THE POTENTIAL OF ENTOMOPATHOGENIC NEMATODES TO CONTROL SOIL- BORN STAGES OF WESTERN FLOWER THRIPS *Frankliniella occidentalis* (PERGANDE) (THYSANOPTERA: THRIPIDAE)

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ABSTRACT

The efficacy of six entomopathogenic nematode (EPN) strains was tested in a laboratory and under semi-field (in pot) conditions against soil-dwelling life stages of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). The EPN strain collections screened in the present study include two *Heterorhabditis bacteriophora* species, i.e. *H. bacteriophora* HK3 (H.b H) and *H. bacteriophora* HB Brecan (H.b B), three *Steinernema feltiae* species, i.e. *S. feltiae* Sylt (S.f S), *S. feltiae* OBSIII (S.f O), and *S. feltiae* CR (S.f C), and the *S. carpocapsae* strain DD136 (S.c D). All soil-dwelling life stages of WFT were susceptible to the tested EPN strains. The most virulent strains were S.f S, S.c D and H.b H. The S.f O strain was highly virulent against late second instar larvae and prepupae of WFT under high soil moisture conditions, but less effective against pupae under comparatively drier soil conditions. Results from dose rate experiments indicate that a comparatively high concentration of 400 infective juveniles (IJs) per cm^2 was needed to obtain high mortality in all soil-dwelling life stages of WFT. However, dose rates of 100–200 IJs/cm^2 already caused 30–50% mortality in WFT. In a mixed population structure of soil-dwelling stages of WFT under laboratory conditions, the proportion of both prepupae and pupae did not affect the efficacy of S.f S. However, the proportion of late L2 in the soil negatively affected the general mortality of WFT by S.f S and it was discussed that the late L2 is less susceptible to EPNs compared to prepupal and pupal stages of WFT. In pot experiment with the presence of host plants of the pest, up to 60% WFT population reduction was obtained at 1000 IJs/cm^2. The chances for combining EPNs with other biological control agents of WFT are discussed.

ZUSAMMENFASSUNG

Die Effizienz von sechs Stämmen entomopathogener Nematoden (EPN) gegen bodenbürtige Entwicklungsstadien des Kalifornischen Blütenthrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) wurde in Labor- und Halbfreiland-Versuchen getestet. Die untersuchten EPN Stämme beinhalteten zwei *Heterorhabditis bacteriophora* Arten, d.h. *H. bacteriophora* HK3 (H.b H) und *H. bacteriophora* HB Brecan (H.b B), drei *Steinernema feltiae* Arten, d.h. *S. feltiae* Sylt (S.f S), *S. feltiae* OBSIII (S.f O), und *S. feltiae* CR (S.f C), sowie den *S. carpocapsae* Stamm DD136 (S.c D). Alle bodenbürtigen *F. occidentalis* Entwicklungsstadien erwiesen sich als empfindlich gegenüber den EPN. Die virulentesten EPN Stämme waren S.f S, S.c D und H.b H. Der S.f O Stamm erwies sich bei hoher Bodenfeuchte als sehr effizient gegenüber dem späten zweiten Larvenstadium und Praepuppen von *F. occidentalis*, war jedoch weniger wirksam bei geringerer Bodenfeuchte gegenüber den Thripspuppen. In Konzentrationsreihenversuchen wurde festgestellt, daß relative hohe Dosen von infektiösen Juvenillarven (IJ) der EPN (400 IJ/cm^2) notwendig waren um eine hohe Mortalität gegenüber allen bodenbürtigen Thripsstadien zu gewährleisten. Allerdings verursachten Konzentrationen von 100–200 IJ/cm^2 schon ein Mortalität von 30–50%. In Laborversuchen mit heterogenen Populationszusammensetzungen der bodenbürtigen Thripsstadien wurde die Effizienz von S.f S nicht durch den Anteil von Praepuppen und Puppen beeinflußt. Allerdings beeinflußte der Anteil des späten zweiten Larvenstadiums in der Populationszusammensetzung die Nematoden-bedingte Mortalität negativ, was darauf schließen läßt, daß das späte zweite Larvenstadium von *F. occidentalis* weniger empfindlich gegenüber EPN ist als Praepuppen und Puppen. In Halbfreiland-Versuchen bewirkte eine Konzentration von 1000 IJ/cm^2 bis zu 60% Mortalität bei bodenbürtigen Stadien von *F. occidentalis*. Die Möglichkeiten eines kombinierten Einsatzes von EPN mit anderen natürlichen Feinden von Thripsen werden diskutiert.

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ABBREVIATIONS USED IN THE TEXT

Abbreviation	Meaning of the abbreviation
ANOVA	analysis of variance
CM	corrected mortality
df	degree freedom
DJ(s)	dauer juvenile(s)
e.g.	for example
Eq.	equation
EPN(s)	entomopathogenic nematode(s)
F	statistical F-value
Fig.	figure
FL	statistical fiducial limits
h	hour
i.e.	that is
IJ(s)	infective juvenile(s)
J1	first-stage juvenile
J2(D)	second-stage (dauer) juvenile
J3	third-stage juvenile
J4	fourth stage juveniles
L1	first instar larvae
L2	second instar larvae
LD	lethal dose
log	Logarithm
MC	moisture content

N	sample size
NE	not estimated
ns	non-significant
Ø	diameter
P	p-value (statistical significance)
R^2	regression/correlation coefficient
RH	relative humidity
SAS	statistical analysis system
SE	standard error of the mean
t	statistical t-value
TSWV	tomato spotted wilt tospovirus
w/w	weight by weight
WFT	western flower thrips
Z	statistical Z-value

1. INTRODUCTION

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important cosmopolitan pest of a wide range of economically important crops, such as vegetables and ornamentals in both greenhouses and in the field (Tommasini and Maini, 1995). WFT directly damage plants by feeding on the leaves, flowers and fruits (Childers, 1997; Higgins, 1992) and indirectly by transmitting plant viral diseases like tomato spotted wilt virus (Allen and Broadbent, 1986).

Eggs are laid in the plant tissue and first and early second instar larvae feed on the plants. A larger proportion of mature second instar larvae display positive geotaxis together with negative phototaxis, moving away from the flower or the leaves towards the soil. Prepupation occurs at a depth of 1.5–2.0 cm where the prepupa develops to pupa. Prepupae and pupae move only upon disturbance and do not feed. Consequently, pupa develops to adult that emerges from the soil (Moritz, 1997; Tommasini and Maini, 1995).

Chemical control of WFT is difficult because of its cryptic feeding behavior (flowers and leaf axis) and life strategy (i.e. pupation in the soil) (Helyer and Brobyn, 1992; Palmer, 1989). Moreover, the high frequency of insecticide applications for WFT control, coupled with the short generation time in *F. occidentalis* has lead to an increasing incidence of insecticide resistance in WFT in recent years (Broadbent and Pree, 1997; Brødsgaard, 1994; Immaraju *et al.*, 1992; Zhao *et al.*, 1995). For biological control, a limited range of natural enemies against the foliage life stages of WFT is available, including several species of predatory bugs of the genus *Orius* (Heteroptera: Anthocoridae) as well as predaceous mites like *Amblyseius barkeri* (Hughes) and *A. cucumeris* (Oudemans) (Acari: Phytoseiidae) (Loomans and van Lenteren, 1995; Riudavets, 1995). Because WFT spends about one-third of its life cycle in the ground,

these predators cannot sufficiently control thrips populations (Loomans and van Lenteren, 1995). Therefore, identification of natural enemies targeting the soil-inhabiting life stages of WFT could substantially improve biological control of *F. occidentalis*.

Entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae) are lethal obligate parasites of a large number of insect species (e.g. Peters, 1996; Smart, 1995), and are suitable biological control agents for soil-inhabiting insects. Even though only limited research has been conducted on the control of WFT by EPNs, virulence of some EPN species was reported (Chyzik *et al.*, 1996; Helyer *et al.*, 1995; Tomalak, 1994). However, no data is available on the relative susceptibility of the different soil-dwelling immature stages of WFT to EPNs. Moreover, little is known about strain differences in EPNs with regard to control of WFT in the soil media.

2. LITERATURE REVIEW

2.1. Western Flower Thrips

Thrips is the common name given to insects of the order of Thysanoptera (thysanos, a fringe; ptera, a wing). The word is both singular and plural (Tommasini and Maini, 1995). They are mainly phytophagous, mycophagous, or predatory thrips that inhabit a wide range of habitats, generally in the tropical, subtropical, and temperate regions. Of the 5000 or so known species only a few hundreds attack cultivated plants (Lewis, 1997c). The most important crop pest thrips species belong to the two genera – *Thrips* and *Frankliniella* (Thripidae). Currently, approximately 175 species belong to genus *Frankliniella*. Western flower thrips (WFT), *Frankliniella occidentalis*, (Pergande) (Thysanoptera: Thripidae) is one of the most phytophagous thrips harmful to vegetables and ornamental crops (Lewis, 1997b). WFT exists as more than one strains or biotypes (Palmer, 1989). Early workers named such colour and structural variants as different species and such practices account for most of the 17 names that are now rejected as synonyms (Nakahara, 1997).

2.1.1. Origin and distribution

WFT was first reported by Pergande in 1895 in California on apricot and potato leaves, on orange flowers and various weeds. Since then it was reported from different parts of the world. In Europe, its first appearance dates back to 1983 and its introduction is supposed to be via plant material from North America. Since its initial detection in Europe, it has spread through the continent (Tommasini and Maini, 1995).

2.1.2. Biology

WFT is very small and light (20–50 μg) insect. Lengthwise, the macropterous adult WFT is characterised by 0.9–1.1 mm from the tip of the antennae to the tip of the abdomen in male and 1.3–1.4 mm in female (Palmer, 1989; Tommasini and Maini, 1995).

The symmetric 'punch and suck' mouthparts together form a ventrally directed mouth cone with only one complete left mandible, two well-developed maxillae, the labrum in front and the labium behind. The right mandible is reduced to a small basal plate and degenerates completely in embryogenesis and metamorphosis. Both maxillary stylets combine to form a sucking tube with a sub terminal opening. The position of the opening and the rapid probing of the paired stylets allow a steady food intake. The strong left mandible is used to make an initial puncture in the plant tissue. The paired maxillary stylets are then thrust into the substrate, the grooved interiors of both forming the food channel. Several salivary glands are associated with the intake and digestion of food. In addition to the unpaired mandibular gland, two paired labial glands discharge their secretion into the pre-oral food cavity, where it mixes with the food as it is ingested (Kirk, 1997b; Moritz, 1997).

WFT lives mainly in flower heads. Adult WFT sometimes enter closed buds, and eggs are laid concealed within such buds in the parenchymatous tissues. The adult also lays eggs in the similar tissues of leaves, flower parts and young fruits using saw-like ovipositor with which it drills holes into the parenchyma tissues. The egg is kidney-shaped opaque and 0.25 × 0.50 mm in size. In most Thysanoptera, reproduction requires copulation between adults of the opposite sex. In WFT reproduction is facultative, i.e., partly bisexual and partly parthenogenetic (Brødsgaard, 1989b; Moritz, 1997). Females lay two kinds of eggs. Those that are fertilised have a full diploid number of chromosomes and produce only females, whereas unfertilised eggs are haploid and give only males (Moritz, 1997).

Its post-embryonic development involves two larval instars, as well as prepupa and pupa stages. As with all exopterigotes, the first and second instars resemble a miniature version of the adult, apart from the absence of wings and genital appendages, and they exhibit reddish eyes and antennae with fewer segments (Tommasini and Maini, 1995). The newly moulted larvae are characterised by a glassy white colour and start feeding immediately, becoming yellowish. Second instar larvae, waxy yellowish in colour, are more active than first instar larvae and feed more voraciously, up to three times more than during the first instar (Tommasini and Maini, 1995). Both stages grow in length and girth as they feed, so late first instar and newly moulted second instar larvae appear very similar. They can be distinguished only by examination of the number and placement of small setae on the abdomen. Ecologically, larval and adult stages occupy similar niches, but adults are more likely to invade new habitats (Moritz, 1997).

Upon maturity, the larvae display geotaxis together with negative phototaxis, moving away from the flower or the plant towards the soil. At a depth of 1.5–2.0 cm, the second instar larvae then develop into the prepupae. Upon developing to a pupa, the insect still remains under the surface and features longer antennae that face backwards towards abdomen. The prepupa has short wing buds while wing buds of pupa extend more than half way along the abdomen. Both prepupa and pupa are immobile unless disturbed, non-feeding and inactive, and are covered by a smooth, membranous, colourless and unsclerotised cuticle. The segmentation of antennae and legs is reduced (Moritz, 1997). Both stages are usually white. At the end of the pupa stage, adult dimensions are achieved. The adult male is smaller than the female, with a narrower abdomen. Shortly after having emerged, the insect begins feeding.

Females usually live about 40 days under laboratory conditions, but can survive as long as 90 days. Males live only half as long as females. Oviposition normally begins 72 h after emergence and continues intermittently throughout adult life.

