Nemaplus® and *S. carpocapsae* strain A1 B5, WFT mortality in all tested EPN species/strains was significantly higher than the control mortality which was 12.5 ± 3.3% (Dunnett test: $P < 0.0001$). Twenty-five percent of the tested species/strains resulted in mean CM values ≥50%. EPN strains that caused mean CM values >45%, i.e., *H. indica* LN2, *H. indica* LN10, *H. bacteriophora* PAL H04, *S. abassi* PAL S09, and *S. bicornutum*, were further evaluated.

Table 2.1. Mean (± SE) corrected mortality (CM) (%) of mixed soil-dwelling life stages of western flower thrips as induced by different entomopathogenic nematode (EPN) species/strains applied at 200 IJs cm^{-2}.

EPN species	Strain name	Origin	CM	Test [c]
Steinernema feltiae (Filipjev)	Nemaplus ® [a]	Europe	2.6 ± 4.6	f
S. carpocapsae (Weiser)	A1 B5	Italy	12.2 ± 5.6	ef
Heterorhabditis bacteriophora Poinar	PS8 hybrid [b]	–	24.3 ± 2.0	de
Steinernema sp.	Morocco	Morocco	30.5 ± 7.4	cde
H. bacteriophora	PAL H05	Palestine	32.0 ± 5.4	cd
S. carpocapsae	S.N2	Egypt	34.2 ± 10.5	bcd
S. carpocapsae	DD136	USA	40.6 ± 6.5	abcd
S. carpocapsae	S.S2	Egypt	40.7 ± 4.9	abcd
H. marelatus Liu & Berry	Strain	USA	41.8 ± 8.6	abcd
H. bacteriophora	HK3	Germany	43.3 ± 7.1	abcd
S. abassi Elawad, Ahmad, & Reid	PAL S09	Palestine	45.0 ± 5.5	abc
S. arenarium (Artyukhovsky)	Strain	Russia	46.4 ± 5.5	abc
H. bacteriophora	PAL H04	Palestine	49.6 ± 4.5	abc
S. bicornutum Tallosi, Peters, & Ehlers	Strain	Yugoslavia	54.1 ± 4.0	ab
H. indica Poinar, Karunakar, & David	LN2	India	57.5 ± 5.5	a
H. indica	LN10	India	60.2 ± 6.3	a

[a] Nemaplus® is a hybrid of several European *S. feltiae* strains and was supplied by E-Nema GmbH (Raisdorf, Germany) in a clay formulation. After estimating the numbers of IJs g^{-1} product (by counting the IJs in a suspension which was prepared from the product), a suspension of the product containing a concentration corresponding to 200 IJs cm^{-2} was prepared by diluting the product in distilled water.

[b] PS8 hybrid is a hybrid from eight strains of *H. bacteriophora*.

[c] CM means followed by the same letters are not significantly different (Tukey's test, $\alpha = 0.05$).

Concentration studies (experiment II)

The effect of increasing EPN concentrations for control of WFT depended on the type of the EPN strain used (EPN*Dose: $F_{20, 121} = 2.78$, $P = 0.0003$). Thus, WFT mortalities across different concentrations for a given EPN strain were compared to the natural WFT mortality in the control treatment. WFT mortality by *H. bacteriophora* PAL H04 and *S. abassi* PAL S09 at 100 IJs cm^{-2} did not differ significantly from the water-treated control (Table 2.2).

Table 2.2. Mean corrected mortality (CM) (%) of mixed soil-dwelling life stages of western flower thrips as induced by five different entomopathogenic nematode (EPN) species/strains at different concentrations (100, 150, 200, 400, and 1,000 infective juveniles cm^{-2}).

EPN species/strains	EPN concentrations				
	100	150	200	400	1000
H. indica LN2	30.6 c [a]	39.0 c	60.9 b	80.5 a	89.7 a
H. bacteriophora PAL H04	12.7 d [ns]	20.3 d	46.3 c	66.9 b	84.6 a
H. indica LN10	28.1 c	36.1 bc	54.6 b	66.0 ab	79.2 a
S. abassi PAL S09	14.7 c [ns]	15.1 c [ns]	32.6 b	42.2 ab	51.9 a
S. bicornutum	20.7 d	30.7 dc	45.1 bc	60.1 b	76.3 a

[a] CM means within a row followed by the same letters are not significantly different (Tukey's test, $\alpha = 0.05$).

[ns] CM mean at the respective concentration did not differ significantly from the control mortality.

The effect of increasing concentrations was additionally analyzed for each EPN species/strain separately. For all strains, a concentration of 150 IJs cm^{-2} did not significantly increase WFT mortality compared to a concentration of 100 IJs cm^{-2}. Similarly, even though highest CM values were recorded at 1,000 IJs cm^{-2}, these values did not differ from CM values obtained at 400 IJs cm^{-2} except for *H. bacteriophora* PAL H04 and *S. bicornutum*. Generally, WFT mortality increased with increasing concentrations (Table 2.2), but the degree of increment differed significantly among the

species/strains, as indicated by significantly different slopes of the linear regression coefficients of the five species/strains (Table 2.3). The three *Heterorhabditis* spp. had significantly greater slopes than the two *Steinernema* spp., indicating that the former ones responded stronger to the increase in concentrations than the latter ones. However, an increase in concentration of *Heterorhabditis* spp. beyond 1,000 IJs cm^{-2} may not result in a further increase in WFT mortality as the quadratic function for the three *Heterorhabditis* species/strains were significant (Table 2.3). Concentrations >1,000 IJs cm^{-2} for the two tested *Steinernema* spp. may result in further increases in CM since the quadratic coefficients of the two *Steinernema* spp. were non-significant. Generally, increasing concentrations resulted in only slight differences in terms of efficacy within the two EPN genera.

Table 2.3. Regression coefficients for the effects of concentrations of entomopathogenic nematodes (EPN) on the mortality of western flower thrips for the regression equation:
$CM = \alpha + \beta C + \gamma C^2$ where *CM* = corrected mortality, *C* = concentrations.

EPN	α	β	γ	*P*	R^2
H. indica LN2	8.20	ab 0.26***	–0.0002***	< 0.0001	0.82
H. bacteriophora PAL H04	–11.55	a 0.28***	–0.0002**	< 0.0001	0.83
H. indica LN10	10.63	b 0.18***	–0.0001**	< 0.0001	0.76
S. abassi PAL S09	17.02	c 0.04***	*ns*	< 0.0001	0.52
S. bicornutum	27.16	c 0.06***	*ns*	< 0.0001	0.67

The test for a null hypothesis that $\beta = 0$ and/or $\gamma = 0$ is indicated by *ns* or *asterisk* showing that *ns* = non significant; **, and *** = significant at $\alpha = 0.01$, and 0.001, respectively.

P and R^2 are the probability level and regression coefficient for the model, respectively. Slopes of the linear coefficient (β) of different EPN species/strains followed by the same letter are not significantly different.

Host density (experiment III)

The three-way interactions (EPN*Concentration*Density: $F_{4, 53} = 2.68$, $P = 0.0413$) were significant. Accordingly, the effect of host density on the efficacy of EPNs was analyzed separately for each EPN species/strain at their respective concentrations.

At all host densities, WFT mortality by *H. indica* LN2 and *S. bicornutum* at all concentrations were significantly higher than the control mortality ($P < 0.05$). However, the effect of host density on the efficacy of EPNs depended on the strains (EPNs*Density: $F_{2, 53} = 11.23$, $P < 0.0001$). For *H. indica* LN2 WFT mortality significantly increased with increasing concentrations at host densities of 10 ($r = 0.83$, $P = 0.0009$) and 20 ($r = 0.9$, $P < 0.0001$) but not significantly at 50 WFT/arena ($r = 0.56$, $P = 0.058$). In *S. bicornutum*, except at the medium host density ($r = 0.68$, $P = 0.022$), no significant correlation of WFT mortality and EPN concentration was observed at the lowest ($r = -0.11$, $P = 0.72$) and highest ($r = -0.83$, $P = 0.43$) host densities. Generally, in *S. bicornutum* the maximum WFT mortality was reached at a concentration of 200 IJs cm^{-2} in both the lowest and highest host density (Fig. 2.1). A further increase in concentration at 10 ($P = 0.007$) and 50 ($P = 0.018$) WFT/arena lead to sharply reduced CM levels (Tukey's test for the comparison of CM values at 200 and 400 IJs cm^{-2}).

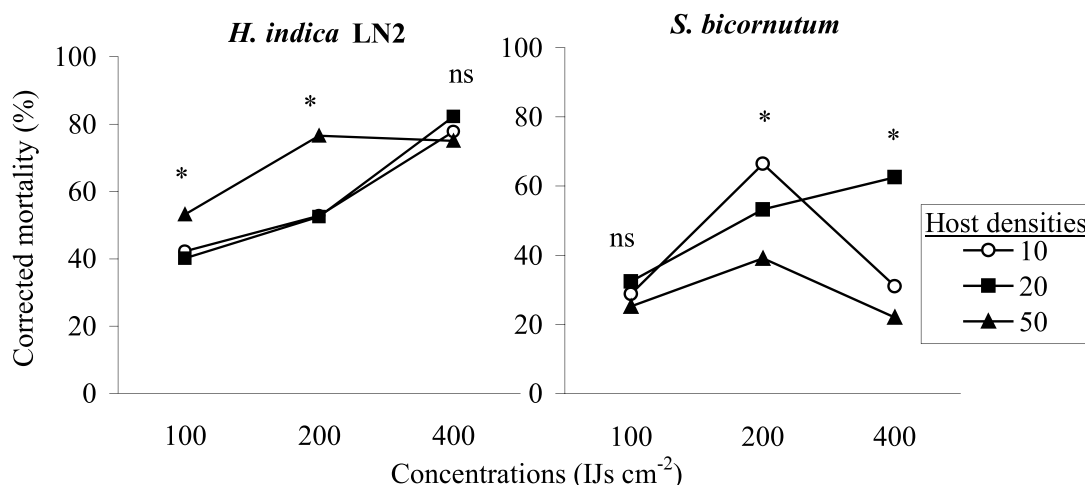

Fig. 2.1. Mean corrected mortality (CM) (%) of western flower thrips (WFT) caused by entomopathogenic nematode (EPN) strains *Heterorhabditis indica* LN2 and *Steinernema bicornutum* at different concentrations applied to varying host densities (i.e. 10, 20, or 50 WFT larvae per arena). CM means at a given concentration for a given EPN strain that differ significantly are indicated by * ($P < 0.05$). Non-significant differences are indicated by ns.

Host density to concentration ratio negatively and significantly correlated with CM in *S. bicornutum*. However, there was no significant correlation in *H. indica* LN2 application (Fig. 2.2). A quadratic model was fitted to assess the combined effects of host density and concentration on the mortality of WFT. Using the lack-of-fit method, higher degrees were discarded from the analysis. Thus, the fitted model was:

$$CM = \alpha + \beta_1 C + \beta_2 C^2 + \eta CH + \gamma_1 H + \gamma_2 H^2$$

where CM = corrected mortality of WFT caused by a given EPN strain; β, γ and η are coefficients of the concentration (C), the host density (H) and the interaction term of the two factors, respectively. The interaction term was not significant for both *H. indica* LN2 (t = 1.98, P = 0.056) and *S. bicornutum* (t = 0.77, P = 0.45). Based on the above equation, host density and concentration of *H. indica* LN2 affected the mortality linearly leading to the following equation:

$CM = 28.9 + 0.1C + 0.28H$ ($F_{2, 33} = 27.7$, $P < 0.0001$, $R^2 = 0.63$).

For *S. bicornutum* the equation turned out to be quadratic:

$CM = -28.1 + 0.54C - 0.001C^2 + 1.9H - 0.04H^2$ ($F_{4, 30} = 7.39$, $P = 0.0003$, $R^2 = 0.50$)

Derivatives of the equations were used to determine a concentration or a host density at which the maximum WFT mortality can be obtained, given one of the two variables. Since the equation was linear for *H. indica* LN2, the maximum CM could not be estimated, indicating that by increasing one of the two variables it may be possible to maximize the control level of WFT. However, for *S. bicornutum*, given a host density between 10 and 50 WFT, the maximum WFT mortality would be attained when a concentration of approximately 270 IJs cm^{-2} is applied. Likewise, given a concentration between 100 and 400 IJs cm^{-2} of *S. bicornutum*, a maximum WFT mortality would be attained when the host density is 23.75 per arena (equals one WFT L2 cm^{-2}).

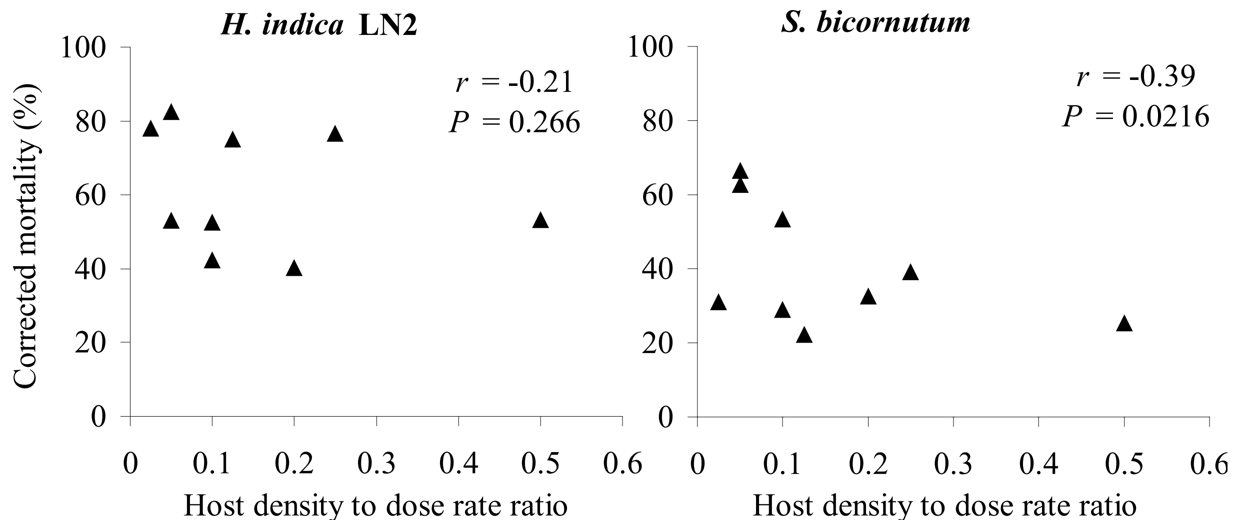

Fig. 2.2. Correlation of host density (10, 20, 50 larvae per arena) to concentration (100, 200, and 400 IJs cm^{-2}) ratio and the corrected mortality (CM) values of western flower thrips caused by an application of entomopathogenic nematode strains *Heterorhabditis indica* LN2 and *Steinernema bicornutum*.

Temperature

Steinernema bicornutum-induced mortalities at a concentration of 100 IJs cm^{-2} at 30 (P = 0.56) and 35 °C ($P > 0.99$), and at 400 IJs cm^{-2} at 35 °C ($P = 0.28$) did not differ from the control mortality. In contrast, for *H. indica* LN2 mortalities at temperatures between 20 and 35 °C were significantly higher than the control mortality.

The two-way interactions (EPNs*Concentration, EPNs*Temperature, and Concentration* Temperature) were significant (Table 2.4), indicating that the two strains differed in their efficacy against WFT depending on their concentrations and on the temperature at which the experiment was carried out.

EPN-induced mortality values by *S. bicornutum*, an EPN species originating from cooler climates, did not differ significantly from *H. indica* LN2, an EPN strain originating from warmer climates, at 20 °C at the lower concentration tested (Table 2.5). On the other hand, at the higher concentration and 20 °C CM values in *H. indica* LN2 were significantly higher than in *S. bicornutum*. The highest CM in both EPNs at the higher concentration was recorded at 25 °C. In *H. indica* LN2 but not in *S. bicornutum*, CM levels at 25 °C did not differ significantly from those recorded at 30 °C. At the lower but not at the higher

concentrations CM by *S. bicornutum* at 25 °C were not different from the one at 20 °C (Table 2.5), indicating that *S. bicornutum* in general was more effective at lower, while *H. indica* LN2 was more effective at higher temperature regimes.

The rate of WFT adult emergence was significantly faster at 30 and 35 °C (Fig. 2.3 A & B). However, the emergence rate was not significantly affected by the concentrations ($F_{2, 96} = 0.16$, $P = 0.856$).

Table 2.4. Summary of ANOVA results for corrected mortality values of western flower thrips caused by entomopathogenic nematode (EPN) strains *Heterorhabditis indica* LN2 and *Steinernema bicornutum* at two concentrations (100 and 400 infective juveniles cm^{-2}) under four different temperature (Temp) regimes (i.e. 20, 25, 30 and 35 °C).

Source of variations	df	*F*	*P*
EPN	1	73.19	< 0.0001
Concentration	1	20.26	< 0.0001
Temp	3	30.12	< 0.0001
EPN*Concentration	1	10.31	0.0024
EPN*Temp	3	9.86	< 0.0001
Concentration*Temp	3	5.43	0.0027
EPN*Concentration*Temp	3	0.26	0.8558

Table 2.5. Corrected mortality (%) of mixed soil-dwelling developmental stages of western flower thrips caused by entomopathogenic nematode species *Heterorhabditis indica* LN2 and *Steinernema bicornutum* at two concentrations (100 and 400 infective juveniles cm^{-2}) as affected by four different temperature (°C) regimes.

Temperature	100		400	
	H. indica	*S. bicornutum*	*H. indica*	*S. bicornutum*
20	23.6 B a	29.8 A a	54.2 B a	34.1 B b
25	48.9 A a	39.3 A b	84.0 A a	47.0 A b
30	36.4 AB a	3.2 B b	77.7 A a	14.1 C b
35	26.4 AB a	0.0 B b	27.2 C a	0.0 D b

Corrected mortality means (%) within a column or a row for a given concentration followed by the same upper or lower case letter, respectively, are not significantly different.

Finally, after checking the data for the appropriate model to be selected using lack-of-fit test, the effect of temperature on the CM of WFT was fitted to a quadratic function ($CM = \alpha + \beta_1 T + \beta_2 T^2$, where β_1 and β_2 are linear and quadratic slopes, respectively, CM is corrected mortality, and *T* is temperature). For *H. indica* LN2, the data from all temperatures were fitted. For *S. bicornutum*, the result showed a zero CM for almost all observations at 35 °C, and hence *S. bicornutum* data at this temperature were excluded from the analysis. The results indicate that in all cases for both EPN species the data fitted to the quadratic equation with high degrees of correlation ($0.79 \leq R^2 \leq 0.99$) and significance ($0.048 \leq P < 0.0001$) (Table 2.6). These data indicate that extreme temperatures beyond the ones tested in this study will result in lower CM of WFT for *H. indica* LN2 and *S. bicornutum* at the two concentrations tested.

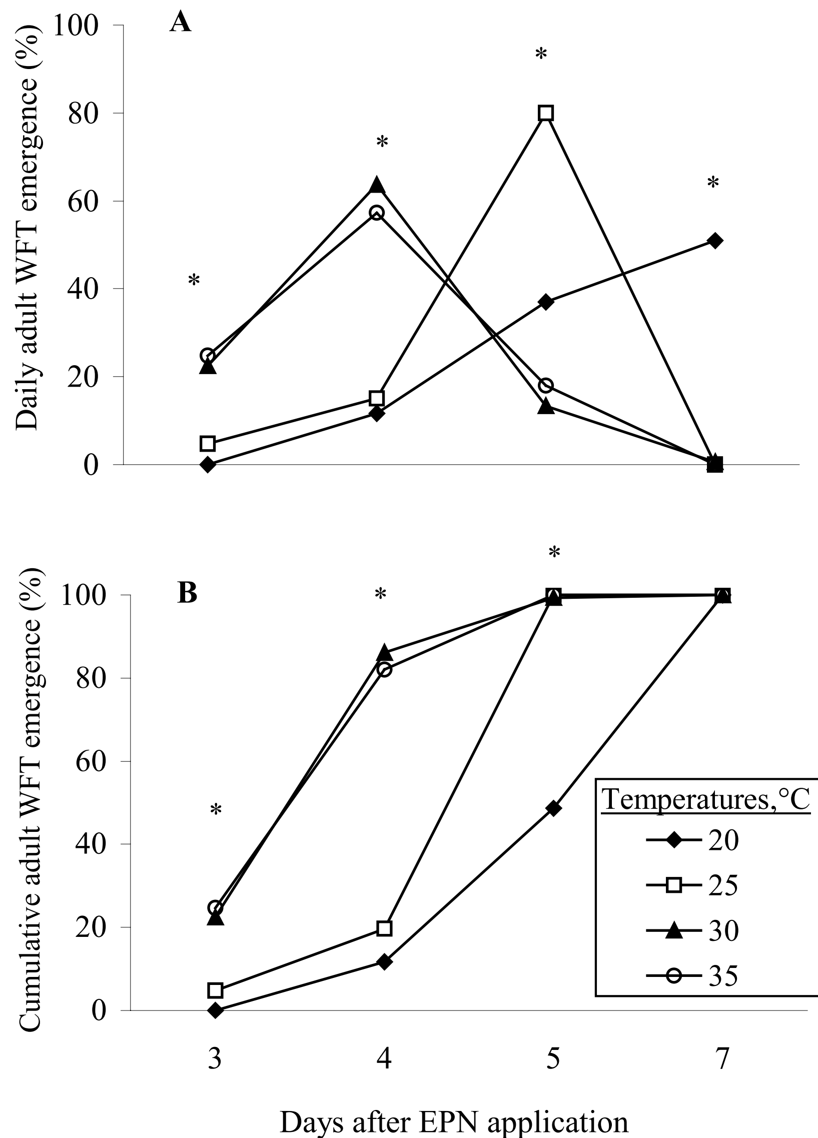

Fig. 2.3. Daily (A) and cumulative (B) adult western flower thrips emergence (%) (previously introduced as L2 to the assay, refer to Materials and Methods part for details) three to seven days after entomopathogenic nematode application at different temperatures (i.e. 20, 25, 30 and 35 °C); * indicate significant differences in adult emergence within the respective temperature regimes tested.

Table 2.6. Regression coefficients of the effects of four temperature regimes (20, 25, 30, and 35 °C) on the efficacy of entomopathogenic nematodes (EPN) at different concentrations (Conc) against mixed soil-dwelling life stages of western flower thrips for the regression equation: $CM = \alpha + \beta T + \gamma T^2$, where CM = corrected mortality (%), and T = temperature (°C).

EPN	[b] Conc	α	β	γ	P_β	P_γ	R^2
H. indica	100	– 219.9	19.3	– 0.35	0.048	0.047	0.7942
	400	– 473.4	42.4	– 0.80	< 0.001	< 0.001	0.998
[a] *S. bicornutum*	100	– 463.5	42.9	– 0.92	0.020	0.015	0.990
	400	– 475.4	43.8	– 0.91	0.004	0.003	0.990

[a] For *Steinernema bicornutum*, data from 35 °C were excluded from the analysis (see text for the details).

[b] Concentrations are given as numbers of infective juveniles cm^{-2}.

The test for a null hypothesis that $\beta = 0$ or $\gamma = 0$ is indicated by their respective *P*-value, i.e., P_β and P_γ and R^2 shows regression coefficient for the model.

2.4. Discussion

Results of this study clearly show that EPNs are efficient control agents of soil-dwelling life stages of WFT, confirming reports of several previous studies (Chyzik et al., 1996; Ebsaa et al., 2001a,b; Premachandra et al., 2003a). However, efficacy of EPNs against WFT largely varied among species and strains, and concentrations. Moreover, environmental factors such as temperature and host density, had a considerable impact on the efficiency of the tested EPN species/strains.

EPN strains (experiment I)

EPNs, even strains of the same EPN species, differ in their pathogenicity to different insect species (Hay and Richardson, 1995). In their study *S. carpocapsae* strain S.S2 from Egypt caused significantly higher CM in WFT than the *S. carpocapsae* strain A1 B5 from Italy, which might be due to the different geographical origin and environmental adaptations of the two strains.

Although we tested more *Steinernema* than *Heterorhabditis* spp./strains, we found more effective strains with *Heterorhabditis* against WFT. Similar results were also reported in other studies (Chyzik et al., 1996; Premachandra et al., 2003a) suggesting that WFT is more susceptible to *Heterorhabditis* than to *Steinernema* spp./strains; thus, in future studies more emphasis should be given to *Heterorhabditis* spp./strains.

Concentration (experiment II)

At 200 IJs cm^{-2}, except for *S. abassi* PAL S09, WFT mortality in this experiment was similar to the one recorded in experiment I. On the other hand, even the most efficient EPN strain in our study, i.e., *H. indica* LN2, yielded only CM levels of up to 40% at 100 IJs cm^{-2}. Yet, for other pests concentrations of 150 IJs cm^{-2} in the field or under semi-field conditions can result in pest population reductions >50% (e.g., McCoy et al., 2000; Samish et al., 1999). In our study, however, an increase in EPN concentration lead to an increased WFT mortality, corroborating results of previous studies (Chyzik et al., 1996; Ebssa et al., 2001a,b; Premachandra et al., 2003a). In experiment I, some EPN strains applied at 200 IJs cm^{-2} did not differ in their pathogenicity against WFT. With increasing concentrations the tested EPN species/strains responded differently in their efficacy against WFT. In general, the tested *Heterorhabditis* spp./strains resulted in significantly steeper slopes than the *Steinernema* spp./strains, indicating that the former responded more to an increase in their concentrations than the latter.

Host density (experiment III)

For a given entomopathogen, there may exist a maximum host density at which the optimum pathogen efficiency is reached (Bellows and Hassell, 1999). In our study, increasing the WFT density from 10 to 20 per arena (i.e., thrips densities of 0.42 and 0.84 cm^{-2}) did not significantly affect the efficacy of *H. indica* LN2. However, at 50 WFT per arena (i.e., 2.1 thrips cm^{-2}), *H. indica* LN2 showed a higher proportion of host mortality than at the lower host densities. This result may indicate that *H. indica* LN2, even at lower concentrations (100 and 200 IJs cm^{-2}), was under utilized at the lower host densities.

Using the methodology described in Ebssa et al. (2001a), *H. indica* LN2 was found to be more cruiser in terms of its foraging behavior than *S. bicornutum* (data not presented). Thus, due to its limited mobility, a further increase in host density to 50 WFT/arena was

probably beyond the capacity of *S. bicornutum* to parasitize its hosts, resulting in significantly lower WFT control at this density compared to the lower host densities. In most cases, the effect of EPN concentration depended on host density, but at high EPN concentrations, intraspecific interactions may negatively affect the host-finding behavior of the nematodes (Lewis et al., 1995b, 1996). In *S. bicornutum*, an increase in concentrations from 200 to 400 IJs cm^{-2} at 10 and 50 L2 WFT/arena, but not at the medium host density, negatively affected the host mortality. In *H. indica* LN2, however, the maximum CM at all host densities was attained at 400 IJs cm^{-2} though it was not significantly higher than at 200 IJs cm^{-2} and the highest host density. In general, the efficacy of EPNs against WFT could be affected by EPN species/strains, concentration, host density, or interactions of these factors indicating that increasing EPN concentrations may not necessarily increase WFT mortality.

Temperature (experiment IV)

Similar reductions and low variability of the moisture content (MC) of the substrate used in the assay arena at the different temperatures tested was recorded (data not presented). This was probably due to the constant relative humidity (rh) of 60–70% maintained in this study. Thus, the differences in EPN efficacy at different temperatures tested can be mainly attributed to the effects of temperature but not to rh and/or MC.

Steinernema bicornutum, a species originating from Yugoslavia, was more effective at lower than at higher temperatures. Likewise, *H. indica* LN2, which originates from a tropical region in India, was more effective at higher than at lower temperatures. These results indicate that efficacy of EPNs may be affected by the origin of the nematodes (Glazer, 2002; Griffin, 1993).

At 20 °C and 100 IJs cm^{-2}, *H. indica* LN2 did not significantly control WFT better than *S. bicornutum.* However, at this temperature, *H. indica* LN2 benefited more from an increase in concentration, and hence, CM levels by *H. indica* LN2 at 400 IJs cm^{-2} were significantly higher than the ones caused by *S. bicornutum* at the same concentration. This might indicate that the comparatively lower efficacy of *H. indica* LN2 at 20 °C was not due to its lower ability to parasitize WFT but due to its limited movement at relatively lower temperatures.

As long as the IJs are active and immature WFT are available as hosts in the arena, the infection process, i.e., searching, infecting, and killing of hosts, can be expected to be continuous. At the end of the experiment, IJs were observed on the top of the substrate in the arena. On the other hand, our results indicate that adult WFT emergence rate is faster at 30 and 35 °C than at 20 and 25 °C. Thus, the time in which both nematodes and immature WFT are present in the same ecological niche was shorter at higher than at lower temperatures. This means that even if the nematodes are capable of infecting and killing their hosts at higher temperatures like 30 °C, overall WFT mortality could be lower compared to a lower temperature like 25 °C due to the reduced contact time at higher than at lower temperatures. Accordingly, WFT could utilize their faster rate of development at higher temperatures as an escape mechanism against EPNs. Alternatively, the lower CM levels at lower temperatures may be due to the direct impact of temperatures on the efficacy of the IJs though under such conditions IJs may have a relatively longer contact time with their hosts.

In summary our results clearly demonstrate the great potential of EPNs as control agents of soil-dwelling life stages of WFT. Yet, generally high concentrations even for the most efficient EPN species/strains were required to achieve high control levels. However, at the comparatively low concentration of 100 IJs cm^{-2} and 23 °C, CM levels caused by *H. indica* LN2 increased from ca. 30% at a density of 20 WFT to ca. 50% at a density of 50 WFT. Thus, in the future more emphasis should be given to study factors that might enhance the efficacy of EPNs for WFT control. Moreover, costs for mass production of EPNs have substantially decreased during the last 10 years and most likely will continue to do so (Ehlers, 2001). Hence in the near future applications of EPNs for WFT control, even at comparatively higher concentrations, may become economical, particularly in high value crops like ornamentals.

3 Effects of Post-Application Irrigation and Substrate Moisture on the Efficacy of Entomopathogenic Nematodes against Western Flower Thrips *Frankliniella occidentalis*

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Abstract

Efficacy of entomopathogenic nematodes (*Steinernema bicornutum* Tallosi, Peters and Ehlers and/or *Heterorhabditis indica* (strain LN2) Poinar, Karunakar and David) against soil-dwelling life stages of western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) was assessed under different moisture conditions in a commercial plant growing substrate in laboratory experiments. In the first experiment both nematode species were tested at substrate moisture ranges of 67, 78, 88, or 95% relative moisture content that were maintained before applying the nematodes at 100 or 400 infective juveniles cm^{-2}. In the second experiment 10, 25, 50, 100, or 120 ml irrigation water, resulting in relative moisture contents of 72, 81, 90, 99%, or more than the saturation level of the substrate, respectively, was applied to the substrate. *Heterorhabditis indica* was applied either in 3 ml water and followed by irrigation, or by suspending the infective juveniles in the water amounts indicated above to apply the nematodes in higher water volume. Results indicated that at the higher application rate, initial moisture content did not significantly affect the efficacy of *H. indica* at the higher application rate. On the other hand, increasing moisture content resulted in improved efficacy of *H. indica* and *S. bicornutum* at lower and higher application rates, respectively. Similar thrips control levels of 44 and 60% at the lower and higher application rate of *H. indica*, respectively, were obtained at 88% relative moisture content. In the second experiment higher and statistically similar thrips mortality of 40 and 50% at lower and higher application rates of *H. indica*, respectively, were obtained when the infective juveniles were applied in a high volume suspension of 100 ml, or when followed by irrigation with 25 ml water, resulting in both cases in 81% relative moisture content. Generally, efficacies of the nematodes for thrips control can be improved by using an appropriate moisture content and/or post-application irrigation. Thus, the high nematode application rates required for successful *F. occidentalis* control can be partly attributed to substrate moisture content and/or post-application irrigation.

3.1. Introduction

Western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is one of the most important insect pests of many important horticultural crops in greenhouses and open fields (Brødsgaard and Albajes, 1999). Chemical control of WFT

is difficult and several pest strains have already developed resistance to many commonly used insecticides (Brødsgaard, 1994; Espinosa et al., 2002). Present biological control strategies, especially inundative releases of predatory mites and bugs, e.g. *Amblyseius cucumeris* Oudemans (Acari: Phytoseiidae) and *Orius* spp. (Hemiptera: Anthocoridae), often do not provide sufficient control of WFT (Castañè et al., 1999), particularly in high value crops like ornamentals because of their low economic threshold levels. Hence the development of alternative biological control strategies for WFT is of paramount importance.

For completing their life cycle the majority of WFT undergoes a soil-passage, where the late second instar larva descends the plants; subsequent prepupa and pupa develop in the soil (Berndt et al., 2004). This opens up the possibility for use of soil-born biocontrol agents. Entomopathogenic nematodes (EPNs) (Nematoda: Heterorhabditidae and Steinernematidae) can successfully attack soil-dwelling life stages of WFT (Chyzik et al., 1996; Ebssa et al. 2001a,b, 2004; Premachandra et al., 2003a,b). Yet so far only few EPN species/strains like *Heterorhabditis indica* Poinar, Karunakar, and David strain LN2 and *Steinernema bicornutum* Tallosi, Peters, and Ehlers caused high mortality in WFT and only when applied at comparatively high application rates (Ebssa et al., 2004a).

Appropriate soil moisture levels (Fujiie et al., 1996; Grant and Villani, 2003; Koppenhöfer et al., 1995) and post-application irrigation (Selvan et al., 1994) can enhance the efficacy of EPNs. High EPN survival and movement require optimum films of free water in soil particles (Glazer, 2002; Kondo and Ishibashi, 1985). At lower soil moisture levels, movement and infectivity of EPN can be inhibited and under dry soil or exposed conditions there is a risk of desiccation (Glazer, 1992; Grant and Villani, 2003). At high soil moisture the host-seeking of nematodes is restricted since all soil pores are filled with water, thereby eliminating the water-film surface tension, which nematodes require to push against for locomotion (Gaugler, 1988). For a better percolation of the EPN suspension the soil has to be moist prior to nematode application. Additionally, to assist nematodes in their search for hosts, EPN applications need to be followed by irrigation to assure that the infective juveniles (IJs) can reach the soil depths where the different hosts are found (Cabanillas and Raulston, 1996a,b; Selvan et al., 1994).

In the last decades peat has increasingly become the substrate of choice in plant pot cultures (Nappi and Barberis, 1993). Thus testing the efficacy of biocontrol agents in such

a media is crucial. Substrates with high proportions of peat have greater total porosity (macro- and micropores), container capacity, and available water (Brückner, 1997). These physical properties of the substrate determine, among others, the mobility of IJs in the media (Kung et al., 1990; Smith, 1999), which in turn affects the efficiency of EPNs to locate and parasitize their hosts. Finally, the amount of irrigation water needed to rinse the nematodes down to their target region in the containers strongly depends on the type of plant growth media used. Hence we tested the efficacy of EPNs for WFT control in a peat-based commercial growth substrate (Fruhstorfer Erde, Archut GmbH, Lauterbach-Wallenrod, Germany). The specific objectives of this study were (i) to test the effects of soil moisture level and irrigation water on the efficacy of two nematode species with different foraging strategies against *F. occidentalis*, and (ii) to determine if soil moisture and irrigation need to be adjusted to reduce the nematode application rates without affecting their efficacies in controlling WFT.

3.2. Materials and Methods

Nematodes and thrips cultures

Heterorhabditis indica and *S. bicornutum* were obtained from the Institute of Phytopathology, Christian-Albrechts University Kiel, Germany. In terms of their foraging behaviour the former is more a cruiser, while the latter is more an ambusher strategist (Ebssa et al., 2004a). The nematodes were reared in the laboratory at 23 ± 2 ºC in greater wax moth larvae *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) following the standard EPN rearing procedures (Kaya and Stock, 1997). *Heterorhabditis indica* (a tropical strain) and *S. bicornutum* (a temperate strain) were stored at 15 and 4 °C, respectively, until use. The nematodes were not more than one month old after harvested from *G. mellonella* larvae and were acclimatized for at least 6 h at room temperature before use. Required application rates of the nematodes were prepared from stock cultures following a quantification and dilution procedure described in Kaya and Stock (1997). Uniform-aged WFT required for the experiments were obtained by rearing the thrips on pods of green beans *Phaseolus vulgaris* L. (Fabaceae) in an incubator (23 ± 2 ºC, 50–60% relative humidity (rh), and L16:D8 h photoperiod) (Berndt et al., 2004).

General methodology

Substrate, i.e., Fruhstorfer Erde, was sieved with a 1.2 × 1.2 mm sieve, and then added to the assay arena in the respective experiments. Plastic pots (5.0, 8.0 and 5.0 cm height, top and base diameter, respectively) were used as arenas. Fruhstorfer Erde is composed of humus, clay and peat in a proportion of 15:35:50, respectively, and has a high water holding capacity (i.e., ca. 480% weight of water to weight of oven-dried substrate) due to its high proportion of peat (Kuntze, 1972). Late second instar larvae (L2) of WFT (8 to 9 days old after the emergence of the neonates) were transferred to the top of the substrate in the arena using a fine camel hairbrush. A Petri dish (diameter 10 cm) was used as a cover for the arena. To allow ventilation, a small hole (diameter 20 mm) was drilled into the centre of the Petri dish and to prevent thrips from escaping, thrips-proof gauze (64 µm pore size) was glued onto the hole. The inner part of the Petri dish, except the hole, was painted with insect glue (Temmen GmbH, Hattersheim, Germany) and served as a ‘sticky trap’ to trap emerging adult thrips. The thrips in the arena were incubated in a growth chamber for two days so that some of the L2 could moult into prepupae and pupae (Premachandra et al., 2003a), thereby providing a population mixture of different developmental stages in the substrate. On the second day after L2 introduction, EPN suspensions were pipetted on the top of the substrate. An equal amount of distilled water to the EPN suspensions was pipetted in the untreated controls. WFT adults started to emerge two days after EPN application. Hence, starting from the second day after EPN application, adult WFT on sticky traps and on the top of the substrate in the arena were removed and counted daily under a binocular for one week until no more adult thrips were observed. In all experiments an assay arena was randomly assigned to a treatment depending on the types of experimental designs used in the respective experiment. The experiments were carried out in a growth chamber (23 ± 2 °C, ca 70% rh, and L16:D8 h photoperiod). All experiments were repeated twice over time, with two and three replicates, respectively, giving a total of five replications per treatment. Levels of efficacy of EPNs in the different treatments were assessed using data on WFT adult emergence.

Experiment I: Effect of substrate moisture levels on the efficacy of EPNs

In a preliminary experiment, the maximum water holding capacity (WHC) of Fruhstorfer Erde, the model substrate used in our study, was determined by the oven-dry method

(Cassel and Nielsen, 1986). The moisture content (MC) at the maximum water holding capacity (MC_{WHC}) is 84.5% (w/w). It was calculated by dividing the amount of water retained in the substrate after drainage ceased by the weight of the fully wet substrate after drainage ceased. To determine the amount of water retained in the substrate after drainage ceased, the weight of the oven-dried substrate was deducted from that of the fully wet substrate. The drainage ceased 6 h after watering the substrate as suggested by Cassel and Nielsen (1986). To minimize the loss of substrate moisture during the course of the experiment, and to keep thrips and EPNs in the arena, the perforated base holes of the pots were fully closed. Ten g of the substrate (MC ca. 55%, i.e., weight of water to weight of the substrate with water) was added per pot. Then, 20 late L2 WFT were introduced to each pot and incubated for two days as indicated above. Two days after L2 introduction 0, 3, 8, or 13 ml water was added per pot. After ca. 30 min, which assured that the water had uniformly moistened the substrate, an EPN suspension in 2 ml water was applied. The different amounts of water were added to the substrate to obtain different MC levels. Based on high natural mortality in thrips and/or low efficacy of EPNs at the two extreme MCs of the substrate, the lowest and highest MCs included in this experiment were determined in preliminary experiments and adjusted to the ones indicated in Table 3.1. A relative moisture content (RMC) of the substrate, a more practical moisture level indicative than the actual moisture content, was used as treatments. The RMC at a given MC of the substrate was calculated as: $\%RMC = \frac{AMC}{MC_{WHC}} * 100$ where $\%RMC$ = relative moisture content of the substrate as a percentage of the actual moisture content (AMC) to the moisture content at the maximum water holding capacity (MC_{WHC}) of the substrate.

Table 3.1. Amounts of water (ml) used for pre-nematode application to establish different moisture contents (%) of the substrate and nematode suspensions (Experiment I), and for nematode suspension and post-nematode application to rinse the nematodes (Experiment II).

	Amount of water [1]	AMC [2]	RMC [3]	Water potential [4]
Experiment I	0 + 2	56.5	67	– 30
	3 + 2	65.5	78	– 8
	8 + 2	74.5	88	– 4
	13 + 2	79.9	95	– 2
Experiment II	3 + 10	61.0	72.0	– 25
	3 + 25	68.5	81.0	– 6
	28 + 0	68.5	81.0	– 6
	3 + 50	76.1	90.1	– 4
	3 + 100	83.9	99.3	– 1
	103 + 0	83.9	99.3	– 1
	3 + 120	More than saturation		

[1] In Experiment I, EPN suspension was applied in 2 ml distilled water after the substrate was moistened with 0, 3, 8, or 13 ml of water. In experiment II, for the amount of water indicated as X + Y, the nematodes were suspended in X ml water and pipetted on the surface of the substrate in the arena before applying Y ml water, which was used for rinsing the nematodes down.

[2] The actual moisture content (AMC, in %) of the substrate was determined through an oven-dried method (w/w).

[3] Relative moisture contents (RMC, in %), a more practical soil moisture parameter than AMC in entomopathogenic nematode studies, was expressed as the ratio of AMC to the moisture content of the used substrate at its maximum water holding capacity (84.5%).

[4] The water potential (kPa) was determined using the filter paper method (Kaya and Stock 1997).

Finally, *H. indica* or *S. bicornutum* at 100 or 400 IJs cm^{-2} in 2 ml distilled water were applied to an arena, resulting in the final MC of the substrate (Table 3.1). EPN species, application rate, and moisture content were combined factorially in a completely randomised block design. Each treatment, i.e., different RMC of the substrate, applied with EPNs had its own untreated control treatment where 2 ml distilled water instead of an EPN suspension were pipetted to the substrate.

Experiment II: Amount of water required for rinsing EPNs down to the WFT pupation depth

The perforated base holes of the pots were closed with thrips-proof gauze (64 µm pore-size) that allowed IJs to pass through. In preliminary experiments, in a similar arena but only with a depth of ca. 0.5 cm, more than 80% of the applied IJs could pass through the gauze. In post-nematode applications, the IJs can be washed down below the depth of host insect if the amount of water used in post-application is higher than the saturation level of the substrate. The arenas were filled with 60 g substrate (MC = 58% ± 2.2), forming a top area of 33.2 cm² and a depth of 4.5 cm. Twenty late L2 WFT were transferred to the arena. Then the arena was covered with a sticky trap as indicated above. Approximately 100 g substrate was added to another plastic pot (10 cm base diameter) and the arenas with WFT were placed individually on the top of the substrate in the second pot. Two days later, i.e., after mixed soil-dwelling developmental stages of WFT had developed, *H. indica* at application rates of 100 or 400 IJs cm^{-2} was pipetted to the top of the substrate in the arena (MC = 54% ± 0.60). Due to decreasing virulence in *S. bicornutum* over several generations in vivo rearing, this nematode was excluded from this experiment. In order to wash the nematodes down to the depth where the thrips pupate, different amounts of rinsing water were used (Table 3.1). Any excess water in the arena could percolate down to the substrate in the bigger second pot. The main factors, i.e., rinsing water levels, and EPN application rates, were combined factorially in a completely randomised block design. For all EPN treatments, equal amounts of distilled water were applied in the untreated controls.

Statistical analyses

WFT mortality data were corrected for control mortality using Abbott's corrected mortality (CM) formula (Abbott, 1925). The efficacy of EPN strains under different conditions was evaluated using the CM data. CM values were arcsine transformed before subjected to

statistical analyses. Data were analysed using SAS version 8 (SAS Institute, 1999). Data of experiments repeated over time were checked for homogeneity of variance using the HOVTEST = LEVENE option in the ANOVA procedure and pooled only when variance homogeneity could be assumed. The data were analysed using the GLM procedure in SAS to determine single or interaction effects of factors. Regression analyses were performed using the REG procedure. Lack-of-fit tests were used to determine the appropriate model to be used in the regression analyses. Whenever significant interactions were observed between factors, treatment means of one factor were compared at each level of the other factor. CM means caused by EPN applications in different treatments were compared to zero (the CM of the control treatment) using Dunnett's two-sided test. When significant factor effects were detected by means of ANOVA, CM means at different levels of the respective factor were compared using least significance difference (LSD) and t-test mean comparison procedure for all pairwise and two sample comparisons, respectively. A significance level of $\alpha = 0.05$ was used in all analyses. Data are presented as means ± SE.

3.3. Results

Experiment I

WFT suffered higher mortality in the untreated control at higher substrate moisture (Table 3.2). The highest proportion of emerged adults in the untreated control treatment was obtained at a RMC of 67%. Except in *S. bicornutum* at 100 IJs cm^{-2} and 67% RMC thrips mortalities in all EPN treatments were significantly higher ($P < 0.05$, Dunnett test) than in the untreated controls.

The effect of the relative moisture content of the substrate on the efficacy of EPNs depended on the nematode species and the application rates (EPN*Concentration*RMC: $F_{3,59} = 2.87$, $P = 0.037$). Thus, the effect of RMC was assessed for each EPN species at the different application rates. Corrected mortality values at different RMCs ranged from 7.4–63.5% depending on the two EPN species and their application rates. At 400 IJs cm^{-2}, RMC did not significantly affect the efficacy of *H. indica* ($F_{3,21} = 0\ 24$, $P = 0.867$). However, at 100 IJs cm^{-2} WFT mortality was significantly reduced at a RMC of 67%. On the other hand, RMC levels significantly affected the efficacy of *S. bicornutum* at both 100 and 400 IJs cm^{-2}. The lowest CM values at 100, and 400 IJ cm^{-2} were obtained at RMCs of 67 and 95, and at 67%, respectively (Table 3.2).

Table 3.2. Mean (± SE) of adult western flower thrips emerged (%) in untreated controls, and corrected mortality (%) values (± SE) of western flower thrips caused by *Heterorhabditis indica* (strain LN2) and *Steinernema bicornutum* at two application rates (100 and 400 IJs cm^{-2}) as affected by the different moisture contents of the substrate (expressed as the relative moisture content (RMC, %) of the substrate; see text for details).

RMC	Emergence	*H. indica*		*S. bicornutum*	
		100	400	100	400
67	94 ± 6.4 a	21 ± 6.7 A b	55 ± 6.0 A a	7 ± 4.7 A b	17 ± 3.6 B c
78	88 ± 4.1 ab	31 ± 3.9 A ab	64 ± 6.9 A a	27 ± 2.4 A a	28 ± 4.0 B bc
88	76 ± 6.0 bc	44 ± 3.9 A a	59 ± 7.7 A a	15 ± 6.3 B ab	35 ± 6.4 B b
95	66 ± 5.0 c	38 ± 9.1 A ab	59 ± 9.3 A a	8 ± 3.4 B b	52 ± 3.8 A a

Means within a column and row (for a given application rate) followed by the same lowercase and uppercase letters, respectively, are not significantly different (LSD-test).

The difference between the two EPN species depended on their application rates and the substrate moisture. At lower moisture levels and lower nematode application rates, and near the saturation level of the substrate at higher EPN application rates, WFT mortality did not significantly differ (Table 3.2).

The negative effect of higher RMC levels on the efficacy of the two nematode species was not evident except for *S. bicornutum* at 100 IJs cm^{-2}, in which the relationship between WFT mortality and RMC lead to a significant quadratic function ($Y = 12.8X - 0.1X^2 - 149.5$, P-value of both terms = 0.0148; Y is thrips corrected mortality, and X is the relative moisture content). At 100 and 400 IJs cm^{-2} of *H. indica* ($Y = 0.71X - 24.2$, $P = 0.036$), and *S. bicornutum* ($Y = 1.2X - 64.9$, $P < 0.0001$) respectively, increase in RMC resulted in significantly higher efficacy of the nematodes as indicated by the significant slopes of the linear functions.

In terms of their efficacy against WFT, *H. indica*, and *S. bicornutum* significantly responded to increases in their application rates only at 67 and 78%, and at 95% RMC, respectively (Table 3.3). Furthermore, considering RMC as a covariate variable, an

increase in WFT mortality due to an increase in EPN application rates from 100 to 400 IJs cm^{-2} was significantly affected by moisture content in *S. bicornutum* ($F_{1, 32}$ = 8.3, P = 0.007) but not in *H. indica* ($F_{1, 37}$ = 1.6, P = 0.213). Moreover, increases in WFT mortality due to an increase in application rate of *S. bicornutum* at 67 and 78% RMC were significantly lower than the ones at other RMC levels except for *H. indica* at 95%.

Table 3.3. Slopes for increase in western flower thrips mortality due to an increase in application rates of entomopathogenic nematodes (EPN) from 100 to 400 IJs cm^{-2} at different relative moisture contents (RMC) of a substrate expressed as the actual moisture content of the substrate to the moisture content of the substrate at its maximum water holding capacity.

EPN	RMC (%)	Slope
H. indica LN2	67	0.110 ab *
	78	0.109 ab *
	88	0.049 b
	95	0.071 abc
S. bicornutum	67	0.032 c
	78	0.001 c
	88	0.067 b
	95	0.148 a ***

Slopes followed by the same letters are not significantly different (adjusted multiple t-test, Zar, 1999). *, *** indicate that a slope at a particular RMC is significantly different from zero at α = 0.05, and 0.0001, respectively.

Experiment II

Rinsing water had a significant impact on adult emergence in WFT ($F_{6, 24}$ = 6.44, P = 0.004). Under the use of excess rinsing water, WFT suffered significantly higher natural mortality than when lower amounts of water were used. Moreover, rinsing water of 100 ml resulted in a lower adult WFT emergence (Table 3.4) than other lower amounts of rinsing water.

Table 3.4. Effect of different water volumes (ml) used for suspending *Heterorhabditis indica* LN2 and as irrigation in post-nematode application on (i) emergence of adult western flower thrips (mean % ± SE) in non-EPN control treatments, and (ii) efficacy of *H. indica* at two application rates for western flower thrips control.

[1] Volume of suspension + irrigation	Adult emergence	Mean (% ± SE) corrected mortality	
		100 IJs cm^{-2}	400 IJs cm^{-2}
3 + 10	94 ± 2.9 a	18.6 ± 3.1 ab	28.7 ± 1.1 bc
3 + 25	86 ± 5.3 a	36.6 ± 6.3 a	49.7 ± 4.0 a [ns]
28 + 0	91 ± 2.9 a	20.7 ± 6.3 ab	43.5 ± 6.4 ab
3 + 50	85 ± 2.0 a	35.8 ± 3.5 a	53.0 ± 1.1 a
3 + 100	62 ± 3.2 b	25.0 ± 3.5 ab	51.4 ± 1.8 a
103 + 0	67 ± 8.0 b	39.0 ± 10.3 a	48.1 ± 2.8 a [ns]
3 + 120	43 ± 4.3 c	13.2 ± 7.1 b	16.4 ± 4.8 c [ns]

[1] For the volume of water indicated as X + Y, the nematodes were suspended in X ml water and pipetted on the surface of the substrate in the arena before applying Y ml irrigation water.

Means within a column followed by the same letter are not significantly different (LSD test).

[ns] Thrips corrected mortality values at 100 and 400 IJs cm^{-2} are not significantly different at the particular treatment (t-test).

The amount of post application water significantly affected EPN efficacy in WFT control ($F_{6, 39} = 6.51$, $P < 0.0001$). On the other hand, the post application irrigation did not depend on the application rate of the nematode suspension (for interaction term: $F_{6, 39} = 0.51$, $P = 0.795$). Except under the use of excess water, i.e., 120 ml water ($P = 0.418$ for both application rates, Dunnett test), corrected mortalities in all post application irrigation treatments for both nematode application rates were significantly higher than the natural mortality in the untreated control treatment. Application of the nematodes in a 3 ml suspension that was followed by 25 or 50 ml post-application irrigation at both application rates and 100 ml at the higher EPN application rate resulted in significantly higher thrips mortality compared to the use of excess irrigation, i.e., 120 ml at the respective application

rates (Table 3.4). Moreover, suspending the nematodes in 103 ml water followed by no additional irrigation caused high thrips control. On the other hand, 10 ml water was not sufficient to rinse the nematodes down; hence, the mortality in this treatment was lower than the one in which sufficient irrigation water was used (Table 3.4). The efficacy of the nematodes did not differ between the use of the nematode in a higher amount of suspension or rinsing the nematodes that had been applied in only a little amount of suspension.