The duration of development from egg to adult and reproductive fitness in WFT is closely related to environmental conditions especially temperature, and quality and type of food (Trichilo and Leigh, 1988). It was estimated that 268 degree-days, above a threshold temperature of 7.9 °C, were required to complete development from egg to adult (MacDonald *et al.*, 1998). Generally, the life cycle may be completed in two to three weeks, particularly under warm conditions in greenhouses. Pollen is important nutrition from reproduction point of view that females fed on pollen have a net reproductive rate of four times more than that of females fed without pollen. Under greenhouses each female lays about 40 eggs and there may be six or seven generations a year (Gaum *et al.*, 1994; Soria and Mollema, 1995).

2.1.3. Behaviour

WFT prefers plants with flowers. Most of the population colonizes the upper parts of the plant, where it settles until the crop is harvested. Peak population is achieved in the hottest months of the year, while in temperate regions WFT tends to go into diapause during the winter months in the field conditions (Kirk, 1997a; Tommasini and Maini, 1995).

Although it preferentially attacks protected crops, WFT can also be found on open field vegetable and ornamental crops. The pest can overwinter under such conditions in a state of quiescence protected under dry vegetable remains or immediately under the upper surface of the soil. In subtropical and Mediterranean areas of infestation, WFT can have continuous generations in the greenhouses and outdoors. In field with a temperature not below 5–6°C, it can overwinter on the wild plants (Kirk, 1997a; Moritz, 1997).

A wide number of studies using chromotropic traps have yielded contrasting results. Brødsgaard (1989a), which agrees with the majorities of the report, found that in a mixture of colours WFT always prefers the light blue flowers. However, in several crops yellow sticky traps are usually employed for capturing WFT as they also capture other pests (Tommasini and Maini, 1995).

Either morphology or chemical constitutes of host plant cultivars appear to affect feeding and development of WFT adversely. WFT larvae generally cause less damage to leaves of cucumber, tomato, and peppers containing low concentration of aromatic amino acids than those with higher concentration of these compounds (de Jager *et al.,* 1995; Mollema and Cole, 1996).

2.1.4. Importance and control

Damage

WFT causes direct damage to plants due to the mechanical action of the mouthparts during feeding and to oviposition. The damages during feeding and egg-laying include perforation of plant tissues as a consequence of the introduction of mouth stylets, injection of saliva into plant tissues and consequent cell lyses, sucking up of cellular contents, and oviposition penetration and egg-laying into plant tissues (Childers, 1997; Kirk, 1997b; Tommasini and Maini, 1995).

In addition to direct damage they impose to different plants, equally importantly, WFT cause indirect damage i.e., they transmit virus-induced diseases. At least four different tospoviruses are known to be transmitted by WFT: tomato spotted wilt tospovirus (TSWV), impatiens necrotic spot tospovirus, groundnut ring spot tospovirus and tomato chlorotic spot tospovirus. The transmission of TSWV is the most important since the virus can affect a wide variety of plants particularly vegetable and ornamental crops (Machoux *et al.*, 1991). TSWV multiplies

in WFT and thus thrips can inoculate plants for the duration of their lives. This indicates that WFT transmits TSWV in a persistent and circulative fashion (Ullman *et al.,* 1997). Only first and second instar larvae acquire the virus. The virus enters the thrips haemocoel at the midgut during larval acquisition but a midgut barrier prevents acquisition by adult WFT (Ullman *et al.*, 1992)

Economic importance

WFT is remarkably versatile and opportunistic. It is a highly polyphagous species with at least 250 plant species from more than 65 families (Anonymous, 1989). Under mild winter in temperate regions and in Mediterranean climates, WFT can over winter on weeds and in the spring feed on tree blossoms (like apricot, peach, palm, and nectarine-blossoms) causing scarring and discoloration of developing fruits (Childers, 1997). In protected crop production in greenhouses, WFT injure seedlings of many crops as they come through the ground. Blossom drop and/or fruit scars of peas, tomatoes, melons, grapevine, eggplant, cucumber, pepper, strawberries and other vegetables are attributed to this pest in many parts of the world (Tommasini and Maini, 1995).

Currently, WFT is cosmopolitan and abundant in greenhouses throughout the world, and is also widespread in subtropical areas including the highlands of eastern Africa (Mound, 1997), thus, indicating its worldwide importance. Until the early eighties, *Thrips tabaci* Lind. (Thysanoptera: Thripidae) was the most prevalent thrips pest in Europe but caused problems only occasionally. Since its accidental introduction in 1983, WFT became the number one key pest in European greenhouses. In the late eighties, it invaded vegetables and ornamentals grown in the plastic tunnels and the field as well as fruit trees in the Mediterranean areas of Europe (van Lenteren and Loomans, 1995).

In addition to yield and market quality reduction WFT cause on commercial plant production, the mere presence of thrips on a crop is used as a reason for denying it entry to a profitable market. However, economic injury levels of many insects depend on seasonal stage and market prices of host plants (Brodersen, 1997). Shipp, Binns *et al.* (1998) reported that a mean density of 10–26 adult WFT/sticky trap in greenhouses would result in the occurrence of unmarketable sweet pepper (*Capsicum annuum*) fruit. However, for impact of WFT on plant growth and photosynthesis Shipp, Hao *et al.* (1998) reported that thrips abundance should be maintained below 1200 larval-days per plant to prevent the negative impact of WFT on plant physiology and yield. Sometimes entire crops are lost due to virus attacks vectored by WFT (Kuo, 1996) and the worst attacks are commonly associated with poor crop hygiene, thus, under such conditions, economic threshold levels of WFT could be low.

Control

Many thrips, including WFT, are difficult to control. Their eggs are protected by the plant tissue in which they are laid, the nymphs are found mostly off their host plant (i.e., in the soil), and the adults are very active. Generally, favouring minute cracks and crevices and pupating mainly in the soil, renders WFT inaccessible to many control agents (Palmer, 1989).

Chemical control: Prior to resistance development to many insecticide groups, chemical control against WFT in both greenhouses and field conditions in different crops was the major control option (Bournier, 1990; Lewis, 1997a). Malathion has been found to be one of the most effective chemicals (Helyer and Brobyn, 1992). In general, chemical control of WFT is very difficult due to its cryptic feeding behaviour, and its short generation time. Moreover excessive pesticide treatments to which WFT are subjected lead to develop resistance to many pesticides (Immaraju *et al.*, 1992; MacDonald, 1995).

Biological control: Because of considerable difficulty in the control of WFT, the introduction of biological control of thrips in many countries was accelerated (e.g. Ramakers *et al.*, 1989). Several efficient predators including mites (*Amblyseius spp.*), and bugs (*Orius spp.*) and parasitoids (*Ceranisus* spp) of WFT have already been described. The predators are used in commercial programs to control the foliage feeding stages of the WFT. Generally, the natural enemies require high density of WFT and the control level is relatively low in spite of low economic threshold level for many crops. Mites consume only small larvae, whereas the bugs feed on either larvae or adults (Loomans and van Lenteren, 1995; Riudavets, 1995).

Other control options: The basis of good IPM strategies is reported to be firstly to produce thrips-free conditions through weed control, host plant screening against the pest, and the production of pest-free mother plants, for instance, in chrysanthemum older mother plant) (CAB intewrnational, 1999; de Jong *et al.*, 1995). On the other hands, contrasting results on the effects of intercropping on WFT infestations have been reported (e.g. Capinera *et al.*, 1985; den Belder *et al.*, 1999). Moreover, the use of soaps and organic products such as extracts of neem trees was reported (Lindquist and Casey, 1990). UV-blocking films are also used to reduce the flight activity of *F. occidentalis* (Antignus *et al.*, 1996).

2.2. Entomopathogenic Nematodes

Nematodes are morphologically, genetically and ecologically diverse organisms occupying more varied habitats than any other animal groups except arthropods (Kaya and Stock, 1997). They may be free living, predacious or parasitic. Many of the parasitic species cause important diseases of plants, animals and humans. Other species are beneficial in attacking insect pests. The only insect-parasitic nematodes possessing an optimal balance of biological control attributes are steinernematids and heterorhabditids.

The two families, Steinernematidae and Heterorhabditidae (Phylum: Nematoda, Order: Rhabditida), are unique because they are the only nematodes which carry and introduce symbiotic bacteria into the body cavity of insects and they are the only insect pathogens with a host range which includes the majority of insect orders and families (Boemare *et al.*, 1996). Because of the rapid killing they cause to their hosts, they are described as entomopathogenic nematodes (EPNs) (Kaya and Stock, 1997). The two genera, *Steinernema* and *Heterorhabditis* are the important EPNs in the two families, respectively. Currently, there are 17 species in *steinernema* and 9 species in *Heterorhabditis* (Hashmi and Gaugler, 1998).

Concern about adverse effects of chemical insecticides on the environment has created a climate in which biological insecticides are regarded as a promising alternative. The situation for soil-born insect pests to be controlled by chemicals is even worsen – the insect may be found deep in the soil and attempts to apply chemicals in such conditions contradict with environmental interest in general. It was after such concern that the research on nematodes for the control of insect pests had got attention as biological control agents (Ehlers, 1996).

2.2.1. Diversity and geographic distribution

In the broader sense, EPNs are wide spread. However, it is not all species which are found everywhere but both families. The biodiversity of steinernematids is probably greater than that of heterorhabditids. Within steinernematids, two species appear to have a global distribution, namely *S. feltiae* and *S. carpocapsae*. EPNs can also exist as strains that differ biologically (Hominick *et al.*, 1996).

2.2.2. Biology and life cycle

EPNs have a simple life cycle that includes the egg, four juvenile stages (separated by moults) and the adult. The infective stage is a special third-stage juvenile (J3) called the infective

juvenile (IJ), or dauer juvenile (larva) (DJ), which is particularly resistant to environmental conditions. An area of interior part of the intestine of the IJ is modified as a bacterial chamber. In this chamber the IJ carries cells of a symbiotic bacterium. *Steinernema* and *Heterorhabditis* nematodes are symbiotically associated with bacteria of *Xenorhabdus* and *Photorhabdus*, respectively (Boemare *et al.*, 1996).

The natural openings of IJs (mouth and anus) are closed (Endo and Nickle, 1994). The infective stage is thus protected from penetration of microbial antagonists as well as from toxic chemicals. IJs are generally ensheathed within the separate but still intact J2 cuticle (Campbell and Gaugler 1991). The sheath may increase desiccation tolerance and protect against biotic factor. They are the only free-living form (outside of the host). They contain carbohydrate energy reserve, are non-feeding and thus can survive in the soil or be stored for extended period until they are able to find a susceptible host (Georgis and Hague, 1991; Glazer, 1996).

The IJs locate a host and enter through natural openings – mouth, anus or spiracles. Unlike steinernematids, however, heterorhabditids possess a tooth with which they may also directly penetrate through the cuticle of a host at the softer intersegmental membrane regions (Bedding and Molyneux 1982). They then actively penetrate through the midgut wall or tracheae into the haemocoel. After arriving the haemocoel of the insect, the IJ releases the bacteria. The bacteria rapidly multiply and cause septicemic death of the insect within 48 hours, and establish favourable conditions for nematode reproduction by providing nutrients and inhibiting the growth of many foreign microorganisms. The J3 feed on multiplying bacteria and dead host tissue and develop to fourth-stage juveniles (J4) (Georgis and Hague, 1991).

Generally, a lethal dose ranging from one to thousands of IJs per insect was reported, thus indicating an enormous variability in the intrinsic virulence of each species and strains. Toxicity in the insect haemocoel is detected a few hours after nematode penetration, reaching a peak just before insect death and declining a few hours later (Simões and Rosa, 1996).

In *Heterorhabditis* species, the J4 then develop further to self-crossing or hermaphroditic females. The J1 hatching from the egg either develop through J2, J3 and J4 stages to males and females, which are amphimictic cross-fertilizing adults, or they develop to pre-dauer stages (J2D) and further to DJs. Favourable conditions enhance the development of amphimictic males and females whereas depleting food resources lead to the development of the DJs, which can further develop to hermaphrodites (Strauch *et al.*, 1994). When the egg production of the hermaphrodite ceases, the remaining eggs develop within the uterus. These individuals develop to DJs and enter into the surrounding medium only after the hermaphrodite has died.

In *Steinernema* species the immature J4 that developed from J3 continue ingesting the bacterial cells and host tissues and develop to adults. The adults are amphimictic in all generations. The progeny of the next or the third generation, in most cases find the food supply depleted, form the new IJs (Woodring and Kaya, 1988).

In both genera, *Steinernema* and *Heterorhabditis*, eventually, the IJs emerge from the cadaver carrying their symbiont bacteria in their gut. At 18–28°C, the nematodes complete their life cycle in most insects in 8–20 days (Georgis and Hague, 1991).

2.2.3. Dispersal and behaviour

The IJs of steinernematids and heterorhabditids disperse vertically and horizontally, both actively and passively (Smart, 1995). Passively, rain, wind, soil, humans, or insects may

disperse EPNs. Adult insect hosts that are capable of flying after infection, especially when factors for the development of the infection are sub-optimal, may be expected to do so over a period of at least one or two days after infection. This type of EPN dispersal could be over long distance if the infected adult insect is wind-transported (Downes and Griffin, 1996).

They possess also different foraging behaviour to infect their insect hosts. Some species, for instance, *Steinernema carpocapsae*, and *S. scapterisci*, have a sit and wait strategy and have been called "ambush" foragers. The majority of the individuals in this strategy tend to remain near or at the soil surface, nictate, and attach to and infect mobile insects that feed at the soil-litter interface. Other species, for instance, *S. glaseri* and *Heterorhabditis bacteriophora*, have an active searching strategy and have been referred as "cruiser" foragers. The majority of nematodes with this strategy tend to be highly mobile and respond to chemical cues from the host, like CO_2 and host excretory products, and are adapted to infect less mobile insects in the soil. There is a continuum between these two extremes (Grewal *et al.*, 1994; Kaya, 1993).

2.2.4. Conditions affecting EPN efficacy

In spite of some successful control programmes, a major problem in implementing EPNs has been their short field persistence, even at high application rates. Pre-application factors associated with production, storage and transport conditions also determine the survival rate and quality of nematodes at the time of application (Glazer, 1996).

The IJs emerging from cadaver or applied as biological control agent and searching for hosts may encounter many potential hazards, including abiotic and biotic factors. The major abiotic factors include extremes in temperature, soil texture, soil moisture, and UV light affecting nematode's persistence and biotic factors antibiosis, competition, and natural enemies (Brown and Gaugler, 1997; Georgis and Poinar, 1983; Kaya and Koppenhöfer, 1996). Mites appear to be especially voracious nematode feeders. The greatest single factor reducing the efficacy of

EPNs in above ground field trials has been found poor nematode persistence on exposed foliage. Surface application of IJs in the evening followed by irrigation, to rinse nematodes down into the soil, thereby increasing both survival and mobility (Selvan *et al.*, 1994) improves efficacy of EPNs.

Though most of the insects tested have shown a higher susceptibility towards a particular nematode species or strain, there is some close relationship between the bacteria, the nematode, and the host insect. The developmental stages of an insect play an important role in susceptibility to the nematodes (Simões and Rosa, 1996). Late instar larvae, pupae in puparium, and adults are less susceptible than the early instar larvae (Lindegren and Vail, 1986; Peters and Ehlers, 1994). On the other hands, the nematode species have differences in infection. *Steinernema glaseri* is able to infect adults of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), but *S. carpocapsae* are not able to do so (Geden *et al* 1985). Variability among strains within the same nematode species is also common: the DD-136 strain is significantly less lethal to *Delia radicum* (Diptera: Anthomyiidae) larvae than are Mexican, Breton and All strains of *S. carpocapsae* (Bracken, 1990). Most of these variability in infectivity were explained either by the intrinsic capacity of each nematode species and strain to overcome adverse environmental conditions or by their ability to find a host.

Insect hosts have defence mechanisms against EPN IJs including sieve plates over spiracles, low carbon dioxide output, high defecation rate, and the ability to push nematodes away from their mouths with the anterior legs. After penetration, some IJs are recognized as non-self and are eliminated by encapsulation (Peters and Ehlers, 1994).

The presence of some plant metabolites (e.g. cucurbitacin) in insect's diet reduces the suitability of insects to EPNs. It was also shown that cucurbitacin D, an oxygenated tetracyclic triterpenoid found in cucurbits, has different effects on the growth of different

isolates of *Xenorhabdus* and *Photorhabdus* symbioant bacteria (Barbercheck and Wang, 1996).

2.2.5. Advantage of EPN

EPNs have some advantages when they are used as one of insect control methods. The major advantages are outlined as follows.

Mass production: EPNs can be mass-produced *in vitro* on solid media or in liquid media. Production in liquid medium can be done in small containers or in fermentation tanks. Greater number of juveniles can be produced per unit area in fermentation tanks, which makes this method especially suited for large-scale commercial production (Ehlers *et al.*, 1998; Surrey and Davies, 1996).

Broad host range but not on non-target organisms: EPN can infect a vast order of insect including agriculturally, medically and ecologically important insects. In the laboratory, a broad spectrum of arthropod species (> 250) of different animal classes (e.g. Chelicerata, Crustacea, Isopoda, Myriapoda and most orders of Insecta) were infected and killed by EPNs (Poinar, 1986). But under field conditions, EPNs are restricted to the soil environment where they are protected against desiccation and UV radiation. Therefore, their host range is restricted to such species, which develop or stay in the upper soil layers for a certain time during their development.

According to Bathon (1996) there is no report that an application of EPNs (indigenous or exotic species) severely affected the non-target fauna in the release area. Despite their broad host range and high virulence for insects, extensive testing has demonstrated a complete lack of mammalian pathogenisity (Gaugler and Bouch, 1979). None of the *Xenorhabdus* species is known to be a pathogen of plants, vertebrates or humans (Obendorf *et al.*, 1983).

Fast pest control: By the use of EPNs as one of pest control tactics, it is possible to control insect and get rid of produce loss within few days after application. The symbiotic bacteria released by the IJs cause up to 100% insect mortality within 24–48 h (Smart, 1995).

Widespread: They are ubiquitous, having been isolated from every inhabited continent from a wide range of ecologically diverse soil habitats including cultivated fields, forests, grasslands, deserts, and even ocean beaches. Moreover, EPNs have the potential to be used in a self-sustaining way, whereby they reproduce in the insect host, thereby providing new IJs to search for new hosts (Parkman *et al.*, 1993; Parkman *et al.*, 1994).

Amenable for genetic improvement: The methodologies of classical genetic engineering can be used for the genetic improvement of EPNs and their symbiotic bacteria. The main targets for genetic improvement in EPNs are increased efficacy, resistance to environmental extremes, and the development of anhydrobiotic strains. There are already reports for the improvements of EPNs in the some of selected genetic traits (e.g., Burnell and Dowds, 1996).

Compatibility with other control measures: There is additive or synergistic effect between EPN species, *H. bacteriophora* and *S. glaseri,* and *Bacillus thuringiensis* subspecies japonensis in controlling scarab grub (Koppenhöfer and Kaya, 1997). The EPNs are compatible with most pesticides (Rovesti and Deseo, 1990) and can also be applied with convectional equipments (Georgis and Hague, 1991). Even if very few studies refer to the combined effects of beneficial insects and EPNs, preliminary reports indicate the potential use of EPNs against soil-dwelling life stages and parasitic wasps against foliar feeding stages of WFT (Greene *et al.*, 1993).

3. RESEARCH OBJECTIVES AND HYPOTHESES

3.1. Objectives

The general objective of this study was to screen a limited number of different EPN strains at various concentrations for control of the soil-dwelling life stages of WFT. More specifically:

1. To select the most effective entomopathogenic nematodes among the test strains against soil-dwelling stages of WFT,
2. To find out the dose of EPN at which maximum pest mortality can be attained,
3. To compare the relative susceptibility of the soil-dwelling stages of WFT to EPNs,
4. To assess the potential of the efficient strains against the mixed soil-dwelling population of WFT in semi-field conditions.

3.2. Hypotheses

Insect pathogenic species of nematodes have a wide insect host range, but the degree of infection of each nematode species/strain for different hosts varies considerably. In addition, there is some close relationship between the pathogen, the nematode, and the host insect. Therefore, we hypothesised that variations among EPN strains exist in pathogenicity to WFT. Moreover, LD_{50} of different strains against WFT could differ considerably (Chyzik *et al.*, 1996). The different soil-dwelling stages of WFT were also expected to be different in their susceptibility to EPNs.

4. MATERIALS AND METHODS

4.1. Nematodes and Thrips Culture

The experiments were conducted in climatic chamber at the Plant Diseases and Plant Protection Institute in Faculty of Horticulture, University of Hanover, Germany. To use different EPNs species/strains on soil dwelling stages of WFT, i.e., late second instar larvae (L2), prepupa, pupa, both nematode and thrips cultures were maintained in the Institute. The rearing procedure of both organisms is described below.

4.1.1. Entomopathogenic nematode

Six strains of EPN (Table 1) were received from Institute of Phytopathology, Department of Biotechnology and Biological Control, University of Kiel, Germany. For convenience, the strains were coded and the acronyms are used throughout this paper.

Table 1. Entomopathogenic nematode strains tested.

Name of the strains	Code
Steinernema feltiae CR (Filipjev) Israel	S.f C
S. feltiae Sylt Germany	S.f S
S. feltiae OBSIII The Netherlands	S.f O
S. carpocapsae DD136 (Weiser) USA	S.c D
Heterorhabditis bacteriophora HK3 Poinar Germany	H.b H
H. bacteriophora Brecon Australia	H.b B

To maintain the nematode culture for the following experiments modified nematode rearing procedure by Woodring and Kaya (1988) was used. All the rearing procedures were carried out at 23 ± 2°C.

Fourth instar larvae of greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), was used to rear the nematodes. *G. mellonella* was reared following Woodring and Kaya (1988) procedure at 30°C.

Ten conditioned *Galleria* larvae were added on a 9.0-cm Whatman #1 filter paper in 100 × 14 mm plastic Petri dish. One ml of nematode suspension (approximately 200–500 IJs/ml of distilled water) was pipetted uniformly on the top of the larvae. For each EPN strain, two to three replications were prepared and stored in a plastic bag to conserve moisture.

Five to seven days after application of EPNs to the *Galleria*, the infected *Galleria* (ten cadavers per White trap) with peculiar characteristics of EPN infection were washed in distilled water and transferred to White trap as described by Woodring and Kaya (1988). A 9.0-cm Whatman #1 filter paper was placed on a watch glass (concave-side-down) in a large plastic Petri dish (150 × 20 mm). Seventy ml 0.1% formaline solution was poured into the Petri dish. The formaline was used to avoid contamination. The filter paper was dragged over the watch glass so that it can come into contact with the liquid surface in the Petri dish. The infected larvae were placed on the filter paper on the edge of the watch glass. Ten to twelve days after infection, the IJs started to exit the cadaver and migrated into the formaline solution in the Petri dish (Fig.1).

For harvesting of the IJs, two types of sieves were prepared from nylon tissue with different diameter of pore-size (i.e., 30 and 15 μm). A "wider sieve cylinder" was prepared by gluing the 30-μm nylon tissue to one end of an acryl-glass cylinder (Ø 84 mm). In similar manner, a

"narrower sieve cylinder" was prepared from the 15-μm nylon tissue. To avoid contamination risk of one nematode strain with the other, the acryl-glass cylinders and the sieves were prepared for individual strains and used constantly only for specified strain during the whole course of rearing.

The wider sieve cylinder was placed in another acryl-glass cylinder (Ø 94 mm), which was closed cylinder from its bottom (and referred to as "closed cylinder"). The migrated IJs in the suspension were observed for their survival under binocular and they were harvested and used for the experiment only if more than about 90% were alive. After the watch glass with cadaver was removed from the Petri dish, the suspension was poured into the wider sieve cylinder, which was in the closed cylinder (Fig. 2A). This is used to allow all IJs to pass through the 30-μm-sieve but to prevent any cadaver parts bigger than the pore size, non-infective juveniles and adult nematodes (as they are bigger than 30 μm in diameter). The sieve was in contact with the suspension in closed cylinder so that migrating IJs could pass through the sieve and be transferred to the closed cylinder through water media.

To facilitate the migration of IJs, the cylinder was shacked gently so that the water above the sieve could give pressure and the IJs can pass through the sieve very soon. To let almost all IJs migrate to closed cylinder, the suspension was kept for 4–6 h. It was observed that IJs stayed for one or more days under high volume of water in such cylinder were died presumably due to lack of sufficient air in the water.

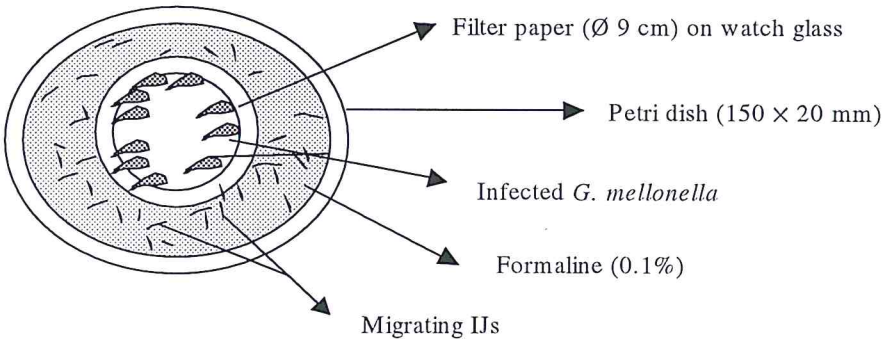

Figure 1. Larvae of *G. mellonella* on White trap after infected by entomopathogenic nematode

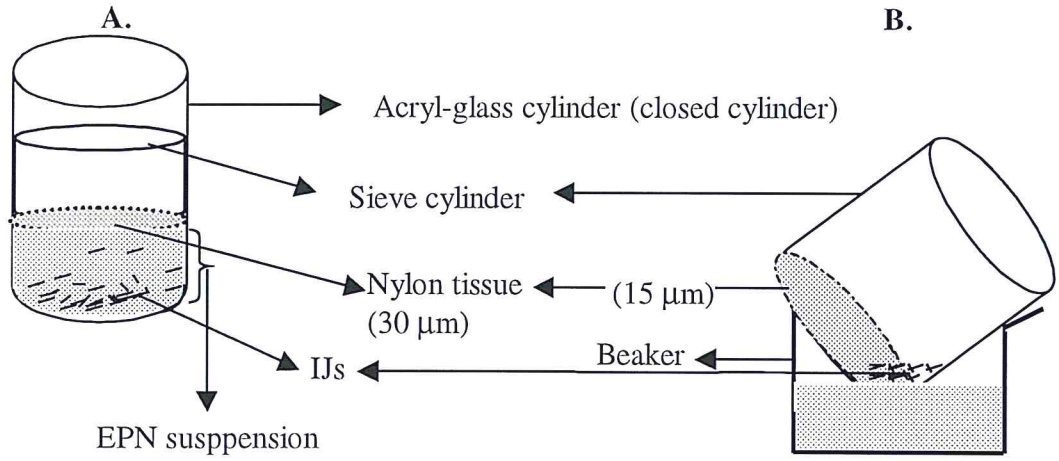

Figure 2. Separation of non-infective juveniles, adult EPN, and cadaver particles from IJs using 30-µm sieve (A) and concentrating the IJs using 15-µm sieve (B).