Increasing the nematode application rates from 100 to 400 IJs cm^{-2} under the use of excess irrigation water did not improve thrips control. However, in all treatments, except at an application of the nematodes in 3 or 103 ml suspension followed by an irrigation of 25 or 0 ml, respectively, thrips mortalities at an application rate of 400 were significantly higher than at 100 IJs cm^{-2} (Table 3.4).

3.4. Discussion

When the substrate was saturated up to 95% of its maximum water holding capacity, as high as 35% thrips natural mortality was recorded, probably due to suffocation of the thrips. Helyer et al. (1995) reported up to 40% thrips mortality in a compost experiment, suggesting that natural mortality in thrips can be rather high. Even if such substrate saturation limits movement of thrips, thereby hampering their potential escape from an IJs attack, migration of the nematodes could be also negatively affected (Molyneux and Bedding, 1984). Consequently we recorded lower thrips mortality at low application rates of *S. bicornutum*, the less mobile nematode species used in our study. On the other hand WFT preferred relatively drier substrate conditions. In such a dry substrate, IJs encounter problems in mobility and persistence (Grant and Villani, 2003). When IJs are able to persist for several days in the substrate, one can presume continuous infection attempts. However, when nematode persistence is interrupted because of drier substrate conditions, the infection process could cease even if WFT in their susceptible developmental stages remain in the substrate. Thus, in our study EPNs caused lower WFT mortalities at lower moisture conditions, possibly because of difficulties in migration and/or persistence of the IJs.

In our study, moisture levels differently affected the ability of the two nematode species to control thrips, corroborating results from earlier studies (Fujiie et al., 1996; Grant and

Villani, 2003; Koppenhöfer et al., 1995; Molyneux and Bedding, 1984). *Heterorhabditis indica* was less affected by different moisture levels than *S. bicornutum*, as indicated by the slope of the regression equation of WFT mortality and moisture levels. In *H. indica* at the lower application rate, the level of the substrate moisture was crucial for an even distribution of the nematodes in the substrate. At higher substrate moisture levels, *H. indica* migration was improved, possibly leading to higher numbers of IJs at the depth of pupation of thrips, and consequently causing higher WFT mortality. Yet levels of moisture content did not affect thrips mortality at the higher application rate. Possibly here already at the lower moisture level a sufficient number of IJs were present at the depth where thrips pupate to cause high mortality in WFT. Increasing the moisture levels might have enabled even more IJs to reach regions where WFT pupates, but as there is no linear relationship between *H. indica* application rates and WFT mortality (Ebssa et al., 2004a) a further increase in IJs did not result in higher rate of host infection.

For a less mobile EPN species like *S. bicornutum*, the advantage of increased substrate moisture could be that the IJs might have been carried to deeper depths with the water used for suspending the nematodes if the substrate is already moist enough. In such situations, when the nematode is applied at a low application rate, most of the IJs could percolate with the EPN-suspending water even to the deeper depth than WFT, though some IJs may be retained on the way down in the substrate. From such a depth ambusher IJs like *S. bicornutum* may not be able to ascend in searching of their hosts, possibly explaining the low efficacy of *S. bicornutum* at 100 IJs cm^{-2} even at an increased moisture content of the substrate. However, at higher application rates more IJs on their way down with the percolating water might stick to substrate particles at various depths. In such a scenario, the number of IJs at the depth of WFT pupation could be high enough for efficient WFT control. Yet in our study similar WFT control was achieved at lower and higher EPN application rates, like in *H. indica*, when the appropriate substrate moisture was maintained. At RMC 88 and 95% high WFT mortality was caused by *H. indica* at 100 and 400 IJs cm^{-2}, with no significant differences between the two application rates. For *S. bicornutum* higher nematode application rates were always required to achieve higher thrips mortality.

For field applications of EPNs most often large spray volumes are required (Shetlar, 1999). When lower spray volumes are used, pre- and/or post-application irrigation may be

required to help nematodes to reach their target hosts in the soil (Downing, 1994; Shetlar, 1999). In our study, similar and higher WFT mortalities were obtained for a given application rate when the IJs were either formulated in a higher amount of suspension (i.e., 100 ml) or when IJs were formulated in a low volume suspension (i.e., 3 ml) but followed by medium to high amounts of post-application irrigation (25–100 ml). Up to 80% of WFT pupate at a depth of 3–5 cm (Ebssa et al., 2004c). Thus, the majority of the applied nematodes need to be washed down to this depth. In over saturated substrates (i.e., 120 ml post-application irrigation) the pores were most likely filled with water, thereby hampering the mobility of the IJs. Moreover, up to 30% of the applied IJs could be recovered from the water that had percolated through the substrate (data not shown). Thus, low WFT mortality in the high amount of post-application irrigation treatments was probably due to lower application rates and/or reduced mobility of the IJs. Use of intermediate amounts of post-application irrigation water (25 ml irrigation) or high volume EPN suspension (103 ml EPN suspension followed by no irrigation) resulted in similar levels of WFT mortality at lower (100 IJs cm^{-2}) and higher (400 IJs cm^{-2}) EPN application rates. However, very low (10 ml) or high (100 and 120 ml) amounts of irrigation water, or low volumes of EPN suspension (28 ml) without any post-application irrigation caused lower thrips mortality at the lower than the higher nematode application rates.

In summary, it is possible to achieve similar levels in WFT mortality using a four times lower application rate of *H. indica*, making the use of EPNs an economically interesting strategy for WFT control. Hence, use of an efficient EPN species/strain, appropriate substrate moisture when applying, and sufficient post-application irrigation are some of the key factors for successful control of WFT by EPNs. However, the exact levels of substrate moisture and/or amount of irrigation water depend on several factors including nematode species/strains used. For Fruhstorfer Erde, our model substrate, a substrate moisture of 88% RMC was the most appropriate pre-application moisture condition. Alternatively, applications of low volume EPN suspensions should be followed by intermediate amounts of irrigation water or the EPNs should be formulated in higher volumes of suspension. In ongoing studies, we are investigating in the greenhouse post-EPN-application irrigation systems at different moisture conditions of several commercial growing substrates to optimise use of EPNs for control of soil-dwelling life stages of WFT.

4 Efficacy of Entomopathogenic Nematodes against Western Flower Thrips *Frankliniella occidentalis* at Different Pupation Depths

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Abstract

To study effects of western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) pupation depth on the efficacy of entomopathogenic nematodes (EPNs), *Heterorhabditis indica* strain LN2 and *Steinernema bicornutum* were applied at concentrations of 100 and 400 infective juveniles (IJs) cm^{-2} to WFT that had pupated at different depths. Additionally, effects of EPN concentrations and thrips densities were tested on the pupation depth of WFT. A higher concentration of *H. indica* was required when the thrips pupated deeper. Yet applications of *S. bicornutum* even at a high concentration resulted in a significantly lower WFT mortality at greater than shallower depths. Generally, WFT control levels of 5–57% were obtained depending on depth of pupation, EPN species, and concentrations. The results clearly indicate that WFT at high EPN concentrations seem to remain at a certain soil depth in order to avoid EPN attraction by moving around. Without or at low EPN concentrations, up to 80% of WFT pupated at the deepest depth of 3–5 cm. However, at higher thrips densities and EPN concentrations, 45–48% of WFT pupated in the medium depth of 1–3 cm. Thus, depth of pupation is an important factor in WFT control using EPNs.

4.1. Introduction

Western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) has become a major pest, especially in vegetable and ornamental crops. It causes considerable crop damage through direct feeding and as a virus vector of for example *Tomato Spotted Wilt Virus* (TSWV) (Kirk, 2002). Several control strategies for WFT have been developed (Jacobson, 1997), but it is often not possible to keep WFT below the economic threshold level, particularly in high value crops such as ornamentals in greenhouses (Sadof and Sclar, 2002). This has been partly attributed to the short life cycle and high fecundity of thrips females, often leading to fast population build-up (Gaum et al., 1994), and the low economic threshold levels in ornamentals (Sadof and Raupp, 1997).

To complete its life cycle WFT undergoes six developmental stages: eggs, first and second instar larvae, prepupae, pupae, and adults. The late second instar larvae (L2) of WFT exhibit positive geotaxis by moving away from plants towards the soil for pupation (Berndt et al., 2004). In compost that was compacted loosely using a spatula, WFT was reported to pupate as deep as 3.5 cm (Helyer et al., 1995). According to van Lenteren et al. (1995) the

majority of WFT pupate at depths between 1.5 and 2.0 cm. Unless disturbed both prepupae and pupae stages are immobile and do not feed. Depending on suitability of the host plants, prepupal and pupal stages last for 1 to 5 days at temperatures between 20 and 30 °C (van Lenteren et al., 1995).

Entomopathogenic nematodes (EPN) are widely used as biocontrol agents against insect pests in the soil (Ehlers, 1996) and on leaves as foliar application (Williams and Walters, 2000) mainly because of their symbiotic bacteria that cause septicemic death to the insect hosts. However, EPNs differ in their efficacy depending on, among other factors, EPN species/strains and susceptibility of host insects (e.g., Hay and Richardson, 1995). Abiotic (such as soil texture and moisture, temperature, aeration, plant root exudates etc.) and biotic factors (such as host density, host size, intra- and inter-specific competition, natural enemies etc.) also affect the efficacy of EPNs (Ebssa et al., 2004a; Kaya and Koppenhöfer, 1996; Koppenhöfer et al., 1995; Zervos et al., 1991). In their foraging behaviour, EPNs are categorized into ambushers, which have a 'sit-and-wait' strategy, 'cruisers', which actively search for their hosts, and intermediates, which show both types of foraging behaviour (Lewis, 2002). Depending on their foraging behaviour, EPN performance may vary under different environmental conditions. Provided sufficient time, cruisers may perform better in finding their hosts at different depths in the soil than ambushers. However, to reach their hosts at deeper soil depths, ambushers often need to be washed down into the soil by means of post-application irrigation (Cabanillas and Raulston, 1996b).

Potential use of EPNs as biocontrol agents against WFT has already been reported (Chyzik et al., 1996; Ebssa et al., 2001a; Premachandra et al., 2003a). All soil-dwelling life stages of WFT are highly susceptible to EPNs, but adult thrips through their negative geotaxis can escape a nematode attack by leaving the soil (e.g. Ebssa et al., 2004a). Yet, EPN species/strains differ in their efficacy against WFT and high nematode concentrations are required for WFT control. *Heterorhabditis indica* Poinar, Karunakar, and David (strain LN2) and *Steinernema bicornutum* Tallosi, Peters, and Ehlers (type strain) are among the most efficient EPN species/strains for control of *F. occidentalis* (Ebssa et al., 2004a). Substrate moisture and the amount of irrigation water used post application can markedly affect the efficacy of *H. indica* and *S. bicornutum* (Ebssa et al., 2004b).

EPNs can be applied where soil-dwelling life stages of WFT are already present in the soil. However, it may take some time until the nematodes reach the depth where the majority of

WFT pre/pupae stay. If the time required for the nematodes to reach these levels is greater than the time the thrips need to complete their life cycle, the majority of the thrips might escape an EPN attack by emerging as adults, thereby leading to decreasing levels of EPN-induced mortality in WFT. Moreover, it is possible that the depth of pupation in WFT is influenced by the thrips density and/or EPN concentrations. Likewise, depth of thrips pupation may affect the efficacy of EPNs. Thus, our objectives were to test the efficacy of EPNs against WFT that pupate at different depths and to assess if the depth of pupation of WFT is affected by thrips density and application of nematodes.

4.2. Materials and Methods

Maintenance of nematodes and thrips

Heterorhabditis indica and *S. bicornutum*, obtained from the Institute of Phytopathology, Christian-Albrechts- University Kiel, Germany, were reared (24 ± 1 ºC) in greater wax moth larvae *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Kaya and Stock, 1997). Until use, *H. indica*, a tropical strain, was kept at room temperatures while *S. bicornutum*, a temperate strain, was stored at 4 °C. The nematodes were a maximum of one month old and acclimatized for at least 6 h at room temperatures before use. From the EPN stock cultures, quantification and dilution procedures described in Kaya and Stock (1997) were used to prepare the required concentration of the nematodes. The WFT culture was maintained by rearing insects on pods of green beans *Phaseolus vulgaris* L. (Fabaceae) at 23 ± 2 ºC, 50–60% relative humidity (rh), and L16:D8 h photoperiod (Berndt et al., 2004).

General methodology

Assay arenas were prepared from plastic pots (top and base diameters 8 and 5 cm, respectively, and height 5 cm). Fruhstorfer Erde, a commercially available growing substrate (Archut GmbH, Lauterbach-Wallenrod, Germany), sieved with a 1.2 × 1.2 mm mesh sieve, was used as a model substrate and added to an assay arena in the respective experiments. This substrate is composed of humus, clay, and peat (15:35:50) and has a water holding capacity of ca 480% wt/dry wt substrate. A Petri dish (diameter 10 cm) was used to cover the arena. A 20 mm diameter hole was drilled into the centre of the Petri dish to allow ventilation into the arena. To prevent thrips from escaping, thrips-proof nylon gauze (64 µm pore size) was glued onto the hole. The inner part of the Petri dish, except

the hole, was painted with insect glue and served as a 'sticky trap' to trap emerging adult thrips. Four days after the introduction of late L2 WFT (8–9 days old after neonate) into the arena, adult thrips started to emerge. Hence, adult thrips were daily counted from sticky traps and the top of the substrate in the arena under a stereomicroscope for one week until no more WFT were observed. To repeat the experiments over time, the trials were carried out in two runs with two and three replicates in the first and second runs, respectively, giving a total of five replicates per treatment. The experiments were conducted in a climate-controlled chamber (23 ± 2 °C, ca. 70% rh, and 16L:8D h photoperiod).

Depth of WFT pupation and EPN efficacy

To prepare assay arenas, the bases of the pots were cut out at different heights serving as different pupation depths of 0.5, 1.0, 2.0, 3.0, and 4.0 cm (Fig. 4.1.1) (and hereafter referred as arena). The bottom of the arena was closed with thrips-proof nylon gauze (64 µm pore size) that allowed water percolation but prevented WFT leaving the arena. Each arena was placed in another similar plastic pot without cutting but filled, except for the volume left for the arena, with the substrate. The substrate in the bottom pot served as a reservoir for any excess percolating water from the arena. Additional substrate, i.e., 1.6 to 2.7 g substrate, depending on the base area of the arena, was added to the arena so that a substrate depth of ca. 0.2–0.3 cm could be maintained before introducing late L2 WFT (Table 4.1). To allow thrips to pupate at any depth within the total 5.0 cm substrate depth, a 'free pupation' depth treatment pot, which was similar to the other pots but without any cutting and fully closed base, was filled with 45 g substrate before introducing the L2 WFT. Each treatment was replicated five times. In the first two replicates, 20 L2 per pot were introduced in all treatments. In the last three replicates different numbers of L2 were introduced to maintain WFT density equal to approx. 0.6 L2 cm^{-2} at the base of the arena at which insects were placed (Table 4.1). Immediately after all introduced L2 had descended into the substrate, the remaining upper part of the arena was filled with substrate to the top of the pot. The total amount of substrate in the bottom pot and in the arena was approximately 45 g for all arenas with different heights. Thus, a similar moisture content of the substrate in the arenas was assured among pots with different treatments. Then, the arena was covered with a sticky trap and incubated for two days to obtain a mixture of late L2, prepupae, and pupae.

Table 4.1. Amount of substrate (g) added to the arena before and after introducing different numbers of late second instar larvae (L2) of western flower thrips (WFT) into assay arenas that vary in their base area (cm^2) and height (cm), and hence allowing WFT to pupate at different depths.

Assay arena		L2 per pot [3]	Amount of substrate added to the arena	
Height	Base area [2]		Before L2 introduction	After L2 introduction
0.5	34.2	20	2.7	7.1
1	31.2	18	2.4	14.3
2	28.3	17	2.1	22.9
3	25.5	15	1.7	28.7
4	22.9	13	1.6	36.2
Free pupation[1]	36.3	21	45.4	–

[1] Arenas for this treatment were prepared from plastic pots (5.0 cm) without any cutting and were filled with the substrate before placing WFT L2 onto the top of the substrate.

[2] This is the top area of the substrate in the pot onto which L2 were placed.

[3] Varying numbers of WFT were used to maintain equal numbers of L2 per area (i.e., 0.6 L2 cm^{-2}) in three out of five replicates. In the remaining two replicates, 20 L2 WFT were used in all treatments.

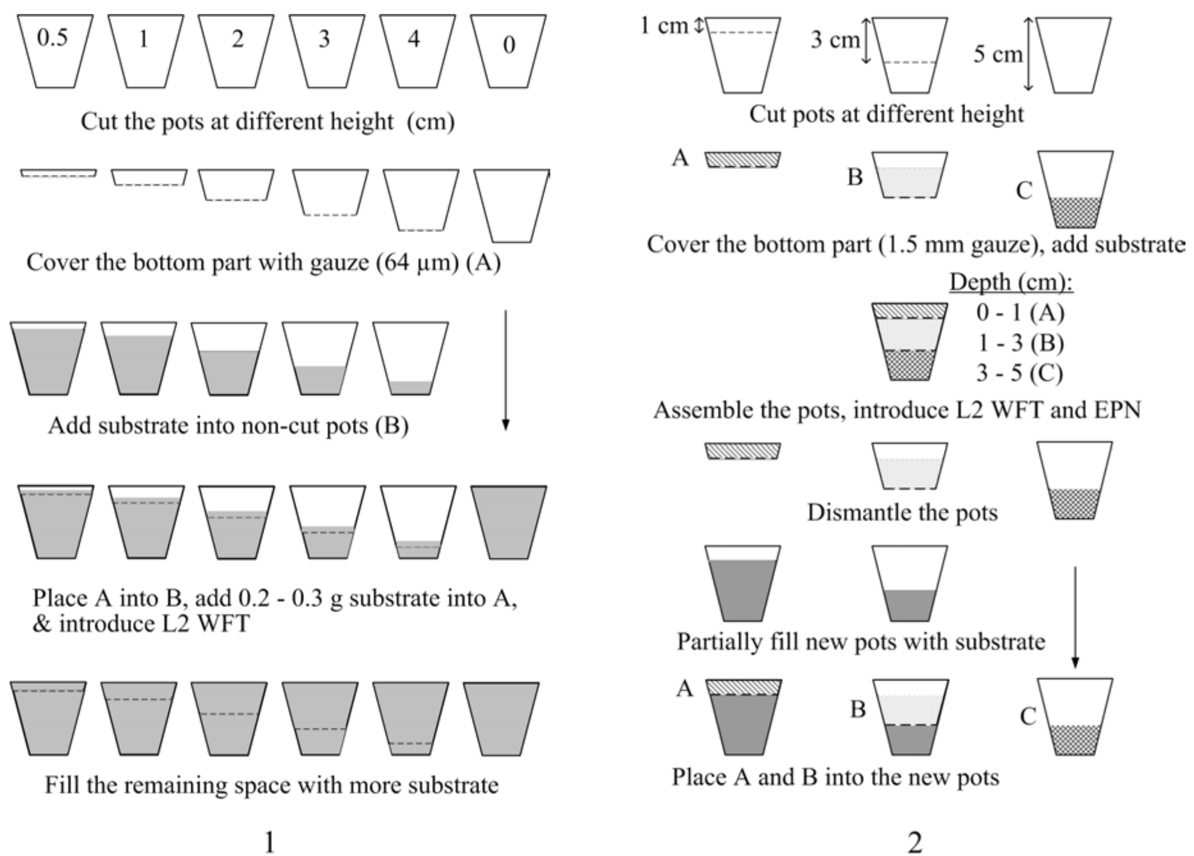


Fig. 4.1. Experimental assay arenas prepared from plastic pots (5.0 cm height): (1) The pots were cut out to create arenas with different heights (0.5, 1.0, 2.0, 3.0, and 4.0 cm) and a non-cut pot (thus, 5.0 cm). The arenas with different heights and non-cut pots but similar to the arenas were filled with the required amount of substrate so that they fitted into one another. Then, second instar larvae of WFT were introduced from the top and the remaining part was filled with the substrate. (2) Plastic pots were cut to create arenas with 1 or 3 cm height that were filled with substrate. The appropriately cut pots were placed into one another so that pupation depths of 0–1, 1–3, and 3–5 cm were established.

Two days after L2 introduction, *H. indica* or *S. bicornutum* were applied to the top of the substrate in the arenas at concentrations of 100 and 400 IJs cm^{-2} in 3 ml distilled water. For all pupation depth treatments, the control treatment was distilled water (3 ml) only. To maintain the final moisture content of the substrate equal to ca. 70% and to wash the IJs down into the substrate, in all treatments 10 ml distilled water was pipetted onto the substrate after application.

Influence of thrips density and EPN concentrations on WFT pupation depth

The lower portion of the plastic pots described above was removed to create pots with heights of 1 or 3 cm, termed pot A and B, respectively (Fig. 4.1.2). Gauze with 1.5 mm diameter pore size was used to close the bottom end of pots A and B allowing both thrips larvae and IJs to easily pass through. Pots A and B were then filled with 9.3 and 25.6 g substrate to maintain 1and 2 cm substrate depths, respectively. Further substrate (22.9 g) was added to form a substrate depth of 2 cm in non-cut pot (pot type C). Pot A then was placed into the empty portion of pot B directly onto the substrate and finally placed into pot C (Fig. 4.1.2; the reassembled three pots are termed an arena). Twenty, 50, or 70 L2 WFT were placed onto the top of the substrate in the arena. The larvae penetrated into the substrate immediately after this transfer. *Heterorhabditis indica* (100 or 400 IJs cm^{-2} in 3 ml distilled water) was then uniformly pipetted on the top of the substrate in the arena, creating a moisture content of ca. 70%. In the untreated control only distilled water was used. An arena was randomly assigned to one of the factorially combined thrips density and EPN concentration treatments. Finally, each arena was covered with a sticky trap and incubated for 3 days in the climate chamber, during which 70% of the larvae develop to prepupae or pupae (Premachandra et al., 2003a). Thus after 3 days the majority of the insects were in an immobile development stage and further movement within the arena would be insignificant. Three pots, (A, B and C), were then dismantled (Fig. 4.1.2). To avoid thrips escape and excessive moisture loss through the bottom part of the pots, A and B were placed separately in another similar plastic pot that was filled with the same substrate. Pots A and B were carefully fitted into the lower pots avoiding any gap between the top and lower pots (Fig. 4.1.2). All pots (A, B, and C, were covered with a sticky trap and returned to the climate chamber. From day 2 onwards, emerging adult WFT were daily counted until no more thrips emerged. The effect of thrips density and EPN concentration on the depth of thrips pupation was determined from the proportion of WFT adult counted from pots A, B, or C, in the different treatments.

Statistical analyses

WFT mortality data were corrected for control mortality using Abbott’s formula (Abbott, 1925). The efficacy of EPN species under different conditions was evaluated using the corrected mortality (CM) data. The CM data were arcsine transformed before statistical analyses. For the experiment on the effects of WFT density and EPNs on pupation depth,

the proportion of adults that had emerged for a given EPN concentration and thrips density at a given depth was calculated as the ratio of number of adult thrips recovered for the treatment combination at the particular depth to the total number of adult thrips recovered for the same treatment combination over all depths. Data of experiments repeated over time were checked for homogeneity of variance using the HOVTEST = LEVENE option of ANOVA procedure in SAS version 8 (SAS Institute, 1999) and pooled only when variance homogeneity could be assumed. Single or interaction effects of factors were determined using the GLM procedure. Regression analyses were performed using the REG procedure of SAS. Lack-of-fit tests were used to determine the appropriate model in the regression analyses. Whenever significant interactions were observed between factors, the level of one factor was compared at each level of the other factor. Mean mortalities caused by EPN applications under different conditions were compared to zero (the CM of the untreated control) using Dunnett's two-sided test. When significant factor effects were detected by means of ANOVA, means at different levels of the respective factor were compared using the least significance difference (LSD) mean comparison procedure. A significance level of $\alpha = 0.05$ was used in all analyses. Data are presented as means ± SE.

4.3. Results

Depth of WFT pupation and EPN efficacy

In the EPN-free control treatments up to 90% of the introduced L2 WFT were recovered as emerged adults at the end of the experiment. The percentage of WFT recovered from different pupation depths did not differ significantly ($F_{5, 38} = 1.62$, $P = 0.179$) (Table 4.2). Using the different numbers of L2 as a covariate variable, L2 densities did not significantly affect the efficacy of the two EPN species at both concentrations ($F_{1, 56} < 0.001$, $P = 0.991$). Thus, all treatments here after were analysed irrespective of the initial numbers of L2 used.

Table 4.2. Mean (± SE) percentage of adult western flower thrips that had emerged from second instar larvae placed at different depths (cm) in a pot in the untreated control.

Depth	Adults emerged *ns*
0.5	81.0 ± 5.3
1	75.0 ± 5.6
2	79.7 ± 5.7
3	75.2 ± 6.2
4	89.0 ± 7.7
Free pupation[1]	77.8 ± 3.5

[ns] The proportion of WFT emerging from different depths of pupation did not differ significantly (ANOVA: $F_{5, 38}$= 1.62, P = 0.179).

[1] L2 were placed on the top of a fully substrate filled pot (see text for details).

Heterorhabditis indica at 100 and 400 IJs cm^{-2} applied to WFT at different pupation depths resulted in significantly higher WFT mortality than in the respective untreated control treatments (P < 0.05). However, *S. bicornutum* at 100 IJs cm^{-2} applied to WFT at pupation depths of 2, 3, and 4 cm, and a 'free pupation' treatment did not cause significantly higher WFT mortality than in the respective untreated control treatments (P = 0.150, 0.310, 0.120, 0.216, respectively; Dunnett test).

Generally, pupation depth affected the efficacy of both EPN species similarly. Moreover, the effect of EPN concentrations did not depend on the depth of pupation (Table 4.3). Except in *H. indica* at 400 IJs cm^{-2}, significantly lower CM was recorded at the deeper pupation depths of 3 and 4 cm than the shallower 0.5 cm (Table 4.4). In general, increase in pupation depth had a negative impact on the efficacy of both EPN species as indicated by negative slopes of regression equations though not significant in all cases (Table 4.5). Yet, when L2 were left free to pupate at any position in a substrate of 5 cm depth, i.e. the 'Free pupation' treatment, the CM did not significantly differ from the one at the

shallowest pupation depth (i.e., 0.5 cm) except for *S. bicornutum* at 100 IJs cm^{-2} (Table 4.4).

Table 4.3. ANOVA table for single and interaction effects of applying two entomopathogenic nematodes (EPN) at two concentrations (Conc) to control western flower thrips that pupated at different depths (Depth).