The suspension of IJs collected by the above procedure was poured to a narrower sieve cylinder (Fig. 2B). This was used to wash and to concentrate the IJs, and to separate any cadaver particles less than 15 µm in diameter. Infective juveniles in such sieve were washed three to four times by distilled water to get clear IJs suspension as described by Woodring and Kaya (1988). At the end, the narrower sieve cylinder was rinsed with Ringer solution to transfer concentrated IJs on nylon tissue to a beaker and finally transferred to "Tissue Culture Flask" (volume = 250 ml) for storage. The bottles were labelled and stored in cold room at 4°C until the time of use. The suspension was neither more than 2 cm in depth nor more than 20,000 IJs/ml in concentration.

Once harvesting of IJs started, it was taking place everyday for each White trap. At every harvest, the watch glass with cadavers was put back into the Petri dish and new 0.1% formaline solution was poured for next harvest (Woodring and Kaya, 1988). This was repeated for three to four days until most of the IJs were harvested from all cadavers.

Before the use of EPNs for the experiments, the concentration of IJs in the storage flasks was determined by counting the sample from the flask. Two hundreds and fifty µl suspension was taken four times from a storage flask and one to two drops of the suspension was pipetted into each quadrate of the nematode counting apparatus (cloning plate). Drops of water were added to the quadrate over the drops of EPN suspension to distribute the IJs through the quadrate for the ease of counting. The nematodes were counted using either inverted microscope or binocular. The total number of nematodes from the four samples was considered as the concentration of the nematode in one ml of the original suspension in the storage flask.

Whenever the concentration in the flask was more than the required concentration for the experiment, new suspension was prepared. New suspension with new concentration was prepared using equation 1. For this:

Take Y amount of original suspension and add to V_R – Y amount of distilled water. Y can be determined as follows,

$$Y = \frac{V_R * C_R}{C_O} \qquad \text{(Eq. 1)}$$

where Y = amount of suspension in ml to be taken from original storage flask,

V_R = final required volume of the new dilution in ml,

C_R = required concentration to be prepared (number of IJs/ml), and

C_O = concentration of the original suspension in the storage flask (IJs/ml).

4.1.2. Western flower thrips

Following rearing protocol by Ullman[α] (Personal Correspondence), WFT was reared in climatic chamber to obtain uniform-aged larvae, prepupae, and pupae for the proceeding experiments. Four to six pods of green beans (*Phaseolus vulgaris* L.) were put in a glass jar whose lid had a hole in its centre. A nylon tissue (64 μm pore size) was glued on this hole as hole-cover to allow ventilation but preventing insect escape. Two hundred to 300 adult thrips were put in the glass jar to let the thrips lay eggs in the tissue of the pods. After 48 h the pods were taken out of the jar by careful removal of all adults that might stick to the pods. The pods were placed in another glass jar in which two layers of a 90-mm filter paper were put to stimulate pupation between the papers. The rearing glass jars were replicated until required number of larvae, prepupae, and pupae for the proceeding experiments could be obtained.

4.2. Pre-experimental Trials

It was observed that the efficiency of EPNs against WFT depends on the methodology used. and, hence, different pre-experimental trials were carried out to develop a better methodology.

Moisture content

A plastic pot (∅ 5.5 cm) was filled with 70 g non-sterilised soil (Fruhstorfer Erde Type P) so that it formed a top area of approximately 20 cm^2. Sixteen pupae or prepupae were applied to the soil in the pot with a moisture content (MC) of about 45, 50, or 55% (w/w). The inner part of a lid of 100-mm Petri dish was painted with insect glue and used as cover for the experimental pot, hence serving as a sticky trap. On daily bases, emerging WFT adults were counted from the sticky traps. The adults were counted for seven consecutive days until no

[α] Ullman, D. 1998. Rearing protocol for western flower thrips. (Personal Correspondence).

more adults emerged from the soil. Number of emerging adult was used as criteria of the best moisture content to work with.

The result indicated that maximum adult emergence was recorded from soil with a moisture content of 55% (for pupae 92%, and for prepupae 80%).

Use of plant leaves for adult emergence

Similar pots as above described were used in this trial. The pots were filled with fine sand (MC = 12%) and a green bean leaf was added to test whether leaves are required as cue for adult WFT emergence. For this a leaf was placed on top of the sand in the pot, with control treatments without leaves. Sixteen WFT immature stages (pupae, prepupae, or late L2) were transferred to the pots. The results indicated that adult emergence did not differ whether leaves were added (65%) or not (68%).

Arena for EPN application

To select the most appropriate assay arena for the subsequent experiments, a trial was carried testing different media of the bioassay. For this, filter paper, 12 g soil, or 9 g sand was placed in a 100-mm Petri dish. EPN suspension (400 IJs/cm^2) or distilled water as control was pipetted on the surface of the media, resulting in a moisture content of about 55% for soil, and 12% for sand. To avoid any standing water in the Petri dish, only one ml of nematode suspension or distilled water was pipetted over the filter paper. Following application of EPN or water, 10 pupae were placed on the top of the media. The lid of the Petri dishes was painted with insect glue and used as sticky trap.

Starting from the third day after application of the pupae, on daily bases emerged adults were counted from the sticky traps. The total number of emerged adults is shown in table 2.

We observed that before they developed into adults, pupae were crawling on the wall of the Petri dish above the soil surface. This most likely reflects a contact-avoidance behaviour of WFT when encountering EPNs, and hence no mortality was recorded on filter paper and only low levels with sand and/or soil as media (table 2).

Table 2. Number of emerged adult western flower thrips and percentage of corrected mortality (CM) in different media (soil i.e. Fruhstorfer Erde, sand, or filter paper) pipetted with water or entomopathogenic nematode (*S. feltiae* OBSIII).

	Soil	Sand	Filter paper
Water	7	8	8
EPN	5	7	9
CM (%)	28.6	12.5	0

When to apply EPN suspension?

In order to obtain the highest efficacy of the EPNs in the bioassays, the question was whether the IJs or the immature WFT should be first introduced into the test arena. To answer this question the following trial was conducted: a Petri dish (Ø 35 mm) was filled with soil and/or sand. Ten pupae or late L2 were transferred to the Petri dish. EPN suspension or distilled water was pipetted to the Petri dish before or after the introduction of immature WFT stages. The results in table 3 indicate that the virulence of EPNs is not very much affected by the timing of the application. Moreover, we observed that larvae clearly preferred soil to sand for pupation. They were crawling on the surface of the sand in the arena avoiding any contact with the EPNs in the sand. However, in the soil the larvae easily descended into the media for pupation. Thus, soil is the preferable media to study the effect of EPNs against L2 stage of thrips.

Table 3. Number of emerged adults and corrected mortality (CM (%)) of western flower thrips for the late L2 and pupae transferred to the sand or soil as affected by time of EPN (*Steinernema feltiae* OBSIII) application.

		Sand		Soil	
		Before [a]	After	Before	After
Larva	Water	8	7	8.5	9
	EPN	6	5	4	5
	CM (%)	25	28.6	52.9	44.4
Pupa	Water	8.5	9.5	8.5	7.5
	EPN	6	7	3	2
	CM (%)	29.4	26.3	64.7	73.3

[a] "Before" and "After" refer to the transfer of WFT to the arena before and after EPN suspension was pipetted, respectively.

4.3. Main Experiments

The experiments were carried out at 23 ± 2°C, 60–90 relative humidity (RH), and 16:8 h light:dark photoperiod. The experiments consisted of four sets:

Set A – Screening of EPN against WFT larva, prepupa, and pupa;

Set B – Dose rate study of EPNs against larva, prepupa, and pupa;

Set C – Effects of EPNs on the population of WFT under laboratory conditions, and under semi-field conditions in pots with green beans as host plants for WFT; and

Set D – Influence of EPN on pupation and adult emergence of WFT.

Except experiments on pupa under set A and on WFT population with the presence of host under set C, all experiments were conducted according to the following assay arena.

Assay Arena

Non-sterilised 2.5 g soil (Fruhstorfer Erde Type P, moisture content (MC) of about 38.4% (w/w as determined by oven drying)), sieved by 2 × 2.5 mm sieve pore size, was added to a plastic Petri dish (35 × 10 mm). The soil is commercially available (Archut GmbH, Lauterbach – Wallenrod, Germany) and used in many German greenhouses. It is composed of humus, clay and peat in the proportion of 15:35:50, respectively. The soil has a pH equal to 5.9 and N:P_2O5:K_2O of 150:150:250 mg/l, respectively. According to the treatment, one ml of the nematode suspension or distilled water (thus, final MC about 67%) was pipetted on the top of the soil after application of immature stages of WFT.

The inner edge of the lid of the Petri dish was lined with modelling clay so that the Petri dish could be tightly closed to avoid any escape of emerging adult WFT. A small hole (∅ 7 mm) was drilled in the centre of the lid of the Petri dish on to which nylon tissue was glued to allow ventilation but preventing thrips from escaping. The inner part of the lid of the Petri dish, except the hole, was painted with insect glue (Temmen GmbH, Hattersheim, Germany) so that emerging adult thrips could get stuck to it (subsequently referred to as 'sticky traps').

The late second instar larvae, prepupae and pupae used in the experiments were collected from the stock culture 14, 16 and 17 days after emergence of neonate larvae, respectively. To avoid the use L2 instar that were not yet ready for prepupation, only larvae that had been staying between the two filter papers in the thrips rearing glass jar were used. All insects were first individually examined under the binocular and then transferred to the top of the soil in the arena using a fine camel hairbrush. Thereafter the nematodes were applied and the Petri dish was tightly closed and kept for one week in a climate chamber. At least, four replications per treatment were used. At the end of the experiments, all WFT adults (dead or alive) and alive prepupae and pupae from the soil in the assay arena (on the surface of the soil and inside

the soil) and adults that got stuck on the sticky traps were counted. Thus, corrected mortality was calculated based on the total number of adults and alive immature stages counted at the end of the experiment.

4.3.1. Screening of EPN strains against immature stages of WFT

All EPN strains listed in table 1 were tested against L2, prepupae and pupae of WFT. For experiments with late L2 and prepupae, the previously described assay arena was used. Ten late L2 were transferred to each arena. The late L2 immediately showed a positive geotaxis and went into the soil just after the transfer to the arena. A nematode suspension at a concentration of 400 IJs/cm^2 or distilled water (for the control treatment) was pipetted on the soil when all larvae had descended into the soil. Experiments with prepupae were conducted in a similar manner except that in the experimental set-up used the prepupae stayed on the surface of the soil and the nematode suspension or distilled water was pipetted directly on the immature.

One of the limiting factors for the use of EPNs for insect control is desiccation of the media where the nematodes are applied (Gaugler, 1988). To screen EPN strains under such conditions for efficacy against WFT pupae, an experiment was carried out at low soil moisture content. A plastic pot (∅ 5.5 cm) was filled with 70 g non-sterilised soil (Fruhstorfer Erde Type P) so that it formed a top area of approximately 20 cm^2. Similar to the experiments with late L2 and prepupae, EPN strains were applied at a concentration of 400 IJs/cm^2 rate just after 16 pupae had been transferred to the top of the soil of each pot. To adjust the soil MC to approximately 45% (w/w), the concentration of the EPNs was adjusted to 1600 IJs/ml so that only 5 ml of the nematode suspension was applied to each pot. The inner part of a lid of a 100-mm Petri dish was painted with insect glue and used as cover for the experimental pot, hence serving as a sticky trap. On daily bases, emerging WFT adults were counted from

the sticky traps. The adults were counted for seven consecutive dates until no more adults emerged from the soil.

Effect of WFT position in the arena on efficacy of EPN

In the present study it was observed that, after transferring the immature WFT to the arena, the L2 penetrated into the soil, whereas the majority of the prepupae and pupae stayed on the top of the soil and hence, the EPNs attacked the insects at their respective position. To assess the effect of position of the soil-dwelling stages of WFT on the efficacy of the EPNs, an additional experiment was carried out. The same assay arena as described above was used. Ten WFT pupae were placed at either of the following positions in the Petri dish: bottom, middle or top. For the bottom position, prior to transferring the pupae the Petri dish was lined with a thin layer of soil (approximately one mm thick). For the middle position, the Petri dish was filled with the soil up to its middle (approximately four mm depth). For both positions, after transferring the pupae, the remaining portion of the Petri dish was filled with the soil gently up to the top (approximately eight mm soil depth). For the third position, the pupae were placed on the top of the soil after the Petri dish was filled with soil up to approximately 8 mm depth. Finally, in all three treatments a S.f S solution at a concentration of 400 IJs/cm^2 was pipetted to the top of the soil and the arena was tightly closed as described above. Each position had its own control treatment where distilled water instead of EPNs was used. Each treatment was replicated five times and data were collected as described above.

4.3.2. Dose rate study of EPN strains against immature stages of WFT

Based on the results of the screening experiments in Set A, four EPN strains, i.e. H.b H, S.f S, S.c D and S.f O, against late L2, and three EPN strains, i.e. H.b H, S.f S, and S.c D, against prepupae and pupae were all farther tested at 0 (control), and concentrations of 100, 200, 400, and 1,000 IJs/cm^2. Each dose rate of a given strain was tested with four replicates. To

investigate the dispersing behaviour of EPN strains in the arena, for experiment on late L2, IJs were counted from the sticky trap and from the bottom of the arena after removing the soil in the Petri dishes.

4.3.3. Effects of EPNs on different population structures of WFT

Under laboratory conditions

To study the effect of EPNs (S.f S at 400 IJs/cm^2) against simulated population structure of soil-dwelling life stages, different proportions of late L2, prepupae and pupae were added to the assay arena (for the different proportions tested, refer to table 4). The total number of individuals transferred to each arena was 21. The proportion represents the three soil-dwelling stages of WFT that could be in different population structure at different times. EPN could be applied at any population structures of WFT in the soil. Here the population is represented as entirely composed of only one of the three stages, entirely composed of two stages (that could be in equal proportion or one stage being half of the other) or composed of all the three stages with equal proportion. The proportions were selected to include the possible extremes (e.g. 100%, or 0%) of a given stage in the population structure. Some representative proportions (%) (e.g. 50:50, 33:67, or 33:33:33) (Table 4) were also included based on the idea that one or more of the stages exist in the arena while the total number of individuals equal to 21. Each treatment, i.e. different proportion of immature stages, had its own control, i.e. pipetted with distilled water instead of the EPN suspension.

Table 4. Number of larva (L), prepupa (B) and pupa (P) of WFT applied to assay arena.

Number of the immature stages of WFT			
Larva	Prepupa	Pupa	Population composition denoted by
0	0	21	P
0	7	14	BP2
0	10	11	BP
0	14	7	B2P
0	21	0	B
7	0	14	LP2
7	7	7	LBP
7	14	0	LB2
10	11	0	LB
11	0	10	LP
14	0	7	L2P
14	7	0	L2B
21	0	0	L

Experiments under semi-field conditions

Seeds of green beans (*Phaseolus vulgaris L.*) were sown and at cotyledon stage, the seedlings were transplanted to a pot (∅ 11 cm) filled with soil (similar to laboratory experiments), forming a top area of approximately 78 cm^2. At the two-leaves stage, the pots were caged using acryl-glass cylinder (∅ 84-mm). Twenty adult females and two males of WFT of the same age were released into the cage, using the same sex ratio as observed in the stock culture. Modelling clay was used to avoid any gaps between the acryl-glass cylinder and the

pot, and hence, to prevent any escape of the thrips from the pot. Likewise, the open end at the top of the acryl-glass cylinder was glued with nylon tissue (64-µm ∅ pore size). For additional ventilation, two side-holes (∅ 3-cm) were drilled in the upper part of the cylinder and covered with the same type of nylon tissue. Two similar sized side-holes in the lower portion of the cylinder (also covered with nylon tissue) were used as 'windows' for releasing the adult WFT to the seedlings and for pipetting the EPN suspensions. Under these conditions, in preliminary experiments, pupation of WFT usually occurred eight to ten days after introduction of the adult thrips. Hence, either EPN suspension (H.b H, S.f S and S.c D at concentrations of 400 and 1,000 IJs/cm^2) or distilled water (as control) was applied to the pots on the 12th day after thrips introduction. Fifteen minutes after the EPN application, the soil was irrigated with 50 ml of distilled water through the lower window to rinse the EPNs on the top of the soil so that the nematodes could reach the pupating WFT in the soil. Completely randomised block design with five replications per treatment was used. The seedlings were treated as normal cases. To provide sufficient time for pupation, the seedlings were kept for two additional days after EPN application, i.e. until the 14th day after introduction of the WFT. Thereafter, the acryl-glass cylinder and the shoot part of the seedling were removed. Then, the pots were covered with the lid of a Petri dish (∅ 100 mm) that had been painted with insect glue to serve as sticking trap for emerging adults.

The fecundity of WFT within 15 days (including the day of introduction) was assessed. For this, pots that were pipetted with distilled water (i.e. control treatment) were sampled and all foliar stages of WFT, i.e., L1/L2, prepupae, and pupae, on the seedling in each pot were counted under binocular. WFT that were in the soil for pupation at the time of data collection (i.e., 15 days after adult introduction) were collected later on as emerged adults. The emerging adults in all treatments were counted daily from the sticky trap for ten consecutive days, starting one day after the removal of the cage. At the end of the experiment, all adults

remaining on the top of the soil in the pots were also counted. Efficacy of the different EPN strains was assessed using corrected mortality values.

4.3.4. Influence of EPN on pupation and adult emergence of WFT

This experiment was conducted to know the effect of EPN on adult emergence and to back up the method of data collection in the study of EPN against WFT. The arena used above (e.g., Set B) was filled with 6.5 g fine sand or 2.5 g soil (similar to assay arena). Ten L2 or pupae were transferred to arena. Suspension of S.f O at 400 IJs/cm^2 was pipetted to the top of the media after the immature WFT were transferred to the arena. Seven days later, all WFT at different positions of the arena were counted. These include adult and immature stages inside the media and on the top of the media, and adults in the sticky trap.

4.4. Statistical Analysis

Mortality data were corrected for control mortality following Abbott's formula (1925) (Eq. 2) and analysed using analysis of variance (ANOVA) (SAS, 1996).

$$CM\% = \frac{C_A - T_A}{C_A} * 100 \qquad \text{(Eq. 2)}$$

where CM = corrected mortality

C_A = control alive

T_A = Treatment alive

Whenever two factors exist (e.g. EPN strains and dose rates) and significantly interacted, means of the levels of one factor were compared at each level of the other factor. Otherwise, when the factors' interaction was not significant, means of the levels of one factor were compared irrespective of the levels of the other (Sokal and Rohlf, 1995).

The corrected mortality means were compared to zero (corrected mortality of control) using Dunnett's two-sided test. When significant factor effects were detected by means of ANOVA, corrected mean mortality of the different levels of the respective factor was compared using Tukey's multiple means comparison procedure. Treatments in a factor only with two levels were compared using t-test (SAS, 1996).

Correlation and regression analyses were performed to investigate the relationships between corrected mortality and the varying concentrations of IJs/cm^2 tested. LD_{50}, LD_{75} and LD_{90} were calculated by means of probit analysis (SAS, 1996). To normalise variance for all analyses, non-continuous data, for instance, the number of IJs counted from the different parts of the assay arena, number of WFT, were square root transformed. Data uniformity was checked and in case of non-uniform data distribution non-parametric test, Wilcoxon, was used (Sokal and Rohlf, 1995). A significance level of $\alpha = 0.05$ was used in all analyses.

5. RESULTS

5.1. Screening of EPNs against WFT

5.1.1. Larvae

The proportion of adult emergence in the control was relatively low (68% (SE = 6.3)). However, all EPN strains tested caused significantly higher larval mortality than natural mortality in the control ($P < 0.001$). Significant differences in larval mortality were observed between the EPN strains tested, with highest mortality recorded in the H.b H, S.f S, and S.f O and lowest in the S.f C treatment, respectively (Fig. 3). However, in all EPN strains larval mortality exceeded 50%.

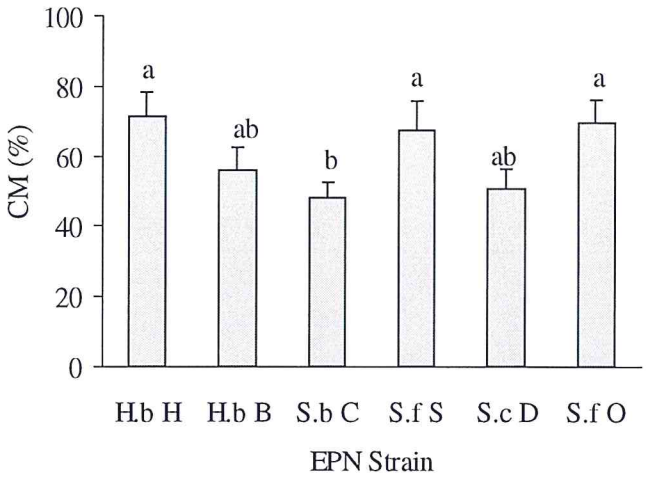

Figure 3. Mean corrected mortality (CM (%)) (±SE) of western flower thrips late second instar larvae caused by different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *H. bacteriophora* Brecon (H.b B), *Steinernema feltiae* CR, *S. feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O)). Bars with the same letters are not significantly different at $P = 0.05$.

5.1.2. Prepupae

In the control 78% (SE = 4.8) of the applied prepupae developed to adults. All EPN strains tested caused significantly higher mortality than in the control treatment ($P < 0.001$). No

significant differences in prepupal mortality were found between the EPN strains (df = 5, 18; F = 1.68; P = 0.1907), with corrected mortality values ranging from 39% (S.c D) to 53.7% (H.b H) (Fig. 4).

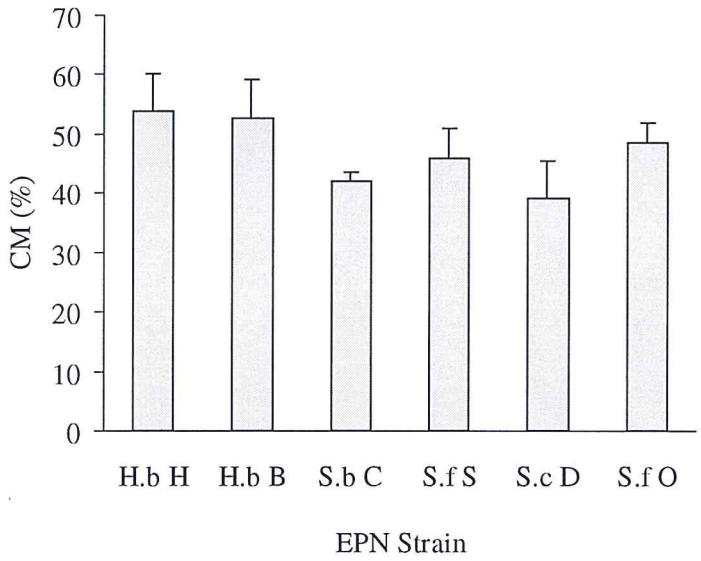

Figure 4. Mean corrected mortality (CM (%)) (±SE) of western flower thrips prepupae caused by different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *H. bacteriophora* Brecon (H.b B), *Steinernema feltiae* CR, *S. feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O)).

5.1.3. Pupae

In the control 89% (SE = 4.7) of the pupae developed to adults. Mortality of pupae in the S.f C (P = 0.208) and in the S.f O treatment (P = 0.07) did not differ significantly from the natural mortality in the control. However, in the four other EPN strains tested, significantly higher pupal mortality was recorded compared to the control (P < 0.001). Moreover, significant differences in pupal mortality were recorded between the strains, with highest mortality in the S.f S (54.5%) and lowest in the H.b H (29.4%) treatment (Fig. 5).

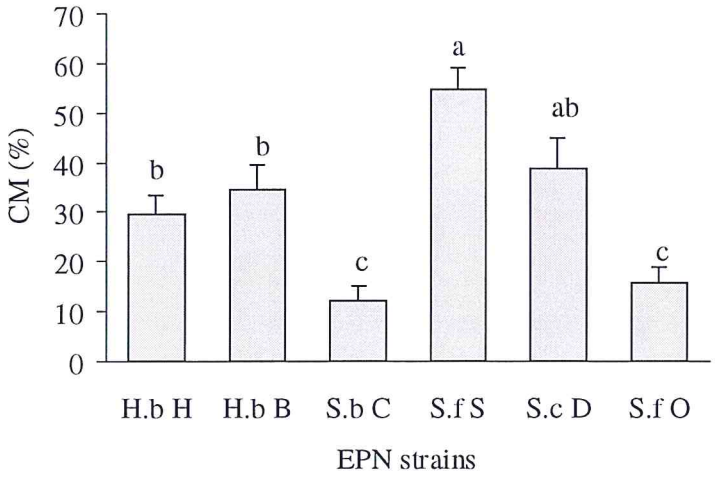

Figure 5. Mean corrected mortality (CM (%)) (±SE) of western flower thrips pupae caused by different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *H. bacteriophora* Brecon (H.b B), *Steinernema feltiae* CR (S.b C), *S. feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O)). Bars with the same letters are not significantly different at P = 0.05.