Source of variations	df	*F*	*P*
EPN	1	46.22	< 0.0001
Depth	5	7.37	< 0.0001
Conc	1	34.73	< 0.0001
EPN*Depth	5	0.62	0.6866
EPN*Conc	1	7.30	0.0086
Depth*Conc	5	1.75	0.1339
EPN*Depth*Conc	5	1.00	0.4225
Error	72	–	–

In *H. indica*, only at the deepest pupation depth did an increase in concentration result in significantly higher WFT mortality (Table 4.4). However, in *S. bicornutum* the higher concentration most often caused significantly higher WFT mortality except at 1 and 4 cm pupation depths (Table 4.4). In general, though not always significant, *H. indica* at both concentrations resulted in higher thrips mortality than *S. bicornutum* at the corresponding concentrations at a given pupation depth. At 4 cm pupation depth, *S. bicornutum* at the higher concentration caused significantly lower mortality compared with *H. indica* at the lower concentration.

Table 4.4. Corrected mortality (%) of western flower thrips (WFT) at different pupation depths (cm) caused by applications of *Heterorhabditis indica* and *Steinernema bicornutum* at concentrations of 100 and 400 infective juveniles cm^{-2}.

Pupation depth	*H. indica*		*S. bicornutum*	
	100	400	100	400
0.5	53.5 a A	57.4 a A	26.7 b A	53.3 a A
1.0	42.8 a AB	54.9 a A	20.2 b AB	28.2 b BC
2.0	34.2 ab BC	42.9 a A	5.1 c C	26.6 b BC
3.0	22.8 a C	37.7 a A	7.3 b C	20.4 a C
4.0	29.5 b BC	42.2 a A	5.9 c C	16.5 c C
Free pupation[1]	36.0 b ABC	54.5 a A	13.4 c BC	42.2 ab AB

Means within a column (row) followed by the same upper (lower) case letters are not significantly different (LSD, $\alpha = 0.05$).

[1] Second instar larvae of WFT were left free to pupate at any position in a substrate of 5 cm depth.

Influence of thrips density and EPN concentrations on WFT pupation depth

Irrespective of the different pupation depths, 73.4% (SE = 6.9) of the introduced larvae were recovered as adult thrips in the untreated control at the end of the experiment. This rate of recovery in the control did not depend on the density of the thrips ($F_{2, 8} = 0.70$, $P = 0.5252$). Thus, the proportion of recovered adults at a given depth was used to assess the effect of host densities and EPN concentrations on the pupation depth of WFT.

Table 4.5. Regression coefficients of an equation CM = $\alpha + \beta$D, where CM is corrected mortality (%), D is pupation depths (i.e., 0.5, 1, 2, 3, and 4 cm), for two entomopathogenic nematodes (EPNs), i.e., *Heterorhabditis indica* and *Steinernema bicornutum*, applied at 100 and 400 infective juveniles cm^{-2} to western flower thrips second instar larvae pupated at different depths.

EPNs	Concentrations	α	$\beta^{ns,\ 1}$	R^2	*P*-value
H. indica	100	51.3	−6.7	0.78	0.0040
	400	58.5	−5.43	0.49	0.0800
S. bicornutum	100	25.4	−4.8	0.50	0.0504
	400	48.9	−9.5	0.75	0.0040

[ns] Linear slopes at different concentrations of the two EPN species are not significantly different (adjusted multiple t-test [Zar, 1999]).

[1] Using lack-of-fit test, the equations fitted only linearly.

In general, 50−80% of the L2 migrated to the bottom part of the arena (i.e., 3 to 5 cm depth) and only negligible proportions (0.6–6.9%) pupated at 0 to 1 cm depth. Analysis of variance indicated that the proportion of thrips pupating at different depths differed significantly. Furthermore, for the proportion of emerged adult WFT from different depths under different treatment, depth significantly interacted with both thrips density and nematode concentration (Table 4.6). Accordingly, the effects of host density and EPN concentration on thrips pupation were assessed separately for the different pupation depths.

Table 4.6. ANOVA table for single and interaction effects of entomopathogenic nematodes (EPNs), concentrations (Conc) and thrips density on the proportion of western flower thrips at different depths.

Source of variations	df	*F*	*P*
Depth	2	397.98	< 0.0001
Density	2	1.09	0.3409
Conc	2	0.43	0.6496
Depth*Density	4	5.16	0.0008
Depth*Conc	4	10.37	< 0.0001
Density*Conc	4	0.41	0.7990
Depth*Density*Conc	8	1.36	0.2252
Error	99	–	–

The proportion of thrips that pupated at 1 to 3 and 3 to 5 cm depths, but not at 0 to 1, differed depending on the concentration of nematodes applied and the density of thrips introduced (Table 4.7). However, the interaction of host density and nematode concentration was not significant at any depth. Except at the higher WFT densities of 50 and 70 L2 per arena and an EPN concentration of 400 IJs cm^{-2}, the proportion of WFT pupating at the intermediate depth (1 to 3 cm), was significantly lower than the ones at 3 to 5 depth (Table 4.8). The proportion of WFT that pupated at 0 to 1 cm depth was usually significantly lower than the ones at 1 to 5 cm depth (Table 4.8) and at 20 L2 per arena, except at a depth of 3 to 5 cm, the proportion of adults that emerged did not depend on nematode concentration (Table 4.8). However, at higher thrips densities significantly higher proportions of thrips pupated at the intermediate depth when 400 IJs cm^{-2} were applied than 0 or 100 IJs cm^{-2}. Similarly, at the deeper thrips pupation depth and higher thrips densities, significantly lower thrips proportions were recovered when 400 IJs cm^{-2} were applied than 0 or 100 IJs cm^{-2}. However, in most cases, the proportion of thrips that

remained on the top part of the arena (0 to 1 cm depth) was not affected by the nematode concentration (Table 4.8).

Table 4.7. ANOVA table for single and interaction effects of entomopathogenic nematode concentrations (Conc) and thrips density on the proportion of western flower thrips at a given pupation depth (in cm).

Depth	Source of variations	df	*F*	*P*
0 to 1	Density	2	2.10	0.1400
	Conc	2	2.98	0.0656
	Density*Conc	4	0.74	0.5708
	Error	31	–	–
1 to 3	Density	2	5.09	0.0123
	Conc	2	5.68	0.0079
	Density*Conc	4	1.50	0.2265
	Error	31	–	–
3 to 5	Density	2	4.86	0.0151
	Dose	2	16.66	< 0.0001
	Density*Conc	4	0.95	0.4482
	Error	29	–	–

The proportion of thrips that pupated at the shallower depth at the different EPN concentrations was not affected by the WFT density (Table 4.9). However, a significantly lower proportion of thrips pupated at the intermediate depth at thrips densities of 20 or 50 compared with 70 for nematode applied treatments. At the lower concentration the proportion of thrips pupating at a depth 3 to 5 cm was also significantly lower at the highest thrips density (Table 4.9).

Table 4.8. Proportion (%) of western flower thrips (WFT) that pupated at different depths (cm) in an assay arena in which different thrips densities (L2 per arena) were established and subsequently treated with 100 or 400 infective juveniles cm^{-2} of *Heterorhabditis indica*. The corresponding overall corrected mortality (CM, in %) of WFT at a given concentration was calculated using the number of survived adult thrips from all depths in the respective EPN treatment and in the untreated control.

Density	Concentration	Proportion of WFT pupated at different pupation depths			Overall CM
		0 to 1	1 to 3	3 to 5	
20	0	3.9 c A	18.3 b A	77.8 a A	0.0 C
	100	2.5 c A	20.6 b A	77.0 a A	19.7 B
	400	6.2 b A	24.4 b A	69.4 a B	41.0 A
50	0	5.4 c A	24.3 b B	70.3 a A	0.0 B
	100	0.6 c B	18.1 b B	81.3 a A	19.1 A
	400	5.3 b A	47.0 a A	47.7 a B	25.2 A
70	0	6.9 c A	24.3 b B	68.8 a A	0.0 C
	100	5.4 c A	29.8 b B	64.8 a A	21.5 B
	400	6.1 b A	45.1 a A	48.8 a B	39.3 A

Means of proportion of WFT pupation within a row (a column) for a given density followed by the same lower (upper) case letters are not significantly different. CM means within a column for a given host density followed by the same letters are not significantly different (LSD, $\alpha = 0.05$).

Since the proportion of thrips that pupated at a given depth depended partly on the nematode concentration (Table 4.7), thrips mortality due to different EPN concentrations at a given pupation depth could not be statistically compared. Thus, efficacy of the nematode in the overall arena, i.e., including all depths, was assessed for a given density. For the overall depth, thrips density did not affect EPN efficacy ($F_{2,\ 35} = 0.7$, $P = 0.5021$). Furthermore, the difference in nematode concentration did not depend on the thrips density

(for interactions Density*Concentration: $F_{4, 35} = 0.55$, $P = 0.7037$). At all thrips densities WFT mortalities at both EPN concentrations were significantly higher than in the untreated control(s) (Table 4.8). The higher nematode concentration caused significantly higher mortality compared with the lower one at the lowest and highest host densities. Generally, low EPN efficacy was recorded even at the higher concentration of IJs (Table 4.8).

Table 4.9. Proportion (%) of western flower thrips (WFT) that pupated at different depths (cm) in an assay arena in which different thrips density (second instar larvae per arena) were established and subsequently treated with 100 or 400 infective juveniles cm^{-2} of *Heterorhabditis indica*.

Depth	Concentration	Proportion of WFT pupated at different WFT densities		
		20	50	70
0 to 1	0	3.9 a	5.4 a	6.9 a
	100	2.5 a	0.6 a	5.4 a
	400	6.2 a	5.3 a	6.1 a
1 to 3	0	18.3 a	24.3 a	24.3 a
	100	20.6 b	18.1 b	29.8 a
	400	24.4 b	47.0 a	45.1 a
3 to 5	0	77.8 a	70.3 a	68.8 a
	100	77.0 a	81.3 a	64.8 b
	400	69.4 a	47.7 a	48.8 a

Means within a row followed by the same letters are not significantly different (LSD, α = 0.05).

4.4. Discussion

The late L2 WFT used in the present experiment were 8–9 days old and ready for pupation. WFT at this developmental stage exhibit positive geotaxis (van Lenteren et al., 1995) and hence only few, if any at all, of the introduced L2 were expected to ascend in the arena after they had been placed at a desired depth of pupation and any descent was limited by thrips-proof gauze. Hence, the majority of the introduced L2 probably remained in the small amount of substrate (0.2–0.3 g) that had been added onto the gauze in the arena before introducing the larvae. Consequently, our assay arena could be used to test the efficacy of EPNs against WFT that pupated at a defined depth in the arena. In our experiments, the substrate in the assay arena was loosely compacted with a moisture content of about 68% (w/w), which is ca. 78% of the maximum water holding capacity of the substrate (Ebssa et al., 2004b). Under such conditions, WFT placed at a substrate depth of 4.0 cm could emerge easily and the depth at which L2 WFT were placed did not affect the proportion of adult thrips that emerged when no EPNs were applied.

Generally, *H. indica* performed better than *S. bicornutum*, however, their specific efficacy differed depending on thrips pupation depth. *Heterorhabditis indica*, a more cruiser type nematode in its foraging behaviour than *S. bicornutum* (Ebssa et al., 2004a), infested a similar proportion of the applied WFT whether the thrips pupated at a shallower or deeper depth, especially at sufficiently high EPN concentration. On the other hand, *S. bicornutum*, a more ambusher type nematode in its foraging behaviour (Ebssa et al., 2004a), could not control WFT when thrips pupated at depths of 2.0 cm or deeper, especially at the lower nematode concentration. Efficacy of *S. bicornutum* at higher concentrations against WFT that were allowed to pupate at any position within the 5.0 cm depth was as good as in those tests in which WFT pupated at shallower depth. Furthermore, at 4.0 cm, *S. bicornutum* at 400 IJs cm^{-2} caused significantly higher mortality in WFT than at 100 IJs cm^{-2} but resulted in significantly lower CM than *H. indica* at 100 IJs cm^{-2} indicating that *S. bicornutum* was less effective when WFT pupate at greater depth.

Koppenhöfer et al. (1996) reported that at shallower depth ambushers infect their hosts more efficiently than cruiser EPN. Our experiment shows that efficacy of an intermediate ambusher EPN species, such as *S. bicornutum* at greater depth may be improved up to a certain level by increasing the concentration of IJs applied. Increasing the concentrations

of *S. bicornutum* IJs at the shallowest pupation depth dramatically increased thrips mortality compared with mortality at greater depths. This is probably due to the intermediate ambusher foraging behaviour of *S. bicornutum* in which a high concentration of IJs may cover most of the thrips pupation zone at the shallower depth and could thus reach and successfully infest a high proportion of the thrips. On the other hand, for the thrips that pupated at the greater depths an increase in IJ concentration may be required not only for the horizontal distribution of nematodes but also for a vertical coverage of the entire thrips pupation zone. Therefore, for insects found at deeper soil depths, only an increase in concentration of an ambusher or an intermediate EPN species may not help much since the IJs need to travel from the point of application to the point of thrips pupation. In a study on vertical distribution of EPNs Campbell et al. (1996) reported that *S. carpocapsae*, an ambusher nematode, was mostly recovered from the first 1 cm of the soil column even at high nematode concentrations while *H. bacteriophora*, a cruiser EPN, was uniformly recovered throughout an 8 cm vertical soil column. In our study out of the 400 IJs cm^{-2} of *H. indica* applied on the top of the substrate in the arena, some of them might have reached even the deeper part of the substrate, resulting in similar WFT mortality to the one at the shallower depth.

Several soil born pests feed on plant roots at deeper depths. Following root exudates and provided sufficient time, EPNs may be successfully used as biocontrol agents in control of such pests that dwell deep in the soil (Boff et al., 2002; Kanagy and Kaya, 1996). However, as in *F. occidentalis* the soil-dwelling stages are non-feeding (van Lenteren et al., 1995), no such plant-derived cues can be expected that might help IJs to locate their hosts. Additionally, because of the rather short duration of the soil phase in WFT (Berndt et al., 2004) even a cruiser nematode has limited time to ‘chase’ and attack its hosts before they can complete their development and emerge as, EPN non-susceptible, adults. According to van Lenteren et al. (1995) WFT can pupate at depth as shallow as 2 cm. Among other factors, the depth of insect pupation depends on soil types, moisture content of the soil, and soil compaction (Dimou et al., 2003). Helyer et al. (1995) reported that WFT pupate as deep as 3.5 cm in a moderately compacted experimental arena. Other thrips (e.g. *Stenothrips graminum* (Uzel)) can pupate as deep as 110 cm in river clay soil (Kirk, 1997). Therefore, for effective use of EPNs in WFT control, use of irrigation in post EPN application may be required to assist the nematodes to reach deeper depth where WFT pupae/prepupae stay (Ebssa et al., 2004b; Selvan et al., 1994).

In our experiment on the effects of EPN concentrations and thrips density on the pupation depth preference of WFT, the introduced L2 could easily pass through the wider gauze and up to 80% of the introduced thrips pupated at 3–5 cm depth. In the control without EPNs of the previous experiment similar numbers of adult WFT emerged from a pupation gradient of 0.5–4 cm depth, indicating that the amount of substrate above the soil-dwelling life stages did not affect their ability to successfully emerge as adults. We assessed the effects of EPN and density of thrips on the pupation depth using the proportion of emerging adults in the different treatments. To avoid any disturbance of thrips and nematodes, the arenas were kept closed throughout the entire experiments. Hence, we did not record the actual numbers of soil-dwelling life stages of WFT and/or IJs in the different zones. Using emergence data for adult thrips to assess where WFT prefers to pupate, one might argue that a low proportion of emergence at a given depth is due to high EPN-induced mortality. However, in such a situation higher proportions of emerging adults should be expected in the controls. Yet, our results clearly show that at the shallower depth, where EPNs are preferably active (as revealed by our previous experiment), proportions of emerging WFT adults did not differ significantly between the control and even high EPN concentration. Thus measuring the proportion of emerging adults enabled us to study the pupation preference in WFT.

The proportion of thrips that pupated at greater depths strongly depended on the density of the thrips and concentrations of EPNs applied. We initially had hypothesised that at the high EPN concentration, WFT may prefer to pupate at deeper depth as a strategy to avoid contact with EPN. However, our results indicate that at no or low EPN concentrations, higher proportions of thrips pupated at deeper depth. On the other hand, in high EPN concentrations and high thrips densities similar proportions of thrips pupated at the intermediate (1–3 cm) and greater (3–5 cm) depths. This probably indicates that at high concentration of EPNs large proportions of WFT prefer to remain at a given pupation depth and avoid moving, thereby possibly minimizing the probability of encountering IJs.

To assure that the applied IJs stayed at the top portion of the arena at least during the first hours after EPN and WFT introduction, no post-application irrigation water was used in experiment 2 though sufficient moisture content was maintained. Hence it is possible that in this experiment lower numbers of IJs of *H. indica* managed to migrate in time to the deepest pupation depth before the thrips could successfully accomplish their development

cycle and emerge as adults, consequently resulting in lower overall WFT mortalities than in a previous study (Ebssa et al., 2004a).

In our experiments the L2 were introduced to the arena immediately before applying the nematodes. If EPNs had been present in the soil prior to introducing the thrips, the decision of the WFT larvae at which soil depth to pupate might have been different. As a contact-avoidance defence mechanism they might avoid zones occupied by the nematodes. However, this hypothesis remains to be investigated. Moreover, data on numbers of IJs at different depth were not recorded in our experiments. Yet, it is evident from the significantly higher thrips mortality at both concentrations compared with control that *H. indica* followed the thrips up to greater depth in the substrate, resulting in high EPN-induced thrips control.

Our results demonstrate that thrips pupation depth is an important factor that needs to be considered in WFT control using EPNs. Thrips that pupated deeper in the substrate may escape an EPN attack when the nematodes are applied in low concentrations, and/or if EPNs with an ambusher foraging behaviour are used. Thus, in substrates, in which WFT pupate at deeper depth, the use of cruiser EPN species/strains and/or post-application irrigation may be required. Our data clearly show when using ambushers for WFT control, high concentrations are required if the thrips pupate at greater depth. In ongoing studies, we are additionally investigating the potential use of foliar applications of EPNs against thrips life stages on the plants.

5 Time and Frequency of Applications of Entomopathogenic Nematodes and Their Persistence for Control of Western Flower Thrips *Frankliniella occidentalis*

Abstract

Post application persistence of two entomopathogenic nematode (EPN) strains, i.e. *Heterorhabditis bacteriophora* Poinar strain HK3 and *Steinernema carpocapsae* (Weiser) strain DD136 was studied against soil-dwelling late second instar larvae (L2) of western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). The nematodes were applied at 200 and 400 infective juveniles (IJs) cm^{-2}, and L2 WFT were introduced at 0 (same day), 3, 6, 9, or 12 days after nematode application (DANA). *H. bacteriophora* caused higher thrips mortality than *S. carpocapsae* in most of the cases and both species persisted at least for 6 days, causing WFT mortality of up to 76 and 37.8%, respectively. In a separate experiment, *H. bacteriophora* and *S. feltiae* (Filipjev) Sylt were applied at 200 and 400 IJs cm^{-2} once (10, 15, or 20 days) or twice (10 and 15, 10 and 20, or 15 and 20 days) after introduction of ten female and two male WFT adults onto bean plants (*Phaseolus vulgaris* L.). An early repeated application of *H. bacteriophora* at 200 IJs cm^{-2} resulted in significantly lower numbers WFT than a single applications at 400 IJs cm^{-2} indicating that higher WFT control can be achieved if the same concentration is split over time. However, an early application of *H. bacteriophora* at 400 IJs cm^{-2} controlled WFT better than late applications indicating that time of EPN application is additionally very crucial in WFT control. For *S. feltiae*, higher WFT mortality was recorded when nematodes were repeatedly applied on the 10th and 15th days than in any other applications at a given concentration. Thus an early application of an efficient and relatively more persistent nematode species/strains at a lower concentration but in a repeated manner can result in higher thrips control than a single application at the higher concentration.

5.1. Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a worldwide pest attacking a wide range of economically important crops in the field and greenhouses (Brødsgaard, 1989; Yudin et al., 1986). The life cycle of *F. occidentalis* consists of the adult, egg, two feeding larval instars (first and second instar larvae), followed by two immobile non-feeding stages (the prepupa and pupa) that both occur predominantly in the soil. The foliar-feeding life stages damage plants through direct feeding and/or ovipositing on leaves, leaf buds, flowers, flower buds and fruits (Childers and Achor, 1995; Rosenheim et al., 1990) and through vectoring tospoviruses such as tomato spotted wilt virus and impatiens necrotic spot virus (Kirk, 2002).

Generally, western flower thrips is difficult to control because of its cryptic feeding habits and life strategy. Repeated applications of insecticides, often considered necessary because of the high fecundity and short generation time of *F. occidentalis*, resulted in the development of WFT strains resistant to many insecticides (Brødsgaard, 1994; Immaraju et al., 1992; Zhao et al., 1995). A limited range of natural enemies, including several *Orius* spp. (Heteroptera: Anthocoridae) and phytoseiids such as *Amblyseius barkeri* (Hughes) and *A. cucumeris* (Oudemans) (Acari: Phytoseiidae), are used mainly against the first instar larvae of WFT on the foliage (van Lenteren et al., 1995); the soil dwelling life stages of WFT are not within reach of these beneficials. Thus, the presently available biocontrol strategies do not suffice to efficiently control western flower thrips, especially on high-value crops like ornamentals (Jarosik and Pliva, 1995).

Entomopathogenic nematodes (EPNs) in the families of Steinernematidae and Heterorhabditidae (Rhabditida) are important biological control agents of a large number of insect pest species in the soil and cryptic habitats (Kaya and Gaugler, 1993). The free living (third juvenile) stage, the so called ‘infective juveniles’ (IJs) of EPNs carry and transmit symbiotic bacteria that are lethal to their hosts (Boemare et al., 1996). Previous studies showed that soil-dwelling life stages of *F. occidentalis* are highly susceptible to different EPN species/strains (Chyzik et al., 1996; Ebssa et al., 2001a,b, 2004a; Premachandra et al., 2003a,b; Tomalak, 1994).

Following application, EPNs are exposed to environmental factors such as radiation, low or high soil moisture, extreme temperatures etc., which may have negative effects on their

persistence and efficacy (Glazer, 2002). EPN species/strains that are sufficiently virulent against WFT and additionally can persist for several days under field conditions are thus of paramount importance for efficient thrips control. A singe treatment with such an EPN species/strain may suffice to control WFT life stages that are already present in the soil at the time of application; moreover, depending on the persistence of the respective nematode species/strain also late L2 that enter the soil for pupation during the subsequent days/weeks can become targets. Recently, Premachandra et al. (2003a) showed that under laboratory conditions EPNs can persist for at least six days without losing virulence against *F. occidentalis*. However, no information is available on post application persistence of EPNs under more practical conditions. EPNs need to be applied early enough to avoid that thrips can escape a nematode attack by developing into adults that are not susceptible to EPNs and start emerging from the soil. Repeated applications of EPNs may be required depending on their persistence and WFT densities in the soil. Consequently EPNs need to be applied timely and at an appropriate frequency to well target the susceptible life stages of the WFT populations in the soil. The rate of development and population build up in WFT may depend on several factors such as temperature and host plant. Therefore, the objectives of the present study were (i) to assess the short-term post application persistence of EPNs under semi-field conditions, and (ii) to investigate time and frequency of EPNs applications following releases of adult thrips to the plants.

5.2. Materials and Methods

Nematode and thrips cultures

Heterorhabditis bacteriophora (Poinar) strain HK3, *Steinernema feltiae* (Filipjev) strain Sylt and *S. carpocapsae* (Weiser) strain DD136, EPN species/strains used in the present study, were obtained from the Institute of Phytopathology, Christian-Albrechts University of Kiel, Germany. The nematodes were reared at 23 ± 2 °C in the last instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), using a modified rearing procedure originally developed by Kaya and Stock (1997). IJs were stored at 4 °C until they were used. The nematodes were acclimatized at room temperature for approximately 12 h before use in the respective experiments. The required EPN concentrations were prepared by quantification and dilution procedures as described in Kaya and Stock (1997).

Western flower thrips were reared on pods of green beans (*Phaseolus vulgaris* L. [Fabaceae]) (Ullman et al., 1997) in a climate-controlled chamber at 23 ± 2 °C temperatures, 50–60% relative humidity (rh) and 16L:8D h photoperiod. Only uniformly aged insects were used in the experiments.

General methodology

For the following experiments bean plants were raised in plastic pots using Fruhstorfer Erde Type P, a commercially available plant growing substrate (Archut GmbH, Lauterbach-Wallenrod, Germany). The substrate is composed of humus, clay, and peat (15:35:50). Due to its high peat content the substrate has a high water holding capacity. The pots had a perforated base and were 7.5 and 8.5 cm in base diameter and height, respectively. A single seedling per pot was caged at the two-leaf stage using an acryl-cylinder (84 mm in diameter) following the methodology developed by Ebssa et al. (2001b). A defined number of a given developmental stage of WFT was introduced to the cage depending on the nature of respective experiments. To prevent escaping of thrips from the cage, the gap between the pot and the acryl-cylinder was filled with modelling clay. To cover the top open end of the cage, a thrips-proof nylon tissue (64 µm pore size) was glued on the edge of the cylinder. Additional ventilation to the cage was provided using two side holes on the upper portion of the cylinder and the holes were covered with the same nylon tissue. Two similar side holes in the lower portion of the cylinder were used as ‘windows’ for releasing *F. occidentalis* larvae and for pipetting nematode suspension. Either 6 ml of an EPN suspension (in the EPN treatments) or only distilled water (in the untreated control) per pot was uniformly pipetted on the top of the substrate in the pot. Thereafter, the pots were irrigated with 40 ml tap water to rinse the nematodes down. At the end of the experiments, the cylinder and shoot parts of the plants were removed. Both the shoots and the cage were examined for WFT. The pot was covered with a “sticky trap”, i.e. a Petri dish (100 mm in diameter) with two central holes (25 mm in diameter). To prevent thrips from escaping, the holes used for ventilation, were covered with thrips-proof nylon tissue. The inner part of the Petri dish, except for the holes, was painted with insect glue. Starting from the next day after removal of the cage, emerging *F. occidentalis* in all treatments were counted daily from the sticky traps as well as from the top of the substrate in the pot for 10 consecutive days until no further adults emerged.

The experiments were conducted in a growth chamber at 23 ± 2 °C temperatures, ca. 70% rh and 16L:8D h photoperiod.