Effect of WFT position in the arena on efficacy of EPN

Results of the additional experiment carried out to study the effect of position of immature WFT in the arena on the efficacy of S.f S are presented in table 5. In the control treatment a significantly higher proportion of adults emerged from the top compared to the two treatments in the soil. However, EPN-induced mortality was not significantly influenced by the position of the pupae ($F = 1.65$; $df = 2, 14$; $P = 0.232$).

Table 5. WFT adult emergence in control treatment and corrected mortality of pupae at different positions in the arena after application of EPNs (*Steinernema feltiae* Sylt).[a]

Position of Pupa	Adult emergence (%)	CM (%)
Bottom	52 b	73 a
Middle	54 b	81 a
Top	74 a	89 a

[a] Means within the same column followed by the same letters are not significantly different at P = 0.05 (Tukey multiple means comparison).

5.2. Dose rate study of EPNs against WFT

5.2.1. Larvae

In the control 78% (SE = 3.4) of the applied larvae developed to adults. All EPN at all dose rates caused a significantly higher mortality than recorded in the control treatments ($P < 0.001$). No significant interaction between dose rates and EPN strains was found ($df = 9, 45$; $F = 0.9$; $P = 0.48$). Therefore, EPN strains were compared irrespective of the different concentrations, and similarly, the dose rates were directly compared irrespective of the EPN strains. Significant differences in mean larval mortality between the four tested EPN strains were recorded, with highest mortality (69%) in the S.f O and lowest (41%) in the S.c D treatment (Fig. 6). Mortality of WFT larvae significantly increased with increasing dose rates up to a concentration of 400 IJs/cm^2 (Fig. 7). A further increase in the concentration to 1,000 IJs/cm^2 did not yield a significantly higher mortality. Except for S.f O, the dose rates significantly correlated with larval mortality (Table 6).

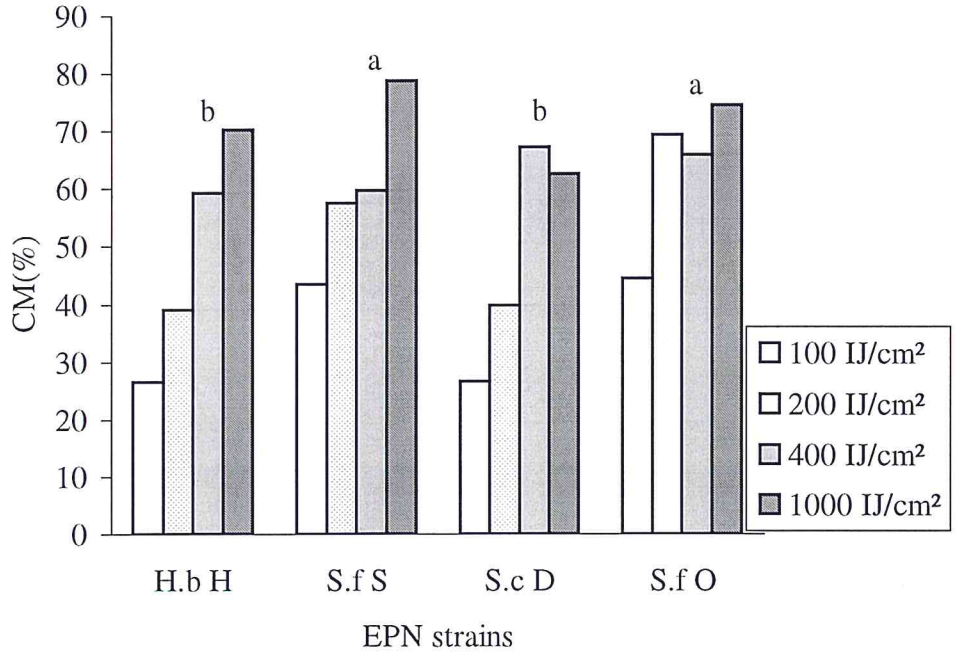

Figure 6. Mean corrected mortality (CM (%)) of western flower thrips late second instar larvae caused by different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O)) applied at 100, 200, 400 and 1,000 IJs/cm^2. The interaction of EPN strains and dose rates was non-significant. Consequently, the EPN strains were compared regardless of the dose rates. Different letters above the four bars of a strain (as means of bars of different dose rates for a given strain) indicate significance differences among the strains at P = 0.05.

Thus, the difference of EPN strains in their ability to move and search for their hosts was compared at each dose level. At all concentration levels, significantly higher numbers of S.c D IJs were recorded on the sticky traps (Table 7). The other three strains did not differ significantly in this respect.

Moreover, fiducial limits for the LD_{50} and LD_{75} values could not be estimated for S.f O strain, indicating that mortality did not depend on dose rate for this strain (Table 6). Virulence was highest in S.f S, even at low dose rates, and lowest in S.c D, with H.b H ranging in-between the two. The IJs applied to the top of the soil in the assay arena penetrated into the soil until the bottom of the arena or left the soil and moved to the sticky trap. The number of IJs of the

different EPN strains counted on the sticky traps differed significantly between the various concentrations tested (df = 9, 45; F = 2.46; P = 0.02).

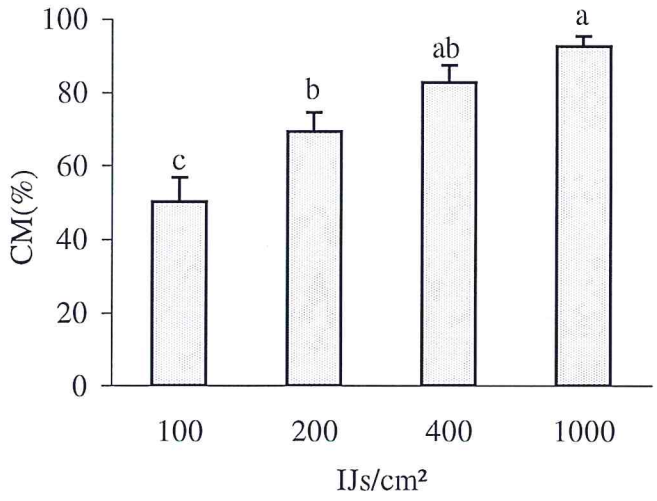

Figure 7. Corrected mortality (CM (%)) (±SE) of western flower thrips late second instar larvae caused by EPN strains applied at 100, 200, 400 and 1,000 IJs/cm^2. The data were pulled from four EPN strains (i.e. *Heterorhabditis bacteriophora* HK3, *Steinernema feltiae* Sylt, *S. carpocapsae* DD136 and *S. feltiae* OBSIII). Mortality values at different dose rates followed by the same letters are not significantly different at P = 0.05.

Table 6. Dose effects of *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O) on the mortality of western flower thrips late second instar larvae.

EPNs	R^2	B [a]	P [b]	LD_{50} (95% FL [c])	LD_{75} (95% FL)
H.b H	0.819	20.1	0.002	184 (74–298)	930 (515–2175)
S.f S	0.908	12.8	0.021	47 (20–119)	407 (195–1592)
S.c D	0.516	18.4	0.013	230 (96–403)	1379 (658–3980)
S.f O	0.488	9.5	0.08	18 (NE)	259 (NE)

[a] B = slope of regression equation: CM(%) = Log(Dose) + C.

[b] P = probability that the slope b is not different from zero.

[c] FL = lower and upper fiducial limits

NE = not estimated

Across all strains tested, increasing the concentrations lead to higher IJs trap catches on the sticky traps, with significantly highest numbers of IJs recorded in the 1,000 IJs/cm^2 treatments (Table 7). Significantly higher numbers of IJs at the bottom of the Petri dishes were found in the H.b H ($P < 0.05$) compared to the S.f S and S.c D treatments, with mean number of IJs/cm^2 of 2.5 (SE = 0.33), 1.4 (SE = 0.25), and 1.3 (SE = 0.30), respectively. The mean number of IJs/cm^2 in the S.f O treatment (1.6 ± 0.25) did not differ significantly from the other three strains. Moreover, the downward movement of the IJs was not affected by the varying concentrations tested (df = 3, 45; F =2.65; P = 0.06).

Table 7. Number of IJs/cm^2 counted on the sticky traps.

	Dose rate (IJs/cm^2) [a, b]			
Strain	100	200	400	1000
H.b H	2.50 Bb	6.17 Bab	9.75 Bab	16.25 Ba
S.f S	4.17 Bb	12.33 Ba	17.33 Ba	14.00 Ba
S.c D	43.58 Ab	50.92 Ab	83.92 Aab	140.08 Aa
S.f O	6.83 Bb	9.08 Bb	22.92 Bab	44.17 Ba

[a] The data was analysed after square root transformation.

[b] Means within the same column (upper case) and row (lower case) followed by the same letters are not significantly different at P = 0.05 (Tukey multiple means comparison).

5.2.2. Prepupae

In the control 74.2% (SE = 4.2) of the applied prepupae developed to adults. In all EPN strains tested at all dose rates studied mortality of prepupae was significantly higher than in the control ($P < 0.001$). No significant interactions between the three EPN strains tested and the four different concentrations were recorded (df = 6, 33; F = 1.26; P = 2.91). Therefore, the EPN strains were compared irrespective of the dosage. Likewise, the dose rates were

compared regardless of the EPN strains. Application of both H.b H and S.f S resulted in a significantly higher mortality of WFT prepupae compared to the S.c D treatment (Fig. 8). For all strains, at the lowest dose rate of 100 IJs/cm^2 already 50% of mortality in WFT prepupae was achieved. Increasing the concentration to 400 IJs/cm^2 lead to a significant increase in prepupal mortality but a higher dose rate did not yield significantly higher mortality (Fig. 9). However, the dose rates significantly correlated with prepupal mortality for all strains (Table 8).

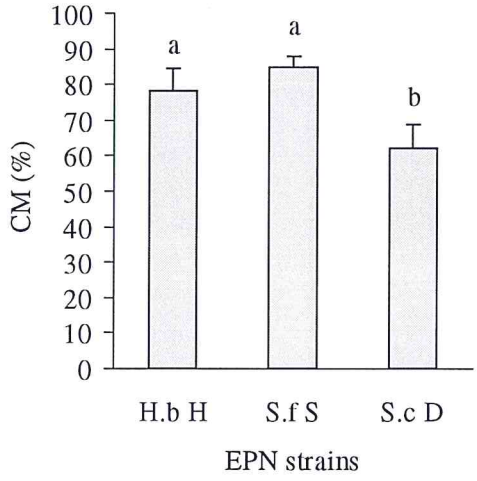

Figure 8. Mean corrected mortality (CM (%)) (±SE) of western flower thrips prepupae as affected by three different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D)). The data were pulled from four different dose rates (i.e. 100, 200, 400, and 1,000 IJs/cm^2). Bars with the same letters are not significantly different at P = 0.05.

Since the corrected mortality of prepupa even at the lowest dose rate was more than 50%, LD_{50} values could not be estimated for all EPN strains used in this experiment (Table 8). However, except for S.f S (R^2 = 0.4, P = 0.136) mortality correlated significantly with dose rates of H.b H (R^2 = 0.79, P = 0.004) and S.c D (R^2 = 0.96, P = 0.024).

Table 8. Dose effects of *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D) on the mortality of western flower thrips prepupae.

EPNs	R^2	B [a]	P [b]	LD_{50} (95% FL [c])	LD_{75} (95% FL)	LD_{90} (95% FL)
H.b H	0.987	20.1	0.001	NE	110 (ns)	320 (224–708)
S.f S	0.682	6.6	0.044	NE	NE	597 (NE)
S.c D	0.883	16.8	0.035	NE	226 (ns)	850 (564–2584)

[a] B = slope of regression equation: CM(%) = Log(Dose) + C.

[b] P = probability that the slope b is not different from zero.

[c] FL = lower and upper fiducial limits

NE = not estimated, ns = non-significant

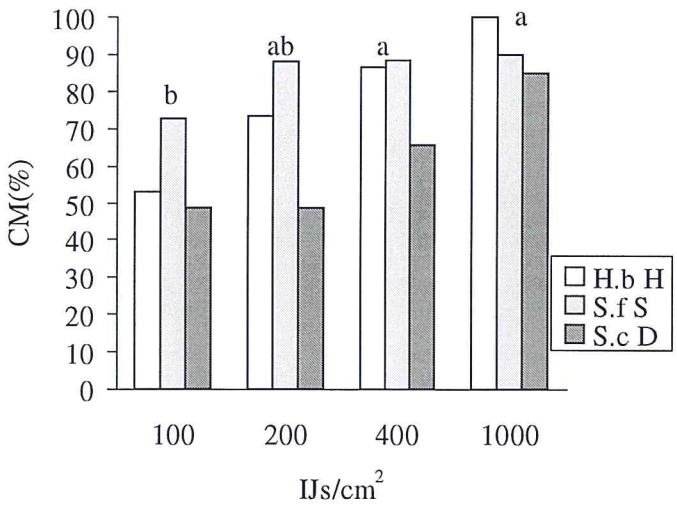

Figure 9. Corrected mortality (CM%) of western flower thrips prepupae caused by EPN strains applied at 100, 200, 400 and 1,000 IJs/cm^2. The interaction of EPN strain and dose rate was non-significant. Hence, dose rates were compared regardless of EPN strains. Different letters above the three bars of one concentration (as means of bars of different EPN strains, i.e., *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D) for a given dose) indicate significance differences among the dose rates (P = 0.05).