Persistence study

Bean plants were caged as described above. Plants were used in this experiment to mimic natural situations in which the soil loses its water through plant evapo-transpiration, which in turn may affect the persistence of EPN. Suspensions of *H. bacteriophora* or *S. carpocapsae*, at a concentration of 200 or 400 IJs cm^{-2}, were applied onto the substrate in the pots. Using a fine camelhair brush, 20 late second instar larvae (L2) of WFT were transferred onto the surface of the substrate in the pot on the following days after nematode application (DANA): 0 (same day), 3, 6, 9, or 12 days. To synchronize the L2 in the thrips rearing culture with the DANA, 9-day-old L2 were used in three out of five replications for treatments 6 and 9 DANA while in the remaining DANA's 8-day-old L2 were used in all repetitions. Each DANA treatment had its own untreated control. The whole experiment was repeated twice over time in which two replications during the first and three replications during the second repetitions were carried out, giving a total of five replications per treatment.

In a similar preliminary experiment, adult WFT started emerging four days after L2 introduction, and hence, the cylinder and shoot parts of the plants were removed in the main experiment three days after thrips introduction and the pot was covered with a sticky trap. Data on emerging adult thrips were gathered daily starting from the next day after removal of the cage until no further adults emerged.

Population dynamics of WFT

This experiment was conducted using bean plants to determine the composition of different developmental stages of WFT at a given time after a release of a defined number of adult thrips. The plants were grown in plastic pots and caged at a two-leaf stage as previously described. Ten female and two male *F. occidentalis* (derived from a cohort 20 days after the emergence of neonate larvae) were introduced onto the caged plant. Both the cage and shoot part of the plant were removed on the 10th, 15th and 20th day after adult introduction and the pots were subsequently covered with a sticky trap as described before. The removed shoot was washed with a soap and water solution to collect all thrips development

stages on the plant. Those thrips that stuck on the inside wall of the cage were collected using a fine camelhair brush. The thrips-containing soap and water solution was first filtered through a sieve (30 µm pore size) and then through a lined Whatman # 1 filter paper (185 mm in diameter) folded like a funnel. Seventy percent ethyl alcohol was added on the filter paper to remove the foam of the soap, and to kill and preserve the thrips until they were counted under the binocular. For each day of cage removal (i.e., 10, 15 and 20 days after adult introduction) the number of thrips on the plant and cage were counted separately as larvae, prepupae/pupae, and adults. The majority of WFT population in the soil at a given time consists of pupae and/or prepupae. Hence, WFT developmental stages in the soil at the time of cage removal were considered to be pupae and/or prepupae and were inferred from the number of emerging adult *F. occidentalis* from the soil (as recorded on the sticky trap). The experiment was repeated twice over time with three replications each, giving a total of six replications per treatment (i.e., days after adult thrips introduction).

Time and frequency of EPN applications

Ten female and two male *F. occidentalis* were released on a caged green bean seedling as described above. Due to its comparatively low efficacy against WFT in the persistence study, *S. carpocapsae* was replaced by *S. feltiae* (see results). Hence, either *H. bacteriophora* or *S. feltiae* at a concentration of 200 or 400 IJs cm^{-2} were uniformly pipetted to the top of the substrate in the pots once (i.e. on the 10th, 15th, or 20th day) or twice (i.e. on the 10th and 15th, 10th and 20th, and 15th and 20th day) after adult thrips introduction. In the corresponding untreated controls distilled water only were applied. In all treatments, plants were kept for 22 days after adult thrips introduction. Then, both the shoot part of the plant and the cage were removed and thereafter the pots were covered with a sticky trap. All thrips development stages on the shoot of the bean plants, and the emerged adults on the sticky trap were collected and counted as previously described. Six replications per treatment were used and the experiment was split over two periods with three replications in each run.

Statistical analyses

To assess the significant effects of the nematode treatments on thrips population reduction, mean numbers of thrips in the different EPN treatments were compared to the number of

thrips in the untreated controls using Dunnett's two-sided test in SAS version 8 (SAS Institute, 1999). Since equal numbers of insects were introduced in all treatments in the persistence study, EPN-induced mortality in the different treatments was corrected for natural mortality using Abbott's formula (Abbott, 1925). In the other experiments, the numbers of thrips recorded in the different treatments were compared. To stabilize variance of percent mortality and thrips count data, the values were arcsine and square root, respectively, transformed before subjected to data analyses. Data from the two periods of repetition of an experiment was pooled only when the variance homogeneity assumption was not violated using the HOVTEST = LEVENE option of the ANOVA procedure in SAS. Individual and interaction effects of factors on thrips mortality and density were analysed using the general linear model (PROC GLM) of SAS. Whenever two factors (e.g. strains and concentrations) showed a significant interaction, means of the levels of one factor were compared at each level of the other factor. In the absence of significant interactions, means of the level of one factor were compared regardless of the levels of the other factor (Sokal and Rohlf, 1994). When ANOVA results indicated significant treatment effects, two and multiple comparisons were performed using the student's t-test and the least significant difference (LSD) mean comparison procedure in SAS, respectively. A significant level of $\alpha = 0.05$ was used in all analyses. Data are presented as means ± SE.

5.3. Results

Persistence study

The emergence rate of adult thrips in the no-nematode treatments ranged between 67 and 95% of the initial number of introduced thrips. At both concentrations and for all DANA, the numbers of emerged adult thrips in the *H. bacteriophora* treatments were significantly lower than in the control treatments ($P < 0.001$, Dunnett's test). Similarly, in the *S. carpocapsae* treatments, except for 6 DANA at 200, and 6 and 9 DANA at 400 IJs cm^{-2}, significantly lower numbers of thrips than the in the corresponding control treatments were recorded.

Thrips mortality differed depending on EPN species and DANA (Table 5.1). Except for Concentration*EPN all two-ways and the three-ways interaction turned out to be not significant. At the lower concentration no significant difference were observed between the

two EPN species across all DANAs except for 9 DANA (Table 5.2). However, at the higher concentrations *H. bacteriophora* resulted in significantly higher thrips mortality than *S. carpocapsae* up to 9 DANA. Furthermore, changing concentrations of the nematodes did not significantly affect thrips mortality for both EPN species on all DANAs (Table 5.1). However, trend of means showed that higher thrips mortalities were obtained at higher and lower concentrations of *H. bacteriophora* and *S. carpocapsae*, respectively (Fig. 5.1).

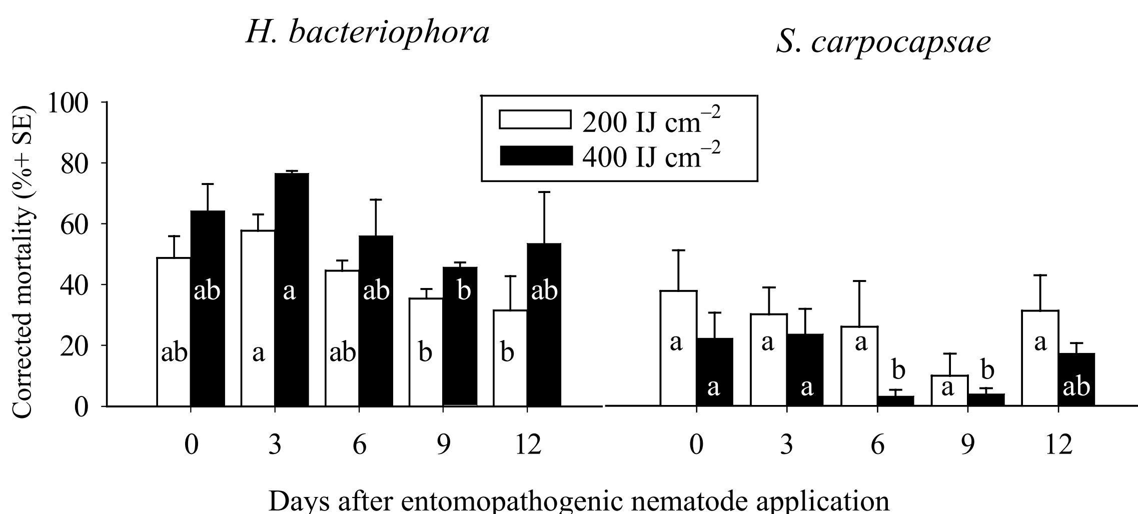


Fig. 5.1. Mean corrected mortality (% + SE) of western flower thrips late second instar larvae introduced on 0 (same day), 3, 6, 9 and 12 days after applications of *Heterorhabditis bacteriophora* or *Steinernema carpocapsae* at 200 and 400 infective juveniles (IJs) cm^{-2}. Means for a given EPN species at a given concentration followed by the same letters do not differ significantly (LSD, $\alpha = 0.05$). Note: Concentration did not differ significantly for a given EPN species at a given DANA.

Moreover, thrips mortality depended on the time between the EPN application and the subsequent introduction of *F. occidentalis*. Generally, the more the introduction of the thrips was delayed after the EPN application, the lower the efficacy of the nematodes recorded (for DANA, $P = 0.0033$, Table 5.1). For instance significantly lower thrips mortality was obtained on 9 DANA than 0 and/or 3 DANAs for both EPN species at high and/or low concentration (Fig. 5.1). For *S. carpocapsae* at high concentration thrips mortality was significantly reduced even on 6 DANA compared to earlier DANAs. However, thrips mortality at 12 DANA was not statistically different from earlier DANAs for *S. carpocapsae* at both concentrations and for *H. bacteriophora* at the higher concentration (Fig. 5.1).

Table 5.1. Single and interaction effects of the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* applied at concentrations (Conc) of 200 and 400 infective juveniles (IJs) cm^{-2} on the mortality of second instar larvae of western flower thrips introduced 0 (same day), 3, 6, 9, or 9 days after nematode application (DANA).

Source of variations	df	*F*	*P*
EPN	1	63.41	< 0.0001
Conc	1	0.06	0.8075
DANA	4	4.37	0.0033
Conc*EPN	1	12.48	0.0007
DANA*EPN	4	1.91	0.1188
Conc*DANA	4	0.20	0.9349
Conc*DANA*EPN	4	0.23	0.9213
Error	70	–	–

Table 5.2. Comparison of efficacy of *Heterorhabditis bacteriophora* vs. *Steinernema carpocapsae* at 200 and 400 infective juveniles cm^{-2} for control of late second instar larvae of western flower thrips, introduced on 0 (same day), 3, 6, 9 and 12 days after nematode application (DANA).

DANA	200		400	
	t	*P*	*t*	*P*
0	0.72	0.472	3.18	0.016
3	2.28	0.056	4.08	0.026
6	1.19	0.311	6.70	0.002
9	3.23	0.018	17.21	< 0.0001
12	0.01	0.996	2.11	0.120

Population dynamics study

Total numbers of WFT per plant increased from 226 on the 10th to 390 on 20th day after adult thrips introduction to bean plants (Fig. 5.2). Composition of thrips developmental stages was significantly affected by the time of data assessment (Stage*time: $F_{4, 45} = 35.04$, $P < 0.0001$). The experiment was stopped before the F1 adults started reproducing and when the majority of the F1 larvae had already developed into prepupae/pupae. Thus, generally, the number of larvae showed a decreasing trend from the 10th to the 20th day after adult introduction. Ten days after the introduction of adult WFT, about 13% of the total immature WFT developmental stages per plant had already left the plant to pupate in the soil (Table 5.3). The proportion of these soil-dwelling stages significantly increased with time, reaching more than 70% of the total immature stages on the 20th day after adult thrips introduction and a density of about 3 thrips cm^{-2} in the soil (Table 5.3). However, the time of introduction of adult thrips did not significantly affect the total percentage of pupation in the soil ($F_{2, 10} = 1.91$, $P = 0.1988$); on average 94.8 ± 1.4% of the thrips left the plant and pupated in the soil. Yet on the 20th day after adult WFT introduction, about 70% of the soil-dwelling life stages that had been recorded on the 15th day already emerged as F1 adult thrips (recalculated from data in Fig. 5.2). However, during the period between 15 and 20 days after adult introduction, more numbers of late L2 had left the plant and entered the soil for pupation.

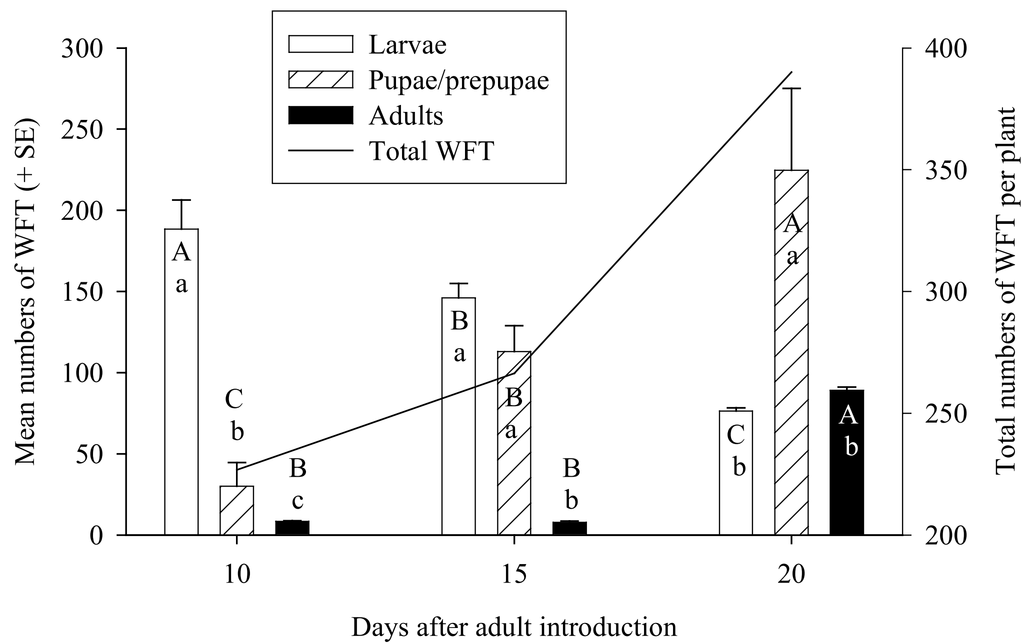


Fig. 5.2. Mean numbers of larvae, adults (both counted from the plant), and pupae/prepupae (counted from the plant and also as emerged adults from the soil) and total numbers of western flower thrips (WFT) per plant on the 10th, 15th and 20th day after introducing 10 female and 2 male adult thrips per bean plant. Different thrips developmental stages on a given day and the same developmental stages across different days followed by different lower and upper case letters, respectively, are significantly different (LSD, $\alpha = 0.05$).

Table 5.3. Mean (% ± SE) proportion (number of prepupae and pupae divided by the total number of thrips, or by the total number of immature developmental stages expressed in %) and density (mean (± SE) numbers of prepupae and pupae cm^{-2}) of western flower thrips (WFT) in the soil on the 10th, 15th, and 20th day after introduction of 10 female and 2 male adult WFT onto bean plants.

Days	[1] Proportion of WFT in the soil:		[2] WFT density
	From total no. WFT	From total no. immature	
10	12.3 ± 2.7 c	12.8 ± 2.8 c	0.39 ± 0.11 b
15	38.9 ± 5.7 b	40.0 ± 5.7 b	1.45 ± 0.37 b
20	56.5 ± 4.5 a	73.5 ± 5.2 a	2.88 ± 0.40 a

Means within a column followed by the same letter do not differ significantly at $\alpha = 0.05$ (LSD).

[1] Numbers of prepupae/pupae in the soil were inferred from the numbers of emerged adults from the soil in treatments in which shoot parts of the plants were removed on 10, 15 and 20 days after adult introduction; see text for details.

[2] The top area of the substrate in the plastic pot, which was used for raising the bean plants, was 78 cm^2.

Time and frequency of EPN applications

The total numbers of adult WFT per plant in the no-nematode treatments at the end of the experiment ranged between 175 and 258, with an average of 228 ± 12.1. In all nematode treatments significantly ($P < 0.001$) lower numbers of adult WFT compared to the corresponding control treatment were recorded. The lowest and highest adult WFT population reductions in EPN treatments compared to the thrips in control treatment were 17.8 ± 2.8 and 62.3 ± 4.5% for *H. bacteriophora* applied once on the 20th day at 200 IJs cm^{-2} and *H. bacteriophora* applied twice on the 10th and 15th day at 400 IJs cm^{-2}, respectively (recalculated from data in Fig. 5.3). The numbers of adult thrips differed significantly between/among the two EPN species, their concentrations, and time of applications with a significant interaction of EPN species and time of applications (Table 5.4).

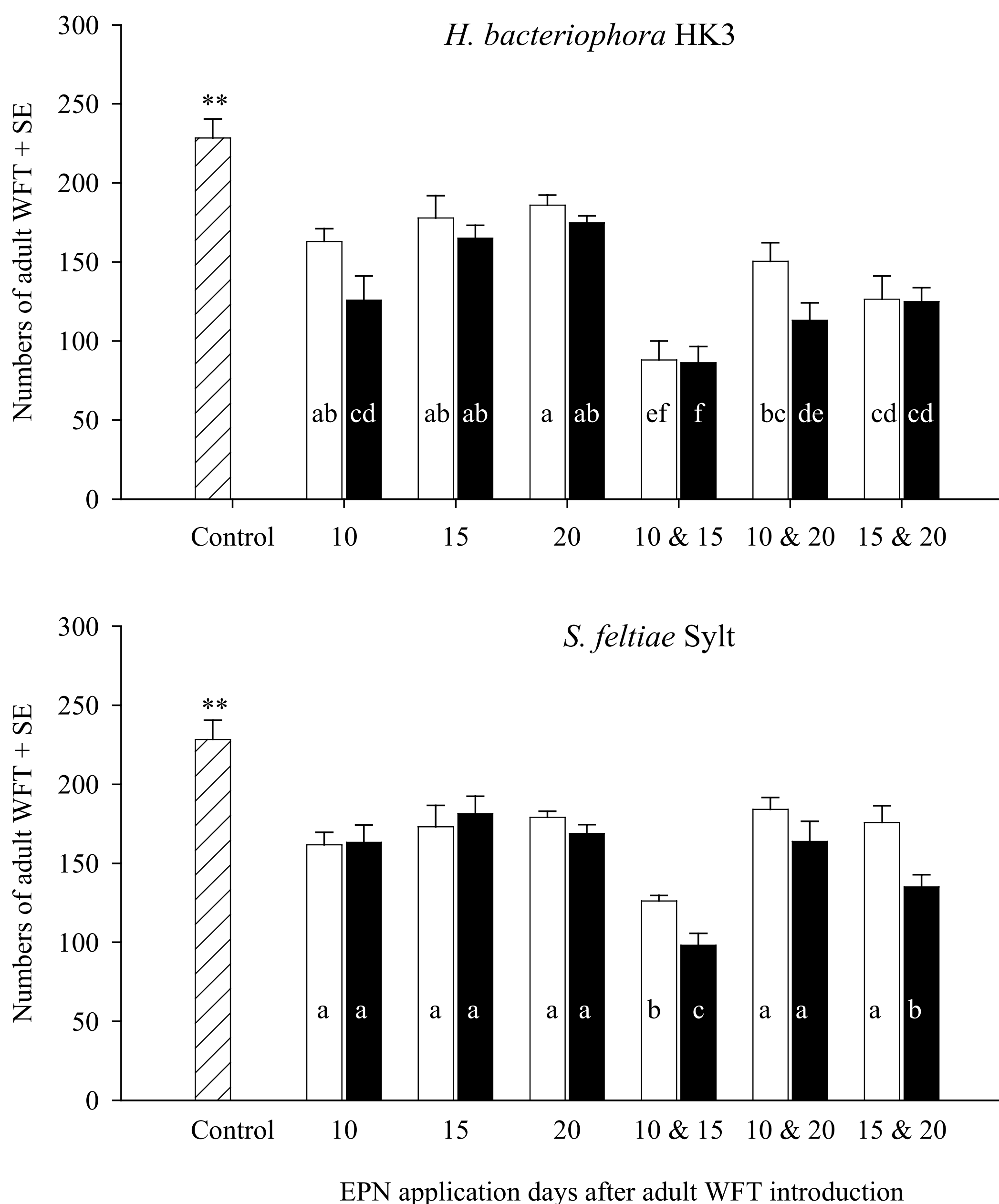


Fig. 5.3. Numbers of adult western flower thrips (WFT) as affected by applications of *Heterorhabditis bacteriophora* and *Steinernema feltiae* at 200 (□) and 400 (■) IJs cm^{-2} once (on the 10th, 15th, or 20th day) or twice (on the 10th and 15th, 10th and 20th, or 15th and 20th days) after adult thrips introduction. Any bars for a given nematode species followed by the same letters are not significantly different (LSD test). ** Denotes that numbers of adult WFT in control treatment is significantly higher than all EPN treatments ($P < 0.001$, Dunnett's test).

In *H. bacteriophora*, except for the single application on the 10th and repeated applications on the 10th and 20th day, increasing concentrations on a given application date did not result in a significant decrease in adult thrips population (Fig. 5.3). At the higher concentration of *H. bacteriophora*, a single early application on the 10th day resulted in significantly lower numbers of adult thrips than single late applications on 15th and on 20th days (Fig. 5.3). However, the effect of a single early application of the lower concentration of *H. bacteriophora* was so low that the nematodes did not significantly result in a better control level than late applications at both concentrations. In a repeated application at a given concentration, delaying the first and/or the second application resulted in higher thrips numbers than early-repeated applications on the 10th and 15th day after adult introduction. Repeated applications of 200 IJs cm^{-2} on the 10th and 15th day after adult introduction caused significantly higher thrips control than single applications at the double concentration irrespective of the day of application (Fig. 5.3).

Table 5.4. ANOVA summary for effects of the entomopathogenic nematodes (EPN) *Heterorhabditis bacteriophora* and *Steinernema feltiae* on the number of adult thrips after an initial introduction of 10 female and 2 male western flower thrips (WFT) to bean seedlings. EPNs were applied at concentrations (Conc) of 200 or 400 infective juveniles (IJs) cm^{-2} at different times (once: 10, 15, or 20 days after introduction of adult WFT, or twice: 10 and 15, 10 and 20, or 15 and 20 days after introduction of adult WFT).

Source of variations	df	*F*	*P*
EPN	1	23.12	< 0.0001
Conc	1	14.07	0.0003
Time	5	32.27	< 0.0001
EPN*Conc	1	0.03	0.8533
EPN*Time	5	2.97	0.0146
Conc*Time	5	0.84	0.5254
EPN*Conc*Time	5	2.2	0.0591
Error	115	–	–

In *S. feltiae* significantly lower adult thrips numbers were recorded by increasing nematode concentrations only in repeated applications on the 10th and 15th, and on the 15th and 20th day after adult introduction (Fig. 5.3). In single treatments, WFT control was affected neither by the time of application nor by the EPN concentration. The significantly highest level of WFT suppression was obtained in repeated applications of the higher concentration on the 10th and 15th day after adult thrips introduction (Fig. 5.3).

As the two-way interaction between EPN species and concentration was not significant (Table 5.4) the two EPN species were compared irrespective of their concentrations. Unlike in single applications on any day after adult thrips introduction, repeated applications of *H. bacteriophora* resulted in significantly lower numbers of thrips than that of *S. feltiae* (Table 5.5).

Table 5.5. Comparison of numbers of western flower thrips after applications of *Heterorhabditis bacteriophora* vs. *Steinernema feltiae* once (on the 10th, 15th, or 20th day) or twice (on the 10th and 15th, 10th and 20th, or 15th and 20th days) after adult thrips introduction.

Days of nematode application after initial introduction of adult thrips		*t*	*P*
Once:	10	1.48	0.153
	15	0.52	0.612
	20	–1.18	0.251
Twice:	10 and 15	2.43	0.024
	10 and 20	3.35	0.003
	15 and 20	2.32	0.030

Note: Since nematode concentrations significantly interacted neither with time nor nematode species (see Table 5.4), data of the two nematode concentrations were pooled for this analysis.

5.4. Discussion

Short-term persistence of EPN

With 5–33% natural mortality we recorded a high variability in thrips emergence in the untreated controls. Ebssa et al. (2001a) and Helyer et al. (1995) discussed that in experiments with WFT high natural mortality is a common phenomenon. In our methodology we mimicked greenhouse conditions in which EPNs may be applied in a prophylactic manner, i.e. before plants are attacked by WFT. Hence it is of paramount to know how long the nematodes can persist in the soil in the absence of suitable host development stages.

For logistical reasons we used in the 6 and 9 DANA treatments of this experiment cohorts of 9-day old L2 thrips whereas in the other treatments cohorts of 8-days old L2 were introduced. This could be one reason for the lower thrips mortality in *S. carpocapsae* at 6 and 9 compared to 12 DANA. Possibly, a higher proportion of the one day older L2 developed into immobile prepupae/pupae, thereby reducing the chances for IJs of a 'sit-and-wait' strategist ('ambushing' behaviour) like *S. carpocapsae* to come into contact with the hosts thrips, leading to a reduced EPN-induced mortality in WFT. In contrast, *H. bacteriophora* has a 'cruiser' foraging strategy and can thus parasitize both sedentary and mobile hosts. These findings corroborate earlier reports of Campbell and Gaugler (1997), Choo et al. (1989), Georgis and Gaugler (1991), and Lewis et al. (1993) that ambushers are more effective against mobile hosts at the soil-litter interface, while cruisers are more effective against less mobile insects in the soil.

In all cases *H. bacteriophora* caused higher mortality at 400 than at 200 IJs cm^{-2}, though not always significant, corroborating previous results by Ebssa et al. (2001a) and Chyzik et al. (1996). However, in *S. carpocapsae* we recorded higher thrips mortality at the lower than the higher concentrations. Differences in EPN-induced mortality in WFT between the two EPN strains at the two tested concentrations might be caused by their specific responses to host cues and in their different foraging strategies. IJs of ambushing nematodes like *S. carpocapsae* respond to host cues only when they come into direct contact with the cuticle of a passing-by host; yet, IJs of cruisers can respond from a distance (Lewis et al., 1993 and 1995b). Moreover, IJs of *S. carpocapsae* remain on the top

region of the soil while IJs of the cruiser *H. bacteriophora* may migrate deeper into the substrate to the levels where WFT prefers to pupate (Campbell et al., 1996; Ebssa et al., 2004c). Hence, if the number of IJs on the top of the soil is very high with respect to the host density, IJs of ambushers will not only encounter passing hosts but also conspecific IJs that make repositioning move (Campbell and Gaugler, 1993), possibly confusing their host recognition capacity (Lewis et al., 1996) and thus resulting in lower EPN-induced host mortality. This could be the most probable reason for having a decreasing trend in mortality of WFT by increasing concentrations of *S. carpocapsae*.

In terms of short-term persistency for *H. bacteriophora*, results at the higher concentration are rather variable. WFT mortality at 9 DANA at both concentrations was significantly lower than the ones at 3 DANA. On the other hand, unlike at 200 IJs cm^{-2}, WFT mortality at 400 IJs cm^{-2} on 12 DANA was not significantly different from the one at 3 DANA at the same concentration. This may indicate that at the higher application rate a larger proportion of the nematodes can persist longer and may result in a similar mortality level to an early application of the same concentration. Yet, this does not explain why mortality at the higher concentration at 9 DANA was significantly lower than at 3 DANA. However, in general *H. bacteriophora* at both the lower and higher concentrations could persist at least for 6 and 12 days, respectively. In *S. carpocapsae* at the higher nematode concentration also significantly lower WFT mortality was recorded at 6 and 9 but not at 12 DANA compared to 0 and 3 DANA, though this might have attributable to one-day age differences in the test larvae (see previous paragraph). Yet these results indicate that, like in *H. bacteriophora*, IJs of *S. carpocapsae* can persist for at least 12 days in the soil without significant loss of their virulence. At the higher concentration *H. bacteriophora* caused significantly higher thrips mortality than *S. carpocapsae* up to 9 DANA; however, no differences between the two species were recorded at 12 DANA suggesting that the persistence of IJs of *H. bacteriophora* was shorter than that of *S. carpocapsae*. Physiological and behavioural differences between the two EPN species might be responsible for the observed differences in persistence. Lewis et al. (1995a) recorded in IJs of *S. carpocapsae* a lower metabolic rate than in *H. bacteriophora*, resulting in longer persistence of the former compared to the latter EPN. Moreover, IJs of cruisers like *H. bacteriophora* consume more energy reserves when actively passing through the soil profile (Aguilar et al., 1999; Molyneux, 1985).

Time and frequency of EPN applications

In this study, the timing of EPN applications had a profound effect on WFT control. For single applications of *H. bacteriophora*, an early application at the higher concentration caused higher WFT mortality than the late application, probably because at this time the host density was near to optimal for the nematodes. Ebssa et al. (2004a) reported that a pupal density of 2.1 cm^{-2} is beyond the capacity of some EPN species like *S. bicornutum* Tallosi, Peters and Ehlers. In the course of the present study the late L2 continuously descended from the plants and penetrated into the soil leading to a population of thrips in the soil up to density of ca. 3 thrips cm^{-2}, which may exceed the control capacity of *H. bacteriophora*. However, as IJs can persist for at least 6 days in the soil, early-applied nematodes may be able to continuously infect new incoming WFT larvae. Yet if EPNs are applied late, thrips that are already present in the soil might escape a nematode attack and successfully emerge as adults. At the lower concentration repeated applications of *H. bacteriophora* within intervals of 5 days resulted in significantly higher WFT control than a single application at the higher concentration. However, the control level decreased when the interval between the first and second EPN applications increased to 10 days, probably because at the time of the second EPN application a high proportion of thrips adults had already emerged, thus successfully had escaped a nematode infection. Moreover, when repeatedly applied, EPN-induced mortality in WFT did not differ between the low and high concentrations of *H. bacteriophora*. Thus, for *H. bacteriophora* it is possible to reduce the EPN concentration if the second application is well timed, i.e. coincides with the appropriate age and density of susceptible thrips life stages in the soil.

At a given concentration of *S. feltiae*, unlike in *H. bacteriophora*, no significant differences in numbers of thrips were found in single applications at different application dates. This may indicate that a single application of *S. feltiae* cannot control thrips over extended periods, and thus irrespective of the time of application a similar proportion of the thrips population is killed. Furthermore, repeated applications of *S. feltiae* at the lower concentration did not result in higher WFT control than a single application at the double concentration. The highest mortality was recorded following early and narrowly timed repeated applications of the higher nematode concentration. Repeated applications of *H. bacteriophora* caused higher levels of WFT control than in *S. feltiae* corroborating results of previous studies in which WFT were found to be more susceptible to

Heterorhabditis than *Steinernema* spp./strains (Ebssa et al., 2001a, 2004a; Premachandra et al., 2003a)

In our experiments EPN-induced mortality in WFT, even at higher concentrations, was lower than levels recorded in previous studies (Ebssa et al., 2001a; Premachandra et al., 2003a). The most likely reason for these discrepancies is the higher host density used in this compared to the previous studies. There is no repeated foraging in an individual EPN (Lewis, 2002). Moreover, in WFT EPNs are incapable of self-perpetuation (Belay, 2003), most likely because of the small host size.

In the population dynamics study 10 days after adult introduction, more than 10% of the immature stages were already in the soil for pupation, and this proportion increased up to 20 days. Hence for WFT control EPNs have to be applied immediately after the first discovery of adult thrips on the plants and/or sticky traps. If the applied EPN species/strain has good persistence abilities and/or the environmental conditions are conducive for the persistence of the IJs (Glazer, 2002), early applied IJs would commence controlling the first pupating thrips and potentially target the larvae that will penetrate the soil during the first consecutive days after the application. Results of the present study and those of previous ones (e.g., Berndt et al., 2004) clearly show that a very high proportion of the thrips pupates in the soil, stressing the great potential of EPNs for control of the soil-dwelling life stages of WFT.

In conclusion we observed that EPNs of the two tested species/strains can persist at least for 6 to 9 days under semi-field conditions without losing their virulence; persistence was longer in ambushers than in cruisers. Moreover, it is possible to reduce the concentrations of EPNs for WFT control if the nematodes are repeatedly applied at lower concentrations and if the timing of the applications coincides well with the presence of susceptible life stages of WFT in the soil.

6 Compatibility of Entomopathogenic Nematodes and Predatory Mites to Control Western Flower Thrips *Frankliniella occidentalis*

Abstract

Single and combined effects of entomopathogenic nematodes (EPNs) [i.e., *Heterorhabditis bacteriophora* Poiner (strain HK3) and *H. indica* Poinar, Karunakar and David (strain LN2)] and predatory mites [i.e., *Amblyseius cucumeris* (Oudemans) (Acarina: Phytoseiidae)] for the control of the western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) were investigated in a climate controlled growth chamber and under greenhouse conditions. The mites and nematodes were tested at different densities and concentrations, respectively, at different WFT densities, using beans (*Phaseolus vulgaris* L.) as model plant. In the growth chamber experiment, the presence of *A. cucumeris* on the plants caused more second instar larvae of WFT to drop off the plants in order to pupate in the soil, thereby increasing the number of available hosts for the EPNs. Single and combined applications of *A. cucumeris* and EPNs always resulted in significantly greater thrips control than in no natural enemies treatments. In general, extent of WFT control depended on the density and concentrations of mites and nematodes, respectively. In no case significantly lower WFT population reduction was recorded in a combined application of both biocontrol agents than in individual applications. In the growth chamber experiment releases of 10 adult *A. cucumeris* per plant and applications of 200 infective juveniles (IJs) cm^{-2} of both EPN species resulted in up to 83% reduction of the thrips, which was significantly higher than individual applications of the natural enemies. Due to high summer temperatures and low relative humidity, weekly applications of EPNs at 50 IJs cm^{-2} failed to significantly reduce WFT populations in the greenhouse experiment. Yet, weekly applications of both EPN species at 200 IJs cm^{-2} and/or releases of 3 *A. cucumeris* per plant significantly reduced thrips populations when applied individually or in combination. However, unlike the results from the growth chamber experiment, no significant differences in WFT control were obtained between single and combined applications of the two natural enemies. The prospects of combined applications of biocontrol agents for control of foliage-feeding and soil-dwelling life stages of WFT are discussed.

6.1. Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a cosmopolitan pest in both protected and open plant production. It is economically one of the most important pests of vegetables and ornamentals in greenhouses (Shipp et al., 1991; van Lenteren et al., 1995). In addition to the quantitative and qualitative damage it causes to plant products through its direct feeding, WFT is also one of the major vectors of tospoviruses like tomato spotted wilt virus (Kirk, 2002). In its lifecycle WFT passes through foliar-feeding stages, i.e., adult, and first and second instar larvae, as well as soil-dwelling developmental stages, i.e., late second instar larvae, prepupae, and pupae. WFT can complete its life cycle in less than two weeks, especially at high temperatures in greenhouses and thus, several generations of *F. occidentalis* can overlap during a production cycle of a given plant (Higgins, 1992; McDonald et al., 1998; Pickett, 1988). Hence, often growers apply several groups of insecticides in a repeated and frequent manner. However, due to their cryptic feeding behaviour and since the majority of the thrips pupate in the soil (Berndt et al., 2004) they are not easily accessible for chemical control. Moreover, because of the repeated and frequent applications of insecticides, strains of WFT resistant to several groups of insecticides have already been reported (Broadbent and Pree, 1997; Immaraju et al., 1992; Jensen, 2000). An ideal biological control strategy would target both the foliar-feeding and soil-dwelling development stages of the pest. However, to date most of the commonly used biocontrol agents like the predatory anthocorid bugs of the genus *Orius* spp. (Hemiptera: Anthocoridae) and phytoseiid mites like *Amblyseius* spp. (Acarina: Phytoseiidae) feed only on the foliar feeding stages of WFT. Yet, according to Berndt et al. (2004) depending on host plant species, up to 98% of WFT pupate in the soil. Moreover, under high temperatures and low humidity the efficacy of anthocorids and phytoseiids is limited (Shipp and van Houten, 1997; Shipp et al., 1996) whereas such environmental conditions favour high reproduction of WFT (McDonald et al., 1998). In addition, several species/strains of *Orius* and *Amblyseius* spp. diapause under short-day length conditions in greenhouses (Morewood and Gilkeson, 1991; Rodriguezreina et al., 1994) whereas WFT continue reproducing under all photoperiods. *Amblyseius cucumeris* (Oudemans) is one of the predators attacking the plant-feeding live stages of WFT. The first and second stage nymphs and adults are predacious but only on the first instar larvae of thrips (Bakker and Sabelis, 1989). Moreover, *A. cucumeris* can be

economically produced in large numbers, making it a suitable species for inundative releases in greenhouses (Jacobson, 1997).

The soil-dwelling developmental stages of WFT can be successfully controlled by applications of entomopathogenic nematodes (EPNs) (Rhabditida: Steinernamatidae and Heterorhabditidae) (Chyzik et al., 1996; Ebssa et al., 2001a,b, 2004a; Premachandra et al., 2003a), predatory mites *Hypoaspis* spp. (Acari: Laelapidae) (Berndt et al., 2004), and combinations of both (Premachandra et al., 2003b). However, high dose rates of EPNs are needed to assure sufficiently high control levels in WFT (Ebssa et al., 2004a; Premachandra et al., 2003a). Thus, the general objective of this study was to test combined applications of *A. cucumeris* and EPNs against WFT under greenhouse conditions. An additional objective was to investigate whether a combination of both biocontrol agents at lower densities/concentrations would result in higher control than individual applications of *A. cucumeris* and EPNs at higher densities/concentrations.

6.2. Materials and Methods

Source and maintenance of thrips, mites, and nematodes

The western flower thrips culture was maintained on fresh pods of green beans *Phaseolus vulgaris* L. (Fabaceae) according to the rearing protocol by Berndt et al. (2004) at 23 ± 2 °C, 50–60% relative humidity, and 16L:8D h photoperiod. Only uniform aged insects were used in the experiment. Specimens of *A. cucumeris* were freshly obtained from Katz Biotech AG (Germany). For the experiments, uniform-sized adult mites were individually selected under a binocular. EPNs, originally obtained from the Institute of Phytopathology, Christian-Albrechts University Kiel, Germany, were reared in greater wax moth larvae *Galleria mellonella* (L) (Lepidoptera: Pyralidae) at 23 ± 2 °C according to the protocol originally developed by Kaya and Stock (1997). Based on the results of previous studies by Ebssa et al. (2001a, 2004a) on the potential of EPNs for WFT control two *Heterorhabditis* spp., i.e., *H. indica* Poinar, Karunakar and David (strain LN2) and *H. bacteriophora* Poinar (strain HK3) were used in the experiments. Infective juveniles (IJs) were stored at 15 and 4 °C for *H. indica* and *H. bacteriophora*, respectively, until used in the experiments. Only fresh nematodes, not older than one month, were used in all experiments. Before application nematodes were acclimatized at room temperature for about 6 hours. The

required concentrations of the nematodes in the respective experiments were prepared by quantification and dilution procedures (Kaya and Stock, 1997).

Growth chamber experiment

One *P. vulgaris* seedling was planted in a plastic pot (11 cm diameter) at the two-leaf stage. The commercially available substrate Fruhstorfer Erde Type P (Archut GmbH, Lauterbach-Wallenrod, Germany) was used as plant growing media. The substrate is composed of humus, clay, and peat in the proportion of 15:35:50, respectively, and due to its high content of peat it has a high water holding capacity. Each seedling was caged with an acryl cylinder, equipped with several ventilations holes covered with thrips-proof gauze (64 µm pore size) as described in Ebssa et al. (2001b), into which 10:2 or 5:1 female:male thrips were released, mimicking the sex ratio in the thrips stock culture. The caged plants were further kept in a growth chamber at L16:D8 h photoperiod (for additional climatic data refer to Table 6.1). Under such conditions eggs of *F. occidentalis* are expected to hatch four days after the release of the adult thrips (Ebssa, 2000). Hence on the fourth day after the adult thrips release 0, 3, or 5 in replication 1 to 3 and 0, 5, or 10 in the replication 4 to 6, uniform-sized female adult *A. cucumeris* were released on each plant. Using a similar experimental set-up Ebssa et al. (2001b) observed that WFT pupation starts eight to 10 days after the introduction of adult thrips. Thus, in the present experiment eight and 13 days after the adult thrips release *H. indica* or *H. bacteriophora* were applied in two repeated applications at 0, 100, or 200 IJs cm^{-2}. The nematode suspensions were pipette in 10 ml distilled water on the top of the substrate in the pot via the lower ventilation hole in the cylinder (Ebssa et al., 2001b). After about 15–30 min 20 ml water was pipetted to the surface of the substrate to rinse the nematodes down. In a preliminary experiment adult thrips, i.e., the F1, started emerging from the soil on the 13th day after the initial adult thrips introduction. Hence, in the main experiment, the plants were cut on the 15th day after adult thrips introduction. To remove the upper plant parts, the cylinder was partially lifted up from the pot and the plant was cut at the base. The shoot part of the plant was placed in the cylinder with the open end of the cylinder sealed with parafilm and kept at 4 °C until counting. The pot was covered with a ‘sticky trap’ prepared from a Petri dish (diameter 10 cm). To trap emerging adult thrips, the inner part of the sticky trap was painted with insect glue. A hole was made into the centre of the sticky trap for ventilation but covered with thrips-proof gauze. Adult thrips emerged from the soil were counted from the sticky trap

and the top surface of the substrate in the pot for ten consecutive days until no more emerging thrips were recorded. The removed shoot was washed with a soap and water solution to collect all development stages of WFT on the plant. Those thrips that got stuck on the inside wall of the cage were collected using a fine camelhair brush. The thrips-containing soap and water solution was first filtered through a 30 µm pore sized sieve and stored in a 30% alcohol solution in glass vials. All foliar feeding life stages of WFT on the plant were counted under binocular and classified into adults, larvae and pre-/pupae. A split-split plot design was used in which the thrips density, mite density, EPN species, and nematode concentrations were assigned to a main plot, sub-plot, sub-sub plot, and sub-sub-sub plot, respectively. The experiment was repeated five times with one replication running at a given time.

Table 6.1. Mean day and night (± SE) temperatures (°C), relative humidity (rh) (%), and vapour pressure deficits (VPD) (kilo Pascal) during experiments conducted in a growth chamber and the greenhouse.

Parameters	Growth chamber		Greenhouse	
	day	night	day	night
Temperature	23.9 a B ± 0.22	23.9 a A ± 0.17	29.2 a A ± 2.42	19.3 b B ± 1.28
rh	67.7 a A ± 0.96	68.7 a A ± 0.45	38.0 b B ± 6.00	63.9 a A ± 12.54
VPD	0.55 a B ± 0.021	0.53 a A ± 0.01	1.56 a A ± 0.15	0.49 b A ± 0.17

Note: Means in a row for a given experimental place and for a given time followed by the same lower and upper case letters, respectively, are not significantly different ($P > 0.05$) (LSD test).

Greenhouse experiment

Similar to the experiments in the growth chamber, two been seedlings were transplanted into a plastic pot at the two-leaf stage. Three pots, individually placed on a tray, were kept in a thrips-proof cage (0.6 m × 0.6 m area and 1.1 m height) in a greenhouse at the Faculty of Horticulture, Hannover University. The greenhouse is equipped with heating and passive-ventilation systems but not with a humidity controller. To ensure sufficient ventilation in the cage but preventing WFT to escape from the cage, two sides were made

of thrips-proof gauze (64 µm pore size) and the remaining sides from transparent acryl plastics. Each cage represented an experimental unit to which one of the different treatment combinations was applied. Fifteen female and 3 male adult WFT were introduced to the cage directly onto the seedlings. Four days later, three unsexed, uniform-sized adult *A. cucumeris* were placed in an Eppendorf tube and attached to one bean plant per pot. Thus a total of nine mites were released per cage/ experimental unit. On average the mites left the Eppendorf tubes and colonised the plant within 15 min. In the control treatment no predatory mites were released. Eight days after the adult thrips release, *H. indica* or *H. bacteriophora* was pipette to the top of the substrate in the pots at concentrations of 0, 50, or 200 IJs cm^{-2}. The pots were irrigated before and after application of the nematode to moist the soil and to rinse the nematodes, respectively. The treatments were arranged in split-split plot design in which *A. cucumeris* releases (with or without), EPN species, and EPN concentrations were assigned to the main plot, subplot, and sub-sub plot, respectively. Both mites and nematodes were applied weekly up to the end of the experiment. Twenty-five days after adult thrips introduction, a blue sticky insect trap card (10 cm $\times$ 20 cm) was hung above the plant canopy inside the cage. Subsequently, every five to seven days adult thrips on the traps were counted, then removed, and the card was returned to the cage. At the end of the experiment, trap data were recorded before the plants were cut at base. The shoot parts of all six bean plants per cage were placed together in a plastic bag for collecting all foliar-feeding life stages of *F. occidentalis* (for details see previous paragraph). The pots remained inside the cage and the blue sticky trap was hung directly above them. Emerging adult WFT from the pots that got stuck on the trap were counted over the following days until no more adults emerged. The experiment was repeated in the same greenhouse but in different months of 2004 (Table 6.2).

Table 6.2. Temperature and relative humidity (rh) recorded inside the insect cages in the greenhouse during the experimental periods in different replications (Reps) in 2004.

Reps	Time, in 2004	Temperature (°C)			rh (%)		
		Min	Max	Mean	Min	Max	Mean
1	Mar 4–Apr 13	14.5	31.4	21.3	20.2	64.8	45.3
2 and 3	Jun 8–Jul 7	19.2	46.6	26.9	16.5	80.0	55.1
4 and 5	Aug 11–Sep 16	8.8	45.4	23.8	13.7	91.2	65.4

Data on temperatures and relative humidity in the growth chamber and greenhouse, for the latter inside and outside the insect cages, were recorded every 30 min using data loggers (Gemini Data Loggers Ltd, UK). The vapour pressure deficit (VPD) was calculated according to List (1984) and Prenger and Ling (2004). VPD is the difference between the amount of moisture in the air and how much moisture the air can hold when it is saturated, and some arthropods like *A. cucumeris* are greatly affected in their performance by the VPD (Shipp and van Houten, 1997). The temperature, relative humidity and VPD for both experiments are presented in tables 1 and 2.

Statistical analyses

Data of an experiment repeated over time were first tested for the variance homogeneity assumption using the HOVTEST = LEVENE option of the ANOVA procedure in SAS version 8 (SAS Institute, 1999) and were pooled only if the assumption was not violated. Except when assessing the influence of *A. cucumeris* on the pupation behaviour of WFT and when evaluating single and combined effects of the two biocontrol agents on the population reduction in *F. occidentalis* in the growth chamber experiment, data on mean numbers of thrips were used to determine the impact of a treatment. For evaluating the influence of the predatory mites on WFT pupation behaviour, the proportion (%) of the number of pre- and pupae on the plants to the total number of pre- and pupae in the system in the no nematode treatment (control) was used. For the experiment in the growth chamber, data on the number of adult thrips in the treatments were corrected for number of adult thrips in the no natural enemy treatment (control) using Abbott's formula (Abbott,

1925). To stabilize the variance, numbers of thrips and percentage values in different treatments were square root and arcsine, respectively, transformed before subjected to any data analyses (Zar, 1999). To assess the significance of a treatment, mean numbers of thrips under single or combined effects of natural enemies were compared to the mean numbers of thrips in the no natural enemy treatment using Dunnett's test (SAS Institute, 1999). Single and interaction effects of factors were detected by fitting the data to a mixed model in SAS (Little et al., 1996) considering repetition time and initial number of adult thrips released (in the growth chamber experiment) and repetition time (in the greenhouse experiment) as random effects. In case of a significant interaction between factors, different levels of a factor were compared at a given level of the second factor; otherwise, data were pooled. Whenever the ANOVA resulted in a significant effect of a given factor, means of different levels of the factor were compared by the PDIFF option on the LSMEANS statement in mixed model of SAS (Little et al., 1996). In all analyses a 0.05 alpha level was used. Mean values estimated in the mixed model of SAS are presented as LSMEANS ± SE.

6.3. Results

Growth chamber experiment

Thrips population on the plant: In the no natural enemy (control) treatment 15 days after adult thrips introduction to the bean plants 68.9 ± 3.5% of the total thrips population were on the plants, with 67.3 ± 3.4, 14.5 ± 2.1, and 18.2 ± 2.1% larvae, pre- and pupae and adults, respectively. On average, in the control treatment 89.9 ± 12.5 and 117.6 ± 9.1 individual WFT per plant were recorded following an initial infestation by 5:1 or 10:2 female and male thrips per plant, respectively. The ratios of WFT on the plants and in the soil were not affected by the initial release density of the thrips ($F_{17, 1} = 0.78$, $P = 0.3908$). Fifteen days after adult thrips 25.4 ± 4.0% of the total number of pre- and pupae were recorded on the plants while the remaining pupated in the soil as inferred from the numbers of adults that had emerged from the soil and were then counted on the sticky traps.

Due to the duration of the experiment no F2 larvae could occur on the plants. Hence, EPNs in their different concentrations did not significantly affect the numbers of immature foliar dwelling life stages of *F. occidentalis* (Concentration: $F_{2, 122} = 0.77$; $P = 0.4641$). Thus, to assess the efficacy of *A. cucumeris* on the number of immature foliar dwelling stages of

WFT at the time of plant cutting, a reduced model was fitted excluding the effect of EPNs and their concentrations. Both the initial density of adult WFT and the release density of *A. cucumeris* had a significant effect on the numbers of immature stages of *F. occidentalis* on the plants (Table 6.3). However, the effects of the mite release densities did not depend on the initial numbers of released thrips.

Table 6.3. ANOVA summary for the effects of the numbers of *Amblyseius cucumeris* (AC) and the initial numbers of adult western flower thrips (WFT) released (Adult0) on the number of immature foliar dwelling life stages of WFT (larvae and pre- and pupae) 15 days after adult thrips release onto bean seedlings in a growth chamber experiment.

Variations	df	*F*		*P*	
		Larvae	Pre- and pupae	Larvae	Pre- and pupae
Adult0	1, 162	43.67	21.68	< 0.0001	< 0.001
AC	3, 164	13.37	15.54	< 0.0001	< 0.001
Adult0*AC	3, 163	0.56	1.55	0.6404	0.203

Releases of *A. cucumeris* always significantly reduced the number of WFT immatures on the plants compared to the no predator treatment, and the highest mite release rate resulted in significantly greater control of *F. occidentalis* larvae and pre- and pupae than the two lower *A. cucumeris* densities (Table 6.4).

On the other hand, using the full model consisting of single and interaction effects of initial number of adult thrips released, release densities of *A. cucumeris*, EPN species, and EPN concentrations, the numbers of adult WFT on the plants were only significantly affected by the concentrations of the EPNs ($F_{2,\ 123}$ = 3.68, P = 0.0279) and the release densities of *A. cucumeris* ($F_{3,\ 124}$ = 7.47, P < 0.0001). Among all interactions, only Adult0*Concentration ($F_{2,\ 123}$ = 3.39, P = 0.0368) turned out to be significant. Hence compared to the no predator treatment, independent of EPN applications, significantly lower numbers of adult WFT were recorded only at the highest *A. cucumeris* release rate (Table 6.4). Furthermore, significantly lower numbers of adult thrips on the plant were recorded in EPN treatment than no nematode treatment only at 10 adult thrips density (Table 6.5).

Table 6.4. Mean numbers (± SE) of immature foliar-dwelling life stages and adults of western flower thrips (WFT) per bean plant 15 days after initial release of adult thrips onto bean seedling as affected by different release densities of adult *Amblyseius cucumeris* (AC) per pot and applications of different concentrations (in number of infective juveniles cm^{-2}) of entomopathogenic nematodes (EPN) in a growth chamber experiment.

AC	Larvae	Pre- and pupae	Adult WFT at three concentrations of EPNs		
			0	100	200
0	75.7 ± 7.4 a	11.6 ± 2.0 a	18.4 ± 2.4 a	13.9 ± 2.4 a	14.49 ± 2.4 a
3	58.2 ± 8.4 b	7.1 ± 2.1 b	17.3 ± 3.3 a	12.7 ± 3.0 ab	11.49 ± 3.0 ab
5	61.6 ± 7.4 b	8.2 ± 2.0 b	14.7 ± 2.4 ab	13.0 ± 2.4 ab	11.69 ± 2.4 ab
10	38.5 ± 8.4 c	5.1 ± 2.1 c	10.2 ± 2.7 b	9.1 ± 2.7 b	8.89 ± 2.7 b

Note: Data in this table are pooled from two initial adult thrips release densities and from two EPN species since all factors, except for EPN concentration * initial numbers of released thrips ($F_{2, 123}$ = 3.39, P = 0.0368), did not interact significantly.

Means within a column followed by the same letters are not significantly different ($P > 0.05$).

Number of adult thrips across different EPN concentration for a given AC densities are not compared since EPN concentrations significantly interacted with initial numbers of released thrips.

Table 6.5. Effect of different concentrations of entomopathogenic nematodes (EPN) on the mean numbers (± SE) of adult western flower thrips (WFT) per plant 15 days after initial releases of 5:1 or 10:2 female:male WFT per plant in a growth chamber experiment.

EPN concentrations	Initial number of female:male WFT released	
	5:1	10:2
0	12.2 ± 2.52 a	18.2 ± 2.50 a
100	12.5 ± 2.51 a	13.0 ± 2.50 b
200	10.2 ± 2.50 a	12.9 ± 2.50 b

Means within a column followed by the same letters are not significantly different ($P > 0.05$).