5.2.3. Pupae

In the control 81.8% (SE = 3.3) of the applied pupae developed to adults. In all EPN strains tested at all dose rates studied mortality of pupae was significantly higher than in the control ($P < 0.001$). No significant interactions between the three EPN strains tested and the four different concentrations were recorded (df = 6, 29; F = 0.24; P = 0.960). Therefore, the EPN strains were compared irrespective of the dosage. Likewise, the dose rates were compared regardless of the EPN strains. No significant differences in pupal mortality were found between the three different EPN strains tested (df = 2, 29; F = 1.58; P = 0.222).

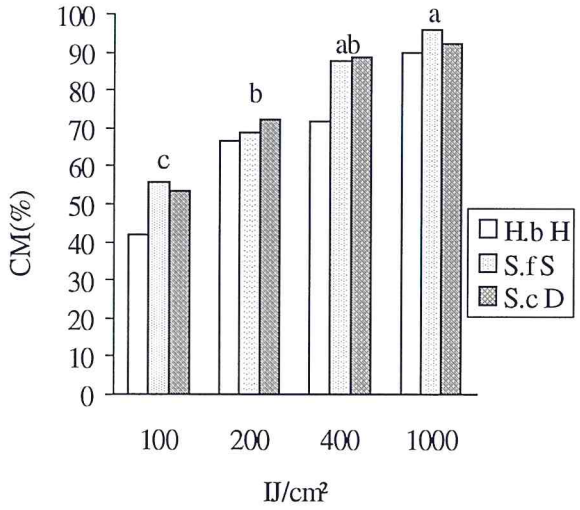

Figure 10. Mean corrected mortality (CM%) of western flower thrips pupae caused by EPN strains applied at 100, 200, 400 and 1,000 IJs/cm^2. The interaction of EPN strains and dose rate was non-significant. Hence, dose rates were compared regardless of EPN strains. Different letters above the three bars of one concentration (as means of bars of different EPN strains, i.e., *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D) for a given dose) indicate significance differences among the dose rates (P = 0.05).

However, mortality in WFT pupae was significantly affected by the EPN concentrations (Fig. 10). Across the three EPN strains tested, the lowest concentration of 100 IJs/cm^2 resulted in approximately 50% mortality of WFT pupae. The significantly highest mortality was recorded at a concentration of 400 IJs/cm^2 and a further increase in the dose rate did not yield

significantly higher mortality rates in pupae (Fig. 10). The slopes of the regression of mortality-dose rate of H.b H (0.05), S.f S (0.04) and S.c D (0.03) did not significantly differ from each other ($P < 0.05$). Thus, the data for the three strains was pooled. The subsequent regression analysis revealed a strong increase in mortality to increasing dosages of IJs (Fig. 11).

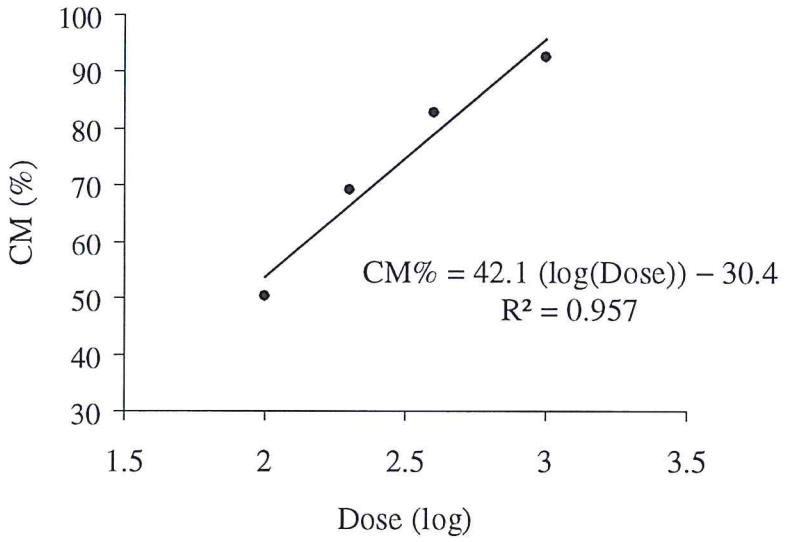

Figure 11. Functional relationship between corrected mortality values (CM (%)) of western flower thrips pupae caused by three different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3, *Steinernema feltiae* Sylt and *S. carpocapsae* DD136) and the logarithms of four different dose rates of IJs (i.e. 100, 200, 400 and 1,000 IJs/cm^2).

5.3. Effects of EPNs on different population structures of WFT

5.3.1. Under laboratory conditions

Different initial proportions of larva, prepupa and pupa did not influence ($df = 12, 24$; $F = 1.61$; $P = 0.47$) the total number of adult WFT emerged in the control treatment (89.2% ± 2.7). Hence, differences in the final number of adult WFT in the EPN treatments cannot be attributed to differing compositions of the population structure at the beginning of the experiments but to the impact of EPN. Consequently, the EPN-induced mortality was directly compared.

Applying EPNs to any of the tested population structures of immature WFT resulted in a significant reduction of the number of emerging adult WFT (N = 78, Z = 8.1 and P = 0.0001). EPN-induced mortality significantly varied in the tested population structures of the immature WFT (df = 12, 24; F = 3.53, P = 0.004). Mortality was significantly lower in treatments with high larval proportion (Fig. 12). Maximum mortality was recorded with high proportions of prepupae and/or pupae.

The proportion of late L2 in the population negatively affected the mortality of WFT by EPNs (R^2_L = 0.69, P_L = 0.0001)[1]. Prepupa and pupa proportions showed a similar trend in their relations to mortality of WFT by EPNs (Fig. 13). The proportion of both stages did not significantly influence mortality (R^2_B = 0.19, P_B = 0.054 and R^2_P = 0.15, P_P = 0.088)[2]. The slopes of the regression lines of prepupa and pupa proportions were not significantly different (df =1, 37; F = 0.07; P = 0.80).

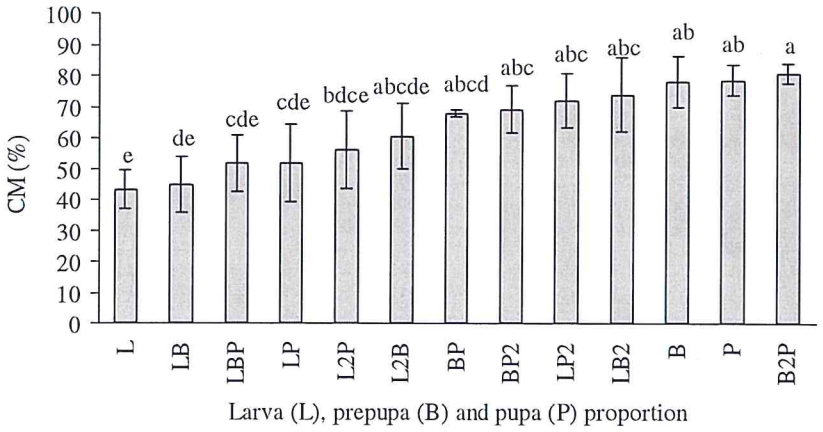

Figure 12. Corrected mortality (±SE) (%) of WFT by *Steinernema feltiae* Sylt as affected by the proportions (%) of second instar larvae (L), prepupae (B) and pupae (P) in the population structure. Refer to table 4 for the acronyms of proportion of immature stages. Bars with the same letters are not significantly different at P = 0.05.

[1] R^2_L, P_L represents correlation coefficient and p-level of larva proportion, respectively.
[2] R^2_B, P_B and R^2_P, P_P represent regression correlation coefficient, P-value of prepupa and pupa proportion, respectively.

Since all the immature stages of WFT exist together in nature and mortality is determined by proportion of all immature stages, a multiple regression analysis was performed. The results from the analysis indicated that unlike that of prepupa and pupa partial regression coefficient was significant for larvae (t = 3.50, P = 0.0013) thus EPN-induced mortality was determined only by the proportion of late L2.

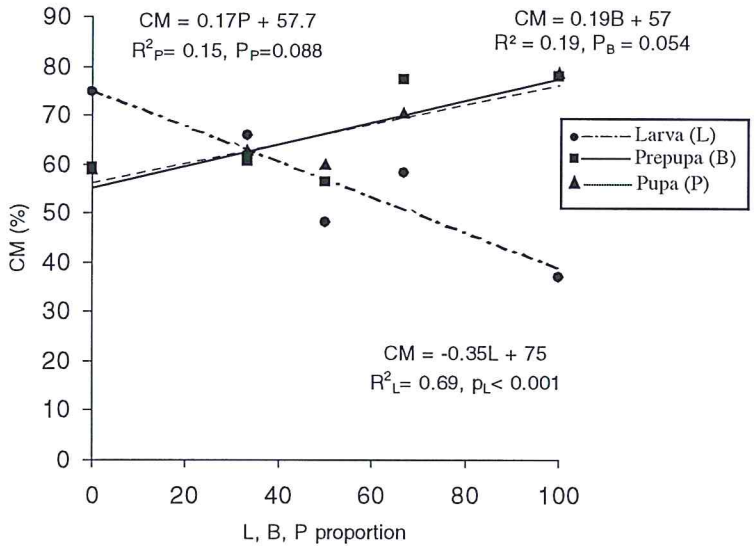

Figure 13. Functional relationship between the proportion of larvae (L), prepupae (B), or pupae (P) in the population composition and EPN induced corrected mortality (CM) (%) in WFT. R^2_L, P_L; R^2_B, P_B; R^2_P, P_P indicate the correlation coefficient, p-value of larva; prepupa; and pupa, respectively.

5.3.2. Under semi-field conditions

Before determining the impact of the three EPN strains on WFT, population growth and pupation of the insect, and method of data collection were assessed.

Population growth

Fifteen days after WFT adults were introduced to the bean seedlings, 73.2% of the total F1 WFT in the control pot was on the plant where most of them (i.e., 94.7% of the foliar life stages) were L1/L2. However, the remaining proportion (i.e., 26.8% of the total F1 WFT in the pot) was in the soil for pupation and was counted as emerged adults (Table 9).

Table 9. Proportion (%) (±SE) of different population structures (i.e. larvae, prepupae, pupae and adult) and pupation of F1 WFT on bean seedlings and in the soil in control treatment.

	WFT on the plant leaves [a]			WFT in the soil [b]
	Larvae [c]	Prepupae	Pupae	Adults
Population structure (%)	69.3 ± 4.0	2.4 ± 0.8	1.5 ± 0.4	26.8 ± 8.6
Pupation (%)[d]	–	8.1 ± 3.7	5.2 ± 2.3	86.7 ± 5.8

[a] Larvae, prepupae, and pupae were counted from sampled plants on 15th day after introduction of adult WFT to bean seedlings.

[b] Adults (that were developed from immature WFT pupated in the soil) were counted from the sticky trap for 9 consecutive days (i.e. from17th to 25th days after introduction of adult WFT to bean seedling) and from the top of the soil in the pot at the end of the experiment (i.e. on 25th day after introduction of adult WFT to the seedling).

[c] Larvae include both L1 and L2.

[d] Pupation proportion was calculated from WFT pupated on the plant leaves and from WFT pupated in the soil (and hence counted as emerged adults from sticky trap and top of the soil in the pot as described in [b]).

The F1 adults started to emerge from the soil 17 days after WFT introduction thus, all adult WFT on the plant at the time of data collection were considered F0 WFT. At the end of the experiment, i.e. 25 days after adult introduction, no more emerging adults were observed. On the 25th day after adult introduction, dead adults, presumably due to starvation, were counted from the top of the soil in each pot. In the control, they account for 31.6% of the total number of emerged adults. The total number of F1 WFT, i.e. L1/L2, prepupae, and pupae (counted from plant leaves on 15th day after adult introduction) and adults (counted from the sticky trap from 17th to 24th day after adult introduction and counted from the top of the soil in the pot at the end of the experiment) per pot in the control treatment was 229.60 ± (SD = 58.57) Out of the total WFT that pupated within fortnight, on the 15th day after introduction of the adults, 86% of WFT intended to pupate left the plant and pupated in the soil while 14% pupated on the leaves (Table 9).

Adult emergence

Considering only those adults that got stuck to the sticky trap; emergence peaked around 20 days after the initial introduction of WFT adults (Fig. 14).

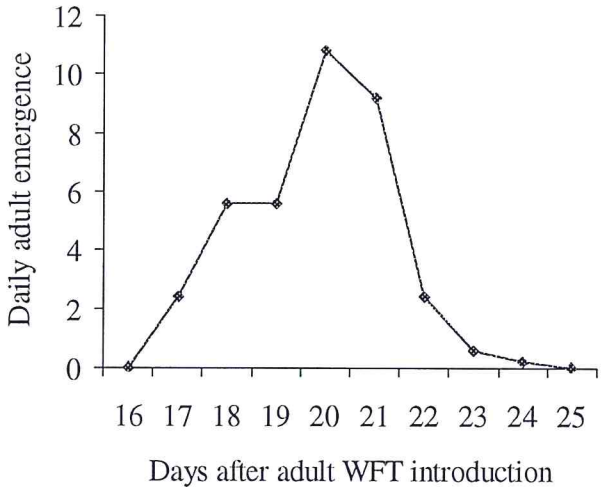

Figure 14. Daily WFT adult emergence counted from the sticky trap.