<u>Effect of *Amblyseius cucumeris* on the pupation behaviour of WFT</u>: In the no nematode treatments, the ratios of WFT pre- and pupae on the plants to the total numbers of pre- and pupae on the plants and in the soil (the latter figure derived from the number of emerging adults from the soil) were significantly affected by releases of *A. cucumeris* ($F_{3, 46}$ = 5.8, P = 0.0018) but not by the initial number of thrips released ($F_{1, 46}$ = 0.30, P = 0.5858) nor by the interaction of the two factors ($F_{3, 46}$ = 0.69, P = 0.6077). Hence, following releases of *A. cucumeris* a significantly lower proportion of the thrips pupated on the plants, though this was not affected by the different mite release densities (Table 6.6).

Table 6.6. Proportion of western flower thrips pupating on the plant at different release densities of *Amblyseius cucumeris* (AC).

AC densities	Pupation on the plant (% ± SE)
0	25.5 ± 3.45 a
3	14.1 ± 4.68 b
5	10.8 ± 3.39 b
10	14.4 ± 4.56 b

Means followed by different letters are not significantly different ($P > 0.05$).

To assess if the presence of *A. cucumeris* and/or higher initial WFT density lead to an earlier pupation in *F. occidentalis*, the proportion of adult WFT in the no EPN treatments that emerged from the soil during the end of the data collection (i.e., from the 6th to the 10th day after the first adult WFT started to emerge from the soil) were compared in the different *A. cucumeris* and initial thrips density treatments. Neither *A. cucumeris* ($F_{3, 43}$ = 1.22, P = 0.3133) nor the initial WFT densities ($F_{3, 46}$ = 0.93, P = 0.3407) significantly affected the proportion of adult thrips that emerged late from the soil. On average 23.4% (SE = 2.7) of all adult WFT in the no nematode treatment emerged from the soil during the last four days of data collection.

<u>Efficacy of *Amblyseius cucumeris* and/or EPNs</u>: In the no natural enemy control treatment 37.8 ± 9.7 and 67.0 ± 12.8 adult WFT emerged from the soil in the low and high initial thrips release density treatments, respectively. These numbers were significantly

higher than those recorded in all EPN alone treatments, the highest *A. cucumeris* release rate, and all combined applications of EPNs and *A. cucumeris* ($P < 0.05$, Dunnett's test). The single and/or combined effects of the predatory mites and EPNs on *F. occidentalis* was assessed after correcting the numbers of adult thrips on the plants and in the soil in the natural enemy treatments with those figures from the no natural enemy treatment (control). Release densities of *A. cucumeris* and EPN concentrations, but not EPN species and initial thrips release density, significantly affected corrected mortality in WFT (Table 6.7). The two-way interaction of *A. cucumeris* release densities and EPN concentrations was significant, whereas all other two-way and the three-way interactions were not.

Table 6.7. ANOVA summary of the effects of three release densities of *Amblyseius cucumeris* (AC) and two entomopathogenic nematode species (EPN) at three different concentrations (Conc) on the corrected mortality of western flower thrips at two initial thrips release densities (Adult0) on bean seedlings in a growth chamber experiment. Note: error df = 101.

Source of variations	df	*F*	*P*
Adult0	1	1.65	0.2019
AC	3	15.07	< 0.0001
EPN	1	1.18	0.2796
Conc	2	82.68	< 0.0001
Adult0*Conc	2	2.36	0.0994
AC*Conc	6	4.96	0.0002
All others			> 0.05

Individual releases of 3 and 5 *A. cucumeris* per plant resulted in lower WFT control than both EPN concentrations alone and all combinations of the two natural enemies (Table 6.8). Increasing the mite density to 10 per plant resulted in a similar control level to all EPN only treatments. However, the highest *A. cucumeris* release rate caused significantly lower thrips control than all combined applications except for the lowest mite density and the combination of the intermediate *A. cucumeris* release rate and the lower EPN concentration (Table 6.8). Furthermore, increasing the EPN concentration in both species

did not result in significantly higher thrips control. In no cases we recorded significantly lower thrips mortality in combined treatments compared to individual applications of the natural enemies. In addition, the combinations of the highest *A. cucumeris* release density and the higher EPN concentration always caused significantly higher thrips control than the respective individual treatments (Table 6.8).

Table 6.8. Percentage population reduction in western flower thrips (WFT) (± SE) by applications of entomopathogenic nematodes (EPN) alone (*Heterorhabditis bacteriophora* (Hb) or *H. indica* (Hi)) at 0, 100, or 200 infective juveniles cm^{-2}, releases of *Amblyseius cucumeris* (AC) only at 0, 3, 5, or 10 adult females per plant, or combined applications of the two natural enemies in a growth chamber experiment.

EPN	Concentrations	AC densities			
		0	3	5	10
Control	0	– *	15.5 ± 9.1 d	24.6 ± 6.3 d	47.1 ± 7.6 c
Hb	100	55.8 ± 6.3 bc	47.2 ± 9.1 c	62.3 ± 6.3 bc	68.0 ± 7.6 ab
	200	64.6 ± 6.3 bc	60.2 ± 9.1 bc	68.2 ± 6.3 ab	82.9 ± 7.6 a
Hi	100	60.1 ± 6.3 bc	67.9 ± 9.1 bc	57.4 ± 6.3 bc	73.2 ± 7.6 ab
	200	64.7 ± 6.3 bc	64.1 ± 9.1 bc	67.9 ± 6.3 ab	83.1 ± 7.6 a

Note: Since numbers of adult thrips initially released did not significantly interact with any other factors, data from the two thrips densities were pooled.

[*] The mean (± SE) number of adult WFT in the no natural enemy control treatment (62.4 ± 8.3) was significantly higher than those at all levels of EPNs alone, at the highest *A. cucumeris* release density, and at all combinations of predatory mites and EPNs (Dunnett's test).

Means in any two cells followed by different letters differ significantly ($P > 0.05$).

Greenhouse experiment

The climatic conditions in the greenhouse during the course of the experiment could not be kept at a comparatively constant level. Temperatures inside the insect cages ranged from as low as 9 °C to as high as 47 °C, and relative humidity levels occasionally dropped to below 15% (Table 6.2). The high summer temperatures were very conducive for a massive

population build-up of WFT. In the no natural enemy treatment 35 days after the initial release 15 female and 3 male *F. occidentalis* a mean number 579.3 ± 102.5 adult and 892.5 ± 130.5 thrips larvae per cage was recorded. However, no nymphal stages of *A. cucumeris*, but adult mites, were observed on the plants. No significant differences in numbers of adult thrips per sticky blue trap were observed between the different treatments ($P < 0.05$ for all trapping times). Thus, the total number of adults recorded throughout the entire experiment (i.e., data from all sticky trap catches and from the plants) and the number of thrips larvae counted on the plants at the end of the experiment were used to assess single and combined effects of *A. cucumeris* releases and EPN applications. Individual weekly treatments of both EPN species at 50 IJs cm^{-2} did not lead to a significantly reduced thrips density compared to the no natural enemy control (Fig. 6.1). However, EPN applications at 200 IJs cm^{-2}, releases of *A. cucumeris* alone, and combined applications of the two EPN species at both concentrations and the predatory mite resulted in significantly lower thrips numbers than in the control treatment. Though, no significance differences were found between the individual and combined natural enemy treatments (Fig. 6.1).

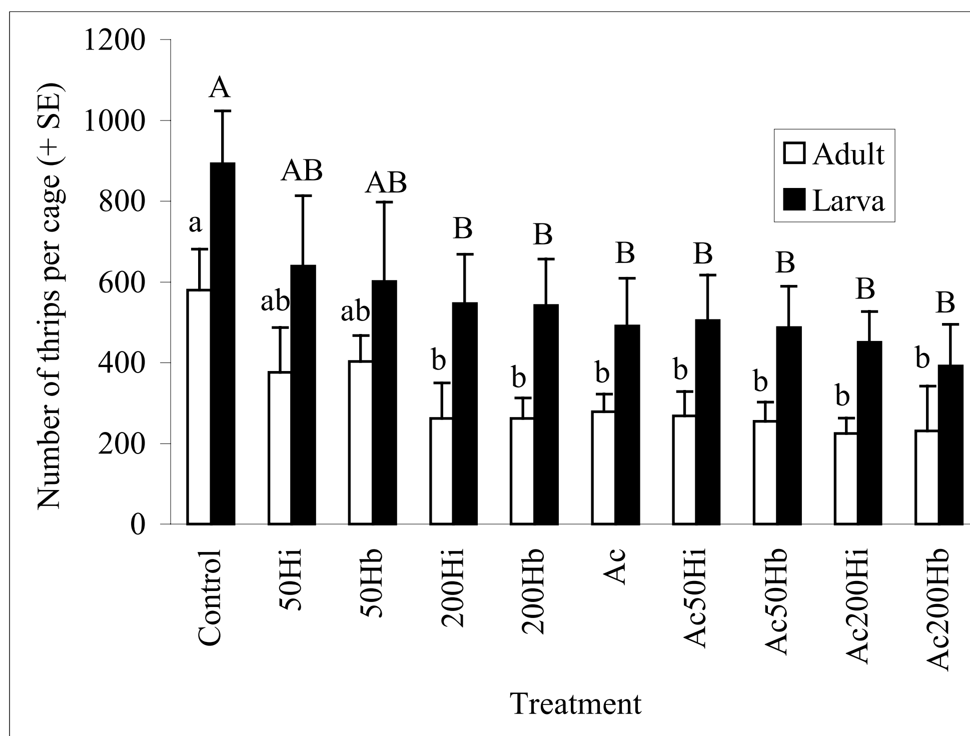


Fig. 6.1. Total number of adult western flower thrips (WFT) recorded in a greenhouse cage experiment on blue sticky traps and number of WFT larvae and adults counted on bean plants. The plants were treated with either no natural enemies (control), or weekly applications of *Heterorhabditis bacteriophora* (Hb) and *H. indica* (Hi) at 50 or 200 infective juveniles cm^{-2}, *Amblyseius cucumeris* (AC) at a release rate of 9 unsexed adults per greenhouse cage, or combinations of the two natural enemies (see materials and methods for details). Bars of a given thrips developmental stage followed by different letters differ significantly ($P > 0.05$).

6.4. Discussion

The relative importance of two natural enemies may depend on their densities/concentrations used and densities of their prey/host (Bellows and Hassell, 1999; Hansen, 1989). In the present study we chose different densities and concentrations of *A. cucumeris* and *H. indica* and *H. bacteriophora*, respectively, to identify the best combination for WFT control, using beans as a model plant. To exclude the effects of environmental factors, such as extreme temperatures and low humidity, the potential compatibility of mites and nematodes was first tested in a climate-controlled growth chamber. To assess the compatibility of the two natural enemies under more practical conditions, where, depending on the weather and the greenhouse architecture, climatic conditions can greatly vary, we conducted a second experiment in a greenhouse.

Growth chamber experiment

Fifteen days after introducing the adult WFT, more than 65% of the foliar feeding life stages were larvae. During this period a great proportion of the thrips were in the L1 stage, on which *A. cucumeris* preferably preys (Bakker and Sabelis, 1989). According to van Lenteren et al. (1995) an adult *A. cucumeris* can consume on average 4.9 L1 per day at 25 °C. In the present study even at the lowest release density of *A. cucumeris* the numbers of immature stages of WFT per plant were significantly reduced. In general, rising mite densities lead to increased levels of WFT control. At the highest *A. cucumeris* release density, both numbers of immature and adult *F. occidentalis* were reduced by approximately 50%.

Similar to previous studies 15 days after introducing the adult WFT about 30% of the total thrips population were in the soil for pupation (Ebssa et al., 2001b; Premachandra et al., 2003b). Thus, at a given time, about one-third of the thrips population are potential hosts for EPNs. Unlike *A. cucumeris*, however, nematodes when applied to the soil only attack the soil-dwelling developmental stages of *F. occidentalis* that have already caused damage to the plants as larvae. Hence, in such a case EPNs reduce the population density of the subsequent generation. Both EPN species at 100 IJs cm^{-2} reduced the number of emerged adult WFT by more than 50% though mortality did not increase at a higher EPN concentration. EPN-induced mortality in WFT as affected by different nematode concentrations may be influenced by several factors such as thrips developmental stages (Ebssa et al., 2001a), host density (Ebssa et al., 2004a), EPN species/strains (Chyzik et al., 1996), moisture level of the substrate (Ebssa et al., 2004b), and pupation depth of *F. occidentalis* (Ebssa et al., 2004c).

The efficacy of *A. cucumeris* was assessed by evaluating its effect not only on the immature foliar-feeding life stages of *F. occidentalis* but also on the number of F1 adult thrips. However, for a comparison of the impact of *A. cucumeris* and EPNs on WFT only the data of emerged adult WFT can be used as in the present study nematodes were only applied to the soil and data collection stopped before F1 adults started to produce offspring. Yet, this may underestimate the efficacy of *A. cucumeris* in comparison to that of EPNs.

Both natural enemies occupy different niches, and hence no antagonistic effects can be expected. Moreover, in the presence of *A. cucumeris* on the plants a higher proportion of late L2 larvae of WFT descends the plants to pupate in the soil, thereby increasing the numbers of potential hosts for EPNs. In a similar study Premachandra et al. (2003b) reported no antagonistic effects between the soil-dwelling predatory mite *H. aculeifer* Canestrini and EPNs although both forage in the soil. Combined applications of the highest *A. cucumeris* release density with both EPN concentrations resulted in significantly higher WFT control than any individual application of the mites or the nematodes, yielding up to 83% reduction in adult WFT.

Our results clearly indicate that in a controlled environment EPNs are highly compatible with *A. cucumeris* for control of WFT. However, the concentrations of EPNs used in this study are comparatively higher than the ones used for control of some other insect pests (e.g., McCoy et al., 2000; Samish et al., 1999). Yet in certain pests very high EPN concentrations are required, for instance in the citrus root weevil *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) (Shapiro-Ilan, 2002). During the last 10 years costs for *in vitro* production of EPNs have substantially decreased and most likely will continue to do so (Ehlers, 2001). Thus, in future applications of EPNs for WFT control, even at similarly high concentrations as in the present study, may become economically feasible, especially in high value crops like ornamentals.

Greenhouse experiment

High temperatures are conducive for the production of many vegetable and ornamental crops (Salunkhe and Kadam, 1998), but WFT populations also build up faster under such conditions (Higgins, 1992). To assess the potential compatibility of EPNs and *A. cucumeris* also under high temperatures, we did not reduce the temperature in the greenhouse during the summer. However, due to technical reasons, the heating system of the greenhouse did not always function properly, leading to the recorded low night temperatures in March and September.

High temperatures and low humidity can limit the virulence of EPNs by influencing their persistence, activity, survival and activity of their symbiotic bacteria (Brown and Gaugler, 1997; Grewal et al., 1994; Griffin, 1993; Kung et al, 1991). In the present study, weekly applications of both tested EPN species at 50 IJs cm^{-2} did not significantly reduce WFT

populations. Moreover, in a preliminary experiment only 10% of the late L2 descended the plants via the stem for pupation, while the remaining ones dropped off the plant canopy to the ground (L. Ebssa, unpublished data). Thus, in our study probably a high proportion of the L2 dropped off the plants in such a manner and hence pupated outside the pots, leading to a reduced impact of the EPNs and possibly explaining the contrasting results between the growth chamber and greenhouse experiments.

According to Shipp and van Houten (1997) survival of *A. cucumeris* decreases rapidly at temperatures ≥25 °C and at a vapour pressure deficit ≥1.0 kPa. Moreover, Shipp et al. (1996) showed that at a constant temperature, the rate of predation of *A. cucumeris* on WFT L1 decreases with increasing vapour pressure deficit, and according to the same authors the maximum rate of predation of the mites in greenhouses is achieved at a vapour pressure deficit of ≤0.75 kPa at the recommended production temperatures. In the present study, the average daytime vapour pressure deficit in the greenhouse over the course of the trial period was 1.56 kPa, with maximum temperatures of up to 45 °C. This is probably the reason why weekly releases of nine adult *A. cucumeris* per cage did not lead to a strong reduction in the WFT population. Furthermore, unlike in the growth chamber experiment, combined applications of mites and nematodes in the greenhouse did not result in greater thrips control than individual applications of the two natural enemies. However, higher concentrations of both EPN species, releases of *A. cucumeris* and their combination significantly reduced WFT densities compared to the untreated control.

In conclusion, results of this study indicate that under controlled conditions, combined applications of *A. cucumeris* and EPNs lead to a significantly higher control of WFT than individual applications of both natural enemies. However, in the greenhouse high temperatures and low humidity can lead to a high population build up of WFT, and at the same time reduce the efficacy of both predatory mites and EPNs.

7 General Discussion

In previous studies the susceptibility of soil-dwelling developmental stages of WFT to selected EPN species/strains has already been demonstrated (Chyzik et al., 1996; Ebssa et al., 2001a; Premachandra et al., 2003a), and up to 90% mortality levels were recorded by these authors. However, high mortality levels were obtained only when high EPN concentrations, i.e., 400 IJs cm^{-2} or more, were applied, and in general mortality levels increased with increasing concentrations for most tested strains. For other insect pests, a population reduction >50% can be obtained with a concentration of <150 IJs cm^{-2} (e.g., McCoy et al., 2000; Samish et al., 1999). With this background the present study was initiated to investigate the potential use of EPNs for WFT control from three different angles: (i) Studying different environmental factors potentially responsible for the high EPN concentrations needed in WFT control (Chapters 2, 3, and 4); (ii) applying EPNS at an appropriate time and frequency to target the majority of the soil-dwelling thrips life stages before they develop into adults and thereby escape the nematodes by emerging from the soil (Chapter 5); (iii) combining EPNs at economically acceptable concentrations with other biocontrol agents that target the foliar-feeding stages of thrips (Chapter 6).

EPN species/strains possess different pathogenicity levels to different insect pests (e.g., Gazit et al., 2000; Mason and Wright, 1997; Wang et al., 2002). According to the present work (Chapter 2) high variability exists among the different EPN species/strains tested in their efficacy against WFT. In general, *Heterorhabditis* spp. are more virulent against WFT than *Steinernema* spp. So far, *H. bacteriophora* strain HK3 and *H. indica* strain LN2 have been identified as the most virulent EPN species against WFT, making them ideal candidates for future investigations on EPNs for WFT control. In studies on foliar applications of nematodes against WFT, *H. indica* was also found to be one of the most promising EPN species (Halaweh, 2004). However, we cannot rule out the possibility of obtaining even more virulent EPN species/strains. Results of the present study suggest that such future screenings should focus on *Heterorhabditis* spp.

The efficacy of EPNs also depends on several factors (Chapter 2, 3, and 4). For instance, higher *H. indica* concentrations are required for high WFT control at low compared to higher thrips densities (Chapter 2). Similarly, high *H. indica* concentrations are required if

thrips pupate deeper (Chapter 4), the substrate is not moist enough, or no or insufficient amounts of rinsing water were used after an EPN application (Chapter 3). On the other hand, *S. bicornutum* that caused similar WFT mortality to *H. indica* in the initial screening experiments proved to be inferior to *H. indica* under most of the before mentioned conditions. Thus, an EPN species/strain that shows high virulence against WFT in screening experiments necessarily needs to be further tested under such different conditions. Moreover, in Europe and North America WFT is primarily a greenhouse pest, and in such greenhouses plants are often produced at comparatively higher temperatures. Thrips population build up accelerates under such warm conditions, especially during the summer (Chapters 2 and 6). Thus, future screening of EPNs for WFT control should focus on nematode species/strains from warmer climates that may be perform better at higher temperatures (Chapter 2).

WFT density in the soil is another factor that proved to be, among others, a reason for the high EPN concentrations needed for high control of thrips (Chapter 2). This could be associated with the behaviour and size of the hosts, as well as the foraging behaviour of the nematodes. The body length of soil-dwelling life stages of WFT is only about twice that of the IJs (van Lenteren et al., 1995; Adams and Nguyen, 2002). Furthermore, the specific foraging behaviour of EPNs affects their locating and invading ability of their host (Lewis, 2002). IJs of cruiser EPN species like *H. indica* in the current study actively move in their search for hosts. However, the fact that the soil-dwelling immature stages of WFT (pre- and pupae) are non-feeding (van Lenteren et al., 1995), and hence do not provoke plants to release chemical cues as a result of the feeding activity of a herbivore (Boff et al., 2001; Lewis et al., 1993), and move upon disturbance, may negatively affect the host-finding and invading capability of the nematodes. IJs of ambushers like *S. bicornutum* in the current study remain near or at the soil surface and nictate to attach themselves to mobile insects (Lewis, 2002). When applying lower or higher EPN concentrations to the soil, a higher proportion of the L2 descends deeper for pupation or pupate at shallower depth, respectively (Chapter 4). Thus, efficacy of ambushers in WFT control may be affected from two different angles: (1) Pupating at deeper levels may enable thrips to avoid contact with IJs of an ambusher. (2) When WFT pupates at a shallower depth, the lack of motion of the hosts may render the nictating behaviour and subsequent attempts of an infective juvenile of an ambusher to attach to a host less successful. Thus, in general, the results of the present study indicate that for WFT control by EPNs limiting factors are more related

to host locating and colonisation, than to penetration and killing. Boff et al. (2001) discussed that once invasion and penetration are successful, only few IJs suffice to kill a smaller than a bigger host; however, a higher EPN concentration is needed to enable the IJs to successfully locate and infect a smaller than a bigger host. This is probably also the case in WFT late L2, prepupa and pupa, which are very small hosts. In laboratory studies, WFT immatures when attacked by few IJs rapidly disintegrated (Belay, 2003). These may explain why the host density in relation to the EPN concentration seems to be one of the limiting factors in WFT control by EPNs. However, these hypotheses need to be verified in future studies.

Results from chapter 3 clearly indicate that the substrate moisture content levels before an EPN application and the amount of post-application irrigation required largely affect the EPN concentrations needed for WFT control. For instance, a higher *H. indica* concentration was needed at a low moisture content of the substrate and inappropriate irrigation levels. An appropriate moisture level may be required not only at the time of nematode application but also during the following days when the IJs are expected to infect the susceptible life stages of their hosts (Georgis and Gaugler, 1991; Shapiro et al., 2002). However, the rate of water loss in substrates depends on several factors, such as temperature (e.g., Lakshmi et al., 2003) and substrate types (Bilderback and Fonteno, 1993). Thus, the initial moisture content of the substrate, which greatly affected the EPN concentrations needed to assure high WFT control, may depend on other factors that were kept constant in the present study (Chapter 3). For instance, under higher temperatures, the substrate probably will loose its moisture faster, thereby affecting the persistence of the EPNs. Hence, depending on the rate of loss of moisture from the substrate repeated post-application irrigations might be required to keep the substrate moist enough during the days following an EPN application (Cabanillas and Raulston, 1996b). Additionally, the amount of irrigation needed for rinsing the IJs down to the horizons where they can encounter their hosts may depend on the application volume (Chapter 3), soil moisture content before the EPN application (Grant and Villani, 2003), and the pupation depth of WFT (Chapter 4).

Results of the present study clearly show that depending on the foraging behaviour of EPNs (i.e., cruisers vs. ambushers), the depth of pupation in WFT is one of the most critical factors explaining the high EPN concentrations presently needed for WFT control

(Chapter 4). Furthermore, it could be shown that depending on EPN concentrations and thrips densities, WFT adjust their pupation depths in the substrate. However, the respective experiment was conducted under rather artificial conditions. Under more practical/ greenhouse conditions, the pupation depth may depend on different characteristics of the substrate such as substrate type, moisture content, level of compaction etc. (Dimou et al., 2003). Depending on the extent of post-application irrigation (Chapter 3) and EPN foraging behaviour (Koppenhöfer et al., 1996), most of the applied IJs may reach the depth where the majority of WFT pupates differently in different substrate types.

Results of chapters 2, 3 and 4 so far have shown that for lowering the EPN concentrations required in WFT control various environmental conditions need to be optimised. In addition, the use of more persisting EPN species and/or split-application of the nematodes proved to be a viable strategy (Chapter 5). Second instar larvae of WFT continuously drop off the plant for pupation in the soil. Thus, the applied EPN should not only target the WFT in the soil at the time of application but also the L2 that will enter the soil during the following days, necessitating a certain level of persistency in the EPN species/strain. In the present study (Chapter 5), applying a more persistent EPN species at a higher concentration resulted in better control of WFT over a longer period than at a lower concentration. However, persistence in EPNs is affected by several external factors like extreme temperatures and desiccations (Glazer, 2002). On the other hand, in less persisting EPN species repeated applications at a reduced concentration might be required. Early and repeated applications of *H. bacteriophora* at a lower concentration caused higher WFT control than a single treatment at twice concentration independent of the time of application (Chapter 5). Hence under uncertain weather conditions that may negatively affect the survival and/or efficacy of the applied EPNs (Glazer, 2002), split applications at a reduced concentration can be a better approach than applying the nematodes once at a higher concentration. Moreover, probably because of its small size, WFT cannot sustain a complete life cycle of an EPN (Belay, 2003). Hence for WFT control by EPNs, by definition, nematodes can be only used in an inundative manner, and especially under unfavourable conditions and for non-persisting EPN species/strains, repeated applications are needed.

Another approach that might enable the use of lower EPN concentrations could be combined applications of nematodes with other biological control agents that target the

foliar-feeding (Chapter 6) or soil-dwelling life stages of WFT (Premachandra et al., 2003b). In the current study the potential compatibility between the predatory mite *A. cucumeris* and EPNs for WFT control was first conducted under controlled conditions in a growth chamber and then under more practical conditions in the greenhouse (Chapter 6). Results from the growth chamber experiment demonstrate for the first time the feasibility of simultaneously using nematodes for WFT control in the soil and releases of predatory mites against the thrips on the plants. As both biological control agents forage in different habitats (soil vs. plant) no antagonistic effects are expected, thus assuring the absence of intra-guild predation (Finke and Denno, 2003) and hence a full compatibility between mites and nematodes. Foliar-feeding stages of WFT that escaped a mite attack were further targeted by EPNs in the soil, resulting in an overall better WFT control compared to the individual applications of mites and nematodes, respectively. In the greenhouse experiment, WFT was significantly controlled only at a high EPN concentration, release of predatory mite, or combinations of the two biocontrol agents. Due to high temperatures and low humidity, the combined application of the two organisms in the greenhouse did not result in a better thrips control than individual releases of the natural enemies. Environmental conditions, such as temperatures, humidity, and soil moisture, are some of the main factors that are often responsible for insufficient pest control levels after treatments with EPNs (Shapiro-Ilan et al., 2002) and/ or releases of *A cucumeris* (Shipp et al., 1996; Shipp, van Houten, 1997) in the field or greenhouses. In the current greenhouse experiment technical problems and insufficient ventilation facilities, resulted in great variations in temperatures and relative humidity, negatively affecting the efficacy of both natural enemies. However, such environmental problems can be overcome by equipping greenhouses with appropriate cooling/heating system, continuous irrigation facilities, and humidity controller, and future studies on the combined use of EPNs and predatory mites should be tested under such conditions.

Because of major technical improvements, costs of *in vivo* EPN production have significantly decreased during the last few decades (Ehlers, 2001) and most likely will do so in the future, thus probably increasing the economic feasibility of a use of EPNs for WFT control. On the other hand, use of an efficient EPN species/strain at 400 IJs cm^{-2}, the concentration that repeatedly provided best thrips control (Chapters 2, 3, 4, and 5), could still be economically feasible in certain high value ornamental crops. Hence in future the use of one of the efficient EPN species/strains identified in this study should be

investigated under practical greenhouse conditions by optimising all environmental factors previously mentioned. Recently foliar applications of EPNs have been proposed for control of foliar-feeding insect pests including WFT (e.g., Broadbent and Olthof, 1995; Piggott and Wardlow, 2002) but with several limitations as discussed in Arthurs et al. (2004). In WFT control, for instance, Wardlow et al. (2001) reported that weekly foliar applications of *S. feltiae* efficiently controlled thrips on chrysanthemums. Foliar applications of EPNs primarily target the foliar-feeding life stages of WFT, however, the run-off of the EPN formulations possibly as well affect the soil-dwelling life stages, though this so far has not been investigated. Moreover, novel adjuvants can improve the formulations used in foliar application of EPNs, resulting in some studies in higher controls levels (Baur et al., 1997; Mason et al., 1998) though in others not (Belair et al., 2003; Halaweh, 2004). Hence, more in-depth research is needed to conclusively evaluate the impact of foliar applications of EPNs on pests like WFT.

In conclusion, in this study several options for improving the efficacy of EPNs for WFT control have been identified, thereby providing a better understanding of the potential use of EPNs as one component in the biological control of *F. occidentalis*. Moreover, their compatibility with foliar acting biocontrol agents, at least under controlled conditions, may open up new prospects for biological control of WFT by targeting simultaneously both foliar-feeding and soil-dwelling thrips developmental stages, possibly leading to increased control levels.

Annex A: Preliminary experiments

1. Substrate moisture content

Three additional pots, along with the other experimental pots to which a treatment was assigned, were used to determine (i) the moisture content of the substrate at the time of pot filling and (ii) moisture loss during the three-day stay of the experimental material in a climate chamber before adding EPNs. The moisture content of the substrate was determined through oven-dry method in the three pots (Fig. 1). On the third day the amount of water or EPN suspension required to obtain an appropriate moisture level was determine (Fig. 1).

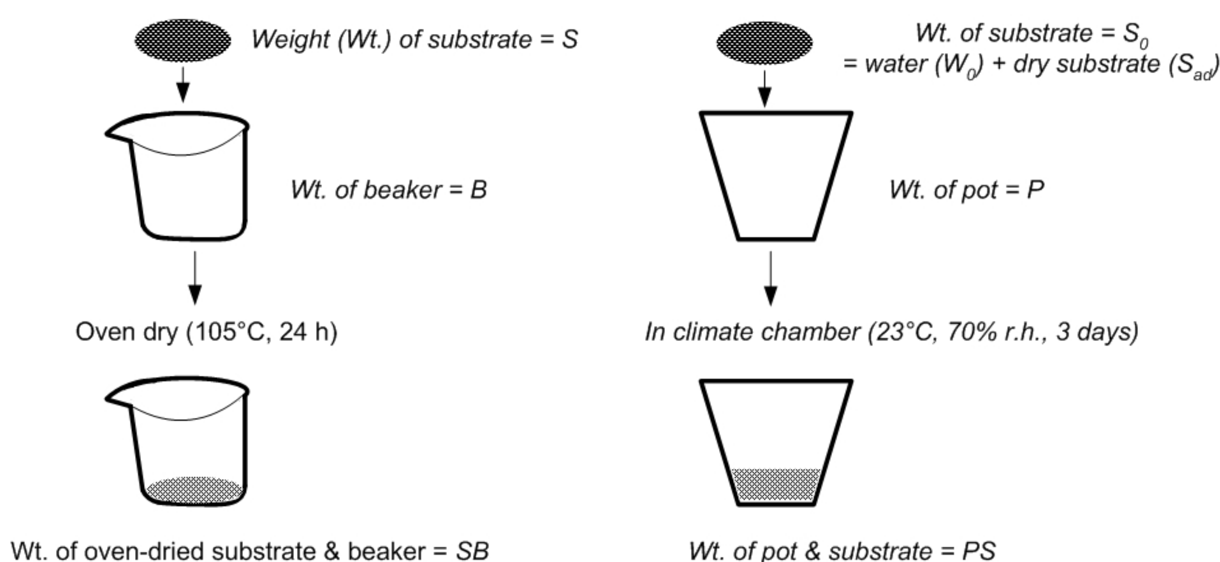


Fig. 1. Schematic representation of a method of moisture content determination. Note: the abbreviations given in this figure are used in different formulas to calculate different parameters (see text).

The original moisture content (MC_0) of the substrate before added to the beaker (or pot) was calculated as[1]:

$$Moisture\ content\ (w/w) = \frac{Weight\ of\ water\ in\ the\ substrate}{Weight\ of\ water\ in\ the\ substrate + Weight\ of\ oven\ dried\ substrate}$$

[1] The abbreviations of different parameters are given in Fig 1.

$$==> MC_0 = \frac{S-(SB-B)}{S} \qquad \text{(Eq. 1)}$$

where MC_0 is original moisture content of the substrate, S is weight of the substrate added to the beaked before oven-dried, B is weight of the beaker, SB is weight of the oven-dried substrate and the beaker (see fig. 1).

The amount of water in the substrate that was added to the pot was obtained from the following equation:

$$MC_0 = \frac{W_0}{S_0} \quad ==> W_0 = MC_0 * S_0 \qquad \text{(Eq. 2)}$$

where, W_0 is amount of water in the substrate, S_0 is weight of the substrate added to the pot (Fig 1). The substrate was kept in the climate chamber and the weight (S_1) was determined three days thereafter as follows:

$$S_1 = PS - P \qquad \text{(Eq. 3)}$$

Where PS is the weight of the pot and the substrate after three days in climate chamber and P is the weight of the pot. Then, water lost during the three days (W_{lost}) was determined as:

$$W_{lost} = S_0 - S_1 \qquad \text{(Eq. 4)}$$

Thus, moisture content (MC_1) of the substrate after three days in the climate chamber is:

$$MC_1 = \frac{W_0 - W_{lost}}{(W_0 - W_{lost}) + S_{ad}} = \frac{W_0 - W_{lost}}{S_1} \qquad \text{(Eq. 5)}$$

where S_{ad} is the theoretical weight of the dried substrate, S_1 is the actual weight of the substrate added as determined in Eq. 3. After adding W_1 ml water (or EPN suspension), the final moisture content (MC_F) of the substrate in the pot was calculated as:

$$MC_F = \frac{(W_0 - W_{lost}) + W_1}{[(W_0 - W_{lost}) + W_1] + S_{ad}} = \frac{(W_0 - W_{lost}) + W_1}{W_1 + S_1} \qquad \text{(Eq. 6)}$$

Conversely, to get a given moisture content (MC_F) of the substrate in which the nematodes were expected to perform best, the required amount of additional water or suspension of EPNs was obtained by rearranging equation 6:

$$W_1 = \frac{W_0 - W_{lost} - MC_F * S_1}{MC_F - 1}$$

To start with moist enough substrate, water was added to the substrate and mixed uniformly before filling the experimental pot. In a pot with six g substrate, the moisture contents under different cases are indicated below (Fig. 2).

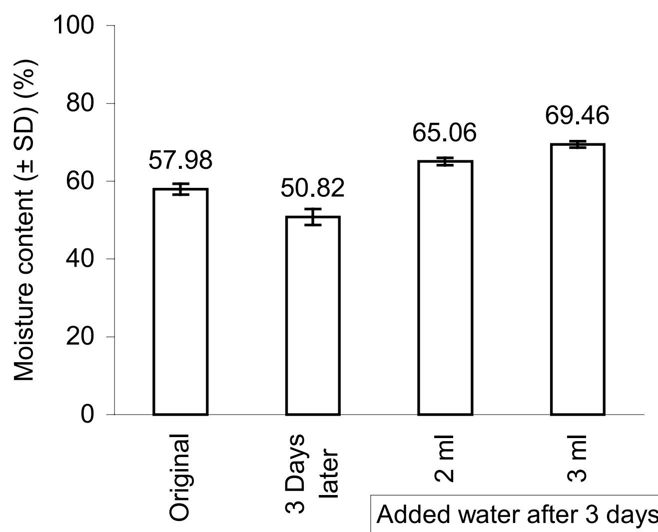


Fig. 2. Moisture content of the substrate at the time of pot filling (original moisture content), three days after stayed in climate controlled chamber in similar conditions to the main experiments, but without adding any water, and after adding 2 or 3 ml water.

2. Water holding capacity of the substrate

Water holding capacity (WHC) of a soil can be determined by the amount of water held in the soil sample vs. the dry weight of the sample. Soil water holding capacity is controlled primarily by the soil texture (particle size distribution) and the soil organic matter content. Fruhstorfer Erde, the substrate used in our experiment, is composed of humus, clay and peat in a proportion of 15:35:50, respectively and hence it holds a good amount of moisture. It can be awkward to re-damp if allowed to get to dry.

Field capacity is the amount of water remaining in a soil after it has been saturated and allowed to drain for 24 hours (Cassel & Nielsen, 1986). Thus, field capacity, which is a term used for soils in the field, is the maximum amount of water that a soil can hold (i.e., WHC) after saturated and allowed to drain for a satisfactory period of time. As *field capacity* is used for field soils, a term '*container capacity'* is used for soils or other growing media (mixture of vermiculite, bark, peat moss, coarse sand, and soils) that are used in pots in greenhouse plant production. The concept is the same in both cases.

Thus, using the following procedure (Cassel & Nielsen, 1986), a *container capacity* (i.e., maximum WHC) of our model substrate was determined before using in EPN experiments (Fig. 3).

A given amount of substrate was added into a plastic pot that had holes at the base. Water was poured to the media until water begins to flow out of the container through the holes at base (Fig 3). The top open part of the pot was covered to prevent evaporation the container was positioned in such a way that water dripped freely into the air from the holes in the base of the container. To make sure that all of the excess water is drained, the container was allowed to drain for 6 h. Normally, drainage cease in <1 h for many potting media. Then, soil samples of a defined weight was taken from the container and oven dried at 105 degree for 24 h, and reweighed.

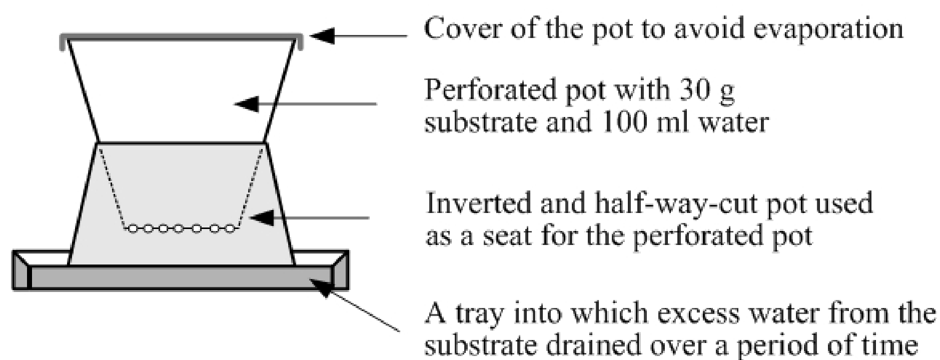


Fig. 3. A pot set-up through which water holding capacity of the substrate (i.e., container capacity) was determined.

Then, container capacity CC (or WHC) was calculated as:

$$CC = \frac{Weight\ of\ water}{Weight\ of\ Oven\ Dry\ Substrate} \quad \text{(Eq. 7)}$$

Table 1 An example of water holding capacity (WHC) of 30 g Fruhstorfer Erde, a model substrate used in the present study, after oven-drying the saturated. Note: 100 ml water was added to the substrate and drained for 1 h.

Rep.	Beaker (B)	Substrate (S)	B+S (oven-dried)	Oven-dried S	Water in S	WHC
1	46.67	18.20	49.53	2.86	15.34	536.36
2	97.42	22.63	101.00	3.58	19.05	532.12
3	41.27	18.72	44.33	3.06	15.66	511.76

Based on the above procedure, WHC was determined (Table 1). To assess the drainage time required in our model substrate and calculate its WHC, the water added to the substrate (Fig. 3) was drained for 1, 2, or 48 h. The results indicated that drainage did not cease before 1 h and hence water remained in the substrate after 1 h drainage was significantly higher than drainage after 2 and 48 h. On the other hand, the water retained in the substrate after 2 and 48 h did not significantly differ, indicating that drainage must have ceased before 2 h (Table 2). Thus, the average WHC was determined after allowing the saturated substrate for 2 and 48 h. The WHC was 484.2% (weight of water in the substrate divided by weight of oven-dried substrate). The moisture content of the substrate at the WHC was calculated according to equation 1 above and was 84.04%.

Table 2. Water retained in the substrate after different drainage time (i.e. 1, 2, 48 h). Note: 100 ml water was added to 30 g substrate and allowed to drain (Fig. 3).

Time (h)	Water retained (%) [b]
1	526.8 a
2	483.2 b
48	478.8 b
48 (Chamber) [a]	490.7 b

[a] The percent water retained was determined for the substrate that stayed in the climate chamber (23°C, and 70% rh) for three days representing the actual experimental setup.

[b] Percentage of water retained in the substrate was calculated in a similar way that WHC was determined (i.e., weight of water retained after a given drainage time divided by oven dry weight of the substrate, Eq. 7).

To test the efficacy of EPNs under different substrate moisture, relative moisture content (RMC) was used instead of the actual moisture content alone. RMC was calculated as the actual moisture content (AMC) divided by the moisture content of the substrate at its maximum water holding capacity (MC_{WHC}). To obtain different RMC (e.g., 60, 65, 70, 75, 80, 85, 90, 95, 100%), first of all the original moisture content of the substrate (and hence water contained in the substrate) was obtained through oven-dry method (Fig. 1). The additional water required to reach the desired AMC was calculated using the following formula (e.g. for AMC at the 60% RMC):

$$Moisture\ content = \frac{Weight\ of\ water\ in\ the\ substrate}{Weight\ of\ water\ in\ the\ substrate + Weight\ of\ oven\ dried\ substrate}$$

$$\frac{W1 + W60}{W1 + W60 + S1} = AMC60 \qquad \text{(Eq. 8)}$$

$$\Rightarrow \quad \frac{AMC60\,(W1 + S1) - W1}{1 - AMC60} = W60,$$

where *AMC60* is the actual moisture content of the substrate at 60% RMC, *W1* is the amount of water already in the substrate before treatment, *S1* is the weight of oven-dried substrate, and *W60* is the additional amount of water required to result in *AMC60*.

The AMC of the substrate was determined according to figure 1 and equation 1 before potted and placed in the climate chamber. Likewise, the AMC of the substrate in the plastic pot that stayed in the climate chamber was assessed before and after adding different amount of water to acquire different level of AMC of the substrate. Then the RMC corresponding to these AMC of the substrate after adding different amount of water was calculated as:

$$RMC = \frac{AMC}{MC_{WHC}} * 100$$

where MC_{WHC} is the actual moisture content of the substrate at the maximum water holding capacity of the substrate, which is equal to 84.04% as indicated above.

An example of different AMC and the corresponding RMC after adding different amount of water to 10 g substrate is given in figure 4.

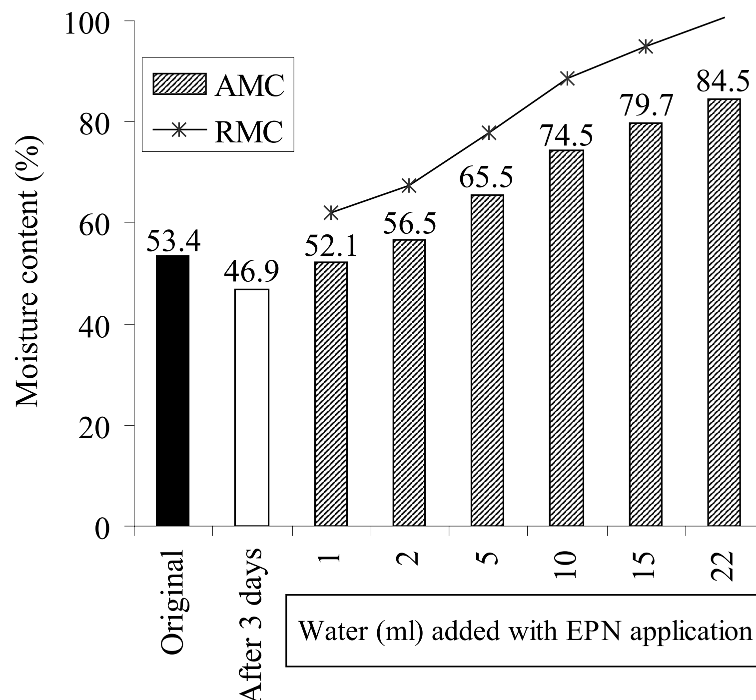


Fig. 4. Actual and relative moisture content (%) of the substrate (Fruhstorfer Erde) before it was added to a plastic pot (Original), after the substrate in the assay arena stayed in a climate chamber for three days (After 3 days), and after adding different amount of water to the substrate that stayed in the climate chamber.

After three days in the climate chamber the substrate lost 6.5% moisture. Application of 1 ml water to the substrate increased the AMC only to 52%, which was 62% RMC. With the application of 22 ml of water (20 ml of distilled water plus 2 ml of EPN suspension), the MC of the substrate reached 84.5%, which was 100.6% of the MC at the maximum WHC.

At 62% RMC, except for *H. indica* at a concentration of 400 IJs cm^{-2} (i.e., CM = 18%), no mortality of WFT by EPNs was recorded. At 100% RMC, WFT suffered high natural mortality (i.e., 80%). Thus, both the lowest and the highest moisture levels were omitted from the treatments in the main experiment hence the treatments were adjusted as indicated in Table 3.

Table 3. Application of different amount of water (ml) to establish different actual moisture content of the substrate (AMC), and hence different relative moisture content (RMC) of the substrate).

Water and EPN suspension [a]	AMC (%)	RMC (%)
0 + 2	56.5	67.3
3 + 2	65.5	77.9
8 + 2	74.5	88.6
13 + 2	79.7	94.9

[a] EPN suspension was applied in 2 ml distilled water after the substrate was moistened with a required amount of water.

3. Water potential of the substrate with different moisture levels

A procedure for water potential determination was adapted from Kaya and Stock (1997) and described as follows:

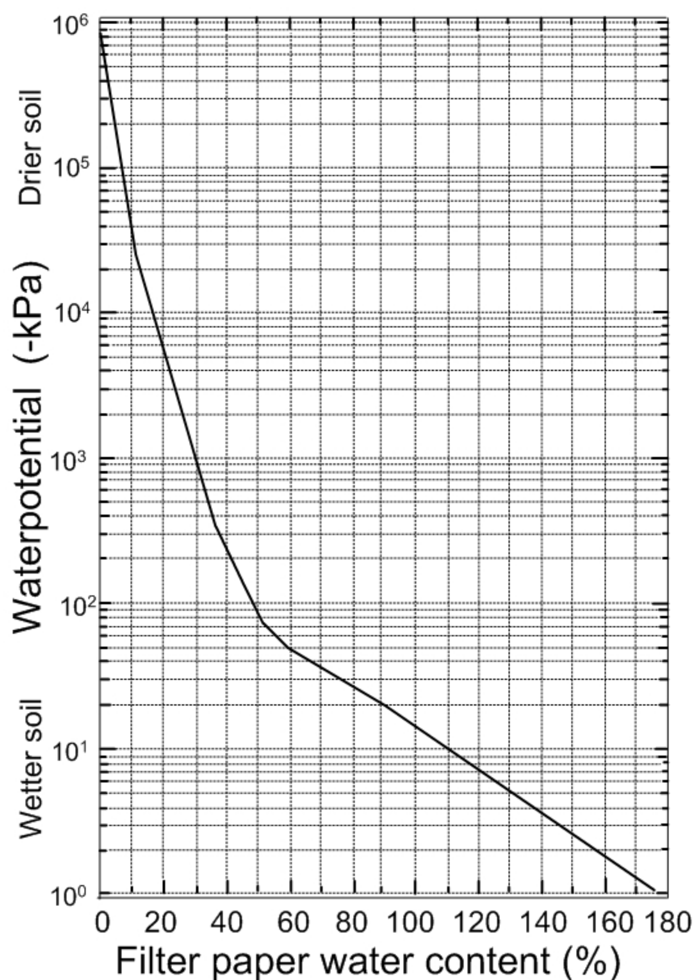
1. obtain the dry weight of the filter paper before placing into the substrate because the weight of the filter paper varies by 10%.
2. sandwich the weighed filter paper between two other pieces of filter paper.
3. add 10 g substrate into a plastic pot and place the sandwiched filter paper in the middle of the substrate in the pot

4. add a required amount of water to obtain the target AMC of the substrate.
5. allow the filter paper to equilibrate with water in the substrate.
6. retrieve the weighed filter paper after 24 h.
7. reweigh the paper and calculate the percentage moisture as follows:

$$\frac{Wet\ weight - Dry\ weight}{Dry\ weight} = \%\ moisture\ of\ \ filter\ paper$$

8. Determine the water potential (kPa) of the substrate by reading off the graph in the figure 5.

Fig. 5. Calibration curve for Whatmann no. 42 filter papers showing water potential in kilopascal



(kPa) of soil against filter paper water content (modified after Kaya and Stock, 1997).

For the moisture levels used in the main experiment, the corresponding water potential was determined according to the above procedure and the result is presented in table 4.

Table 4. Water potential of Fruhstorfer Erde, a model substrate used in the present study.

Water per pot (ml)	RMC (%)	Moisture of filter paper (%)	Water potential (kPa)
2	67	80	- 30
5	78	115	- 8
10	88	140	- 4
15	95	156	- 2

Note: The moisture content of filter paper was calculated after placing sandwiched filter paper in the substrate with a required level of moisture. The water potential corresponding a filter paper moisture level was read from a graph in figure 5 (see text for detail).

4. Do thrips crawl on the plant or jump down to pupate in the soil? An implication for nematode applications in potted plant production

Four concentric circles with radius of 5, 10, 13, and 16 cm were marked on a blue sticky trap (40 cm × 26 cm). A hole (2 cm in diameter) was drilled at the centre of the circles. The card was opened by cutting from the outer edge of its longest side up to the central hole. A bean plant seedling at two-leaf stage was transplanted into a plastic pot (11 cm in diameter). Throughout the experimental period, the seedlings were kept upright by clipping them to a stalk in a greenhouse at temperature ranges of 18–26°C and L16:D8 h photoperiod. Inserting the seedling and the stalk through the opened side of the card, the prepared blue card was horizontally placed on the top of the pot. The horizontal opening of the card was fitted edge-to-edge to avoid any gap. A black sponge was used to close the central hole of the card through which the seedling and the stalk have been inserted. The sponge was painted with insect glue to trap any WFT crawling down on the plant during pupation. The pots were placed individually on a tray and watered accordingly. The seedlings were kept for one additional week until more numbers of leaves emerged and the lower two leaves were removed so that the canopy of the leaves remains only hanging over the circles. Then, 10 L2 of WFT, four-day-old after emergence of neonate larvae, were transferred to the centre of the upper most leaves of the seedlings. Ten replications were run. Five days later, all the introduced larvae left the plant for pupation and hence counted from the different circles on sticky card.

Table 5. Percentage of western flower thrips (WFT) counted from different concentric circles on the sticky blue card.

Radius of the circle (cm)	Percent WFT counted (Mean ± SE)
0	10 ± 4.7 a
5	40 ± 3.7 b
10	30 ± 6.2 b

Note: 0 cm radius is WFT counted on the central hole closed with sticky sponge. Means within a column followed by same letters are not significantly different (P >0.05, Tukey's test)

During the experimental period, the outer most part of the leaf canopy was hanging over only up to the 10 cm radius mark. About 90% of the introduced thrips were recovered from the sticky cards. Ten percent of the thrips counted on the card were recovered from the central hole closed by sticky sponge indicating that at least 90% of thrips drop down from the leaves to pupate. Since there were no thrips found outside the canopy of the plant, it is not evident that thrips jump down for pupation.

Annex B: Data steps in SAS

```
DATA name;
     INPUT
     Replication    FactorA   FactorB   FactorC
     Thrips_number Mortality Varabl3;

/* transformation */
Thrips_numberY = SQRT(Thrips_number + 0.5);
MortalityY = ARSIN(SQRT(Mortality/100)) * 57.3;
/* 'Mortality', e.g., Corrected mortality, expressed in % */

/* Any calculations, e.g., ratio of emergence */
Ratio = Thrips_number/Varabl3 x 100;

CARDS;
/* Data go here… */
;

PROC PRINT DATA = name;
RUN;

PROC MEANS DATA = name CV VARDEF = DF MEAN STD STDERR;
     CLASS FactorA FactorB FactorC;
     VAR Thrips_number   Mortality Varabl3;
RUN;

/* For variance homogeneity test between several repetitions
over time */
PROC ANOVA;
     CLASS Repetition;
     MODEL MortalityY = Repetition;
     MEANS Repetition/HOVTEST=LEVENE;
RUN;


/* For ANOVA summery using GLM*/
PROC GLM DATA = name;
     CLASS Replication FactorA FactorB FactorC;
     MODEL Thrips_numberY MortalityY Ration = FactorA
          FactorB FactorC FactorA*FactorB FactorA*FactorC
          FactorB*FactorC FactorA*FactorB*FactorC;
     MEANS FactorA/LSD;
     MEANS FactorA/TUKEY;
     LSMEANS FactorA/PDIFF = ALL;
RUN;

PROC MIXED DATA =name;
     CLASS Replication FactorA FactorB FactorC;
     MODEL MortalityY = FactorA FactorB FactorC
```

```
          FactorA*FactorB FactorA*FactorC FactorB*FactorC
          FactorA*FactorB*FactorC/ DDFM=SATTERTH ;
      RANDOM Replication FactorA * Replication;
       LSMEANS FactorB /PDIFF ;
       LSMEANS FactorA* FactorB /SLICE = FactorB DIFF;
       LSMEANS FactorB * FactorC/SLICE = FactorB DIFF;
RUN;
```

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Publications

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