The numbers of adults counted only from the sticky trap and the one counted both from the sticky traps and the top of the soil were significantly different (Table 10). However, the difference did not depend on the different treatments (i.e. control, and EPN strains at higher and lower dose rates) (df = 6, 52; F = 0.07; P = 0.998, data analysed after square root transformation). In general, 28.7% ± 13.8 of the total adult WFT stayed on the top of the soil after emergence while the remaining 71.3% ± 13.8 left the soil and subsequently were caught in the sticky traps. The proportion of adults that stayed on the soil was not influenced by the application of different EPN strains or distilled water (Fig. 15). Hence, corrected mortality values did not differ significantly whether they were based on adult counts in sticky traps or both from sticky traps and soil counts (Table 10).

Table 10. Number of adult WFT counted in sticky traps only and from both sticky traps and the top of the soil. Corrected mortality (CM (%)) (± SE) of WFT caused by were calculated based on the number of adults counted using both methods. [a]

Adult count from	Number of adults [b]	CM (%) [c]
Sticky trap	22.1	41.9 ± 4.0
Sticky trap and soil	31	46.4 ± 3.5
Z (N)	2.63 (35)	0.72 (35)
P	0.0085	0.47

[a] Means of adult counts and corrected mortality for both approaches were compared using Wilcoxon rank test (SAS, 1996). P, N, and Z represent probability level, number of samples and Z-value, respectively.

[b] Data analysed after square root transformation

[c] Data pulled over all EPN treatments, i.e. application of *Steinernema feltiae* Sylt, *S. carpocapsae* DD136 and *Heterorhabditis bacteriophora* HK3 at 1,000 and 400 IJs/cm^2

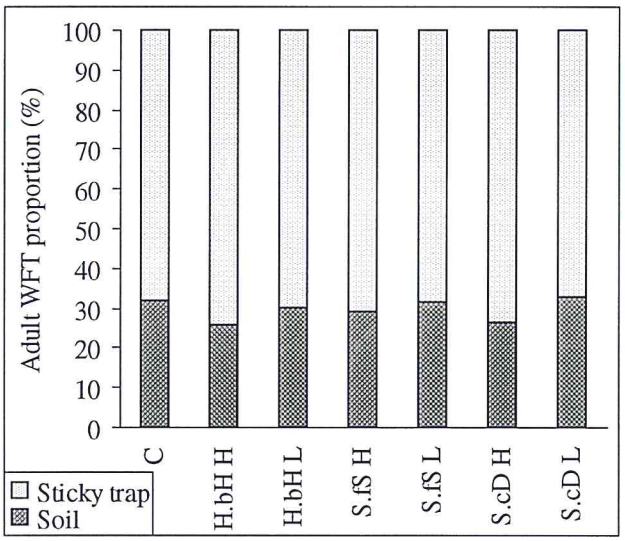

Figure 15. The proportion (%) of WFT adults counted from sticky traps and from the top of the soil in the control (C), and in the different EPN treatments (*Steinernema feltiae* Sylt (S.fS), *S. carpocapsae* DD136 (S.cD) and *Heterorhabditis bacteriophora* HK3 (H.bH)) applied at 1,000 (H = high) and 400 (L = low) IJs/cm^2

Mortality

The two factors, dose level and EPN strains, did not interact significantly (df = 2, 20; F = 0.62; P = 0.547) and hence, the effect of EPN strains or the use of different doses of IJs was analysed irrespective of the level of the other factor. Compared to the control treatments, all tested EPN strains at 400 and 1,000 IJs/cm^2 significantly reduced the population of WFT (P < 0.001).

Increasing the dose rate from 400 to 1,000 IJs/cm^2 significantly increased mortality in WFT (df = 1, 20; F = 10.28; P = 0.004). For the higher concentration maximum mortality was recorded for H.b H (61.8%) and lowest mortality in S.f S (< 50%) (Fig. 16). However, for the two dose rates together EPN-induced mortality did not differ significantly among the three tested strains (df = 2, 20; F = 2.05; P = 0.155).

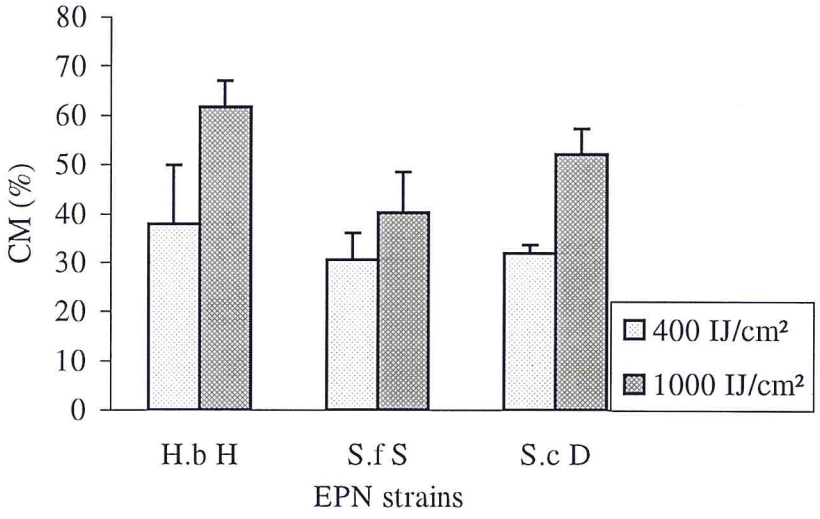

Figure 16. Corrected mortality (CM (%)) (± SE) of WFT caused by EPN strains (*Steinernema feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *Heterorhabditis bacteriophora* HK3 (H.b H)) at 400 and 1,000 IJs/cm^2.

5.4. Influence of EPN on Pupation and Adult Emergence of WFT

Adult Emergence

The proportion of adult emergence did not depend on the application of EPNs or distilled water (df = 1, 25; F = 0.42; P= 0.522), neither on the media of pupation (i.e. soil or sand – and hereafter referred to as media) (df = 1, 25; F = 0.46; P= 0.502). That means, out of the total alive WFT counted at the end of the experiment, almost equal proportion of the adults were recorded in both soil and sand in both the EPN and the control treatments.

Late second instar larvae in the experiment were from the same-aged rearing culture. However, the rate of development of the immature stages to adult was not uniform. Seven days after application, out of the total count, 31.2% of WFT were at either prepupal or pupal stage inside the media (9.9%) or on the top of the media (22.9%) for experiments with larva. However, for experiments where pupae were used, at the end of the trial only 2% of the insects were still immature and all were found on the top of the media.

At the time of data collection, about 25% of the adults had already died, either on the top of the media or on the sticky traps. These dead adults were most likely the first ones that had emerged and died due to lack of food.

WFT distribution in the arena

At the end of the experiment, adults that had developed from the initially introduced immature stages at the beginning of the trials and the remaining alive immatures were distributed throughout the experimental set-up and counted from different positions in the assay arena. The distribution pattern depended on the development stage that was initially introduced (Table 12). The adults remained inside or on the top of the media or left the media and got stuck to the sticky traps. The alive immature stages, i.e. prepupae and pupae stayed on

the surface or inside the media. The proportion of WFT found at different positions within the arena (inside or on the top of the media, or on the sticky trap) was calculated for each arena according to the following (Eq. 3):

$$Proportion\ (\%) = \frac{Total\ number\ of\ WFT\ at\ a\ given\ position}{Total\ number\ of\ WFT\ in\ the\ arena} * 100 \qquad \text{(Eq. 3)}$$

For treatments with late L2, 18.2, 50, and 31.8% of the adults stayed in the media, on the top of the media, and got stuck to the sticky trap, respectively. However, for treatments with pupae, only 2.1, and 31.3% stayed inside and on the media, respectively, but 66.7% left the media and got stuck to the sticky trap.

Table 11. Acronyms and their respective meaning for proportion of adult and immature stages of WFT that were counted from different positions in the arena.

Acronym	Explanation:
1. *InmediaA*	Adult WFT inside the media (soil or sand)
2. *Inmedia*	Immature WFT, i.e., prepupa and pupa, inside the media
3. *OnmediaA*	Adult WFT on the surface of the media
4. *OnmediaI*	Immature WFT on the surface of the media
5. *TrapA*	Adult WFT in sticky traps
6. *TA*	Total adults in the arena (1 + 3 + 5) [a]
7. *TI*	Total immatures in the arena (2 + 4)
8. *TmediaA*	Total adults both inside and on the surface of the media (6 – 5)
9. *Tinmedia*	Total WFT in the media (7 + 8)
10. *T*	Total WFT in the arena (5 + 9 = 6 + 7 = 100%)

[a] Functions in the bracket indicate sum of the 1st, 3rd, and 5th acronyms in the first column and similar for other.

The proportions of immature and adult WFT at different positions in the experimental set-up were compared to one another and to the proportion of total immatures, total adults, and finally to the total proportion of WFT in the arena. The proportion of WFT that were compared is indicated in table 11.

Summary of ANOVA for the comparison of the proportion of WFT at different positions in the arena is given in table 12. The factors that were considered in the analysis include APPLY (distilled water or EPN suspension), STAGE of the immature WFT (L2 or pupa) to which the nematode was applied, MEDIA in which EPN and WFT were applied (sand or soil), and POSITION of the arena from where different proportions of WFT were counted (Table 11).

The proportion of WFT at different positions in the arena differed significantly. It depended also on the development stage of WFT at the beginning of the experiment. Therefore, the proportion of WFT at different positions in the arena was compared for the different development stages (i.e., L2 and pupa).

For treatments with larvae, except for the total proportion of WFT inside the media (*Tinmedia*) all other proportions were significantly lower than the total WFT in the arena (*T*) (Table 13), indicating that the majority of the insect remained in the media. For treatments with pupae, most of the immatures already developed to adults, and thus the proportion of total adults in the arena (*TA*) was not significantly lower than the total number of WFT in the arena (*T*). However, in both cases the proportion of adults in the sticky traps (*TrapA*) was significantly lower than the total proportion of WFT in the arena (*T*) (Table 13). Thus, most likely, instead of using the numbers of all immature and adult WFT in the media for corrected mortality calculation, depending only on the numbers of adult WFT in the sticky traps affects the actual values of EPN-induced mortality, especially if few replications are used.

Table 12. Summary of ANOVA for the proportion of WFT adults and immatures counted from different positions in the arena.

Source of variation	DF	F Value	P > F
APPLY	1	0.04	0.8419
STAGE	1	7.72	0.0058
MEDIA	1	0.26	0.6125
PORTION	9	54.4	0.0001
APPLY*STAGE	1	0.56	0.4568
APPLY*MEDIA	1	0.01	0.9416
APPLY*PORTION	9	0.26	0.9846
STAGE*MEDIA	1	< 0 0001	0.9982
STAGE*PORTION	9	12.24	0.0001
MEDIA*PORTION	9	0.79	0.6261

Mortality calculation

The proportion of emerged WFT adults in EPN treatments was not significantly different from that in the control treatments (Table 12), indicating that the use of EPN did not affect the rate of development of the immatures to adults. Thus, the EPN-induced mortality was not different whether it was calculated using only the emerged adults or both the adults and the immatures that did not yet developed to adults ($t = 0.10$, $df = 43$, $P = 0.92$).

Table 13. Proportions of adult and immature WFT staying at different positions in the arena.

Proportion of WFT at different portions of the arena [a]	Stage of WFT to which EPN was applied [b c]	
	Larva	Pupa
InmediaA	12.3 GF	2.6 D
InmediaI	9.9 G	0 D
OnmediaA	39.1 DE	25.8 C
OnmediaI	22.9 GEF	2.1 D
TrapA	15.8 GF	69.5 B
TA	67.2 BC	97.9 A
TI	32.8 DEF	2.1 D
Tinmedia	84.2 AB	30.5 C
TmediaA	51.4 DC	28.4 C
T	100.0 A	100 A

[a] Refer table 11 for the acronyms.

[b] Data were analysed after arcsine transformation.

[c] Means within the same column followed by the same letters are not significantly different at P = 0.05 (Tukey multiple means comparison).

6. DISCUSSION

6.1. Rationale of the Methodology

Generally, in the experimental set-up used in most of the experiments, the number of WFT recovered after one week in the control treatment was about 70% of the applied larva. For prepupae and pupae, it was about 80–90%. During the development of WFT, mortality is highest in the L2 stage (Soria and Mollema, 1995) and Helyer *et al.* (1995) also reported 68% adult emergence from WFT pupae in a control treatment in compost. Carmen and Mollema (1995) also reported that the L2 stage of WFT is the most critical one in which most individuals die; they reported only 60% survival on a susceptible cucumber genotype. Hence, the relatively low control mortality allowed us to study the effects of EPNs on the different developmental stages of WFT.

If a non-appropriate experimental set-up is used, a potentially effective EPN species/strain against a given insect pest can wrongly be considered as non-efficient (Grewal *et al.*, 1999). In WFT, this could partly be attributed to the behaviour and the size of the insect. In pre-experiments, WFT larvae that were transferred to filter paper in a Petri dish (Ø 100 mm) moved out of the place of application. Gaum *et al.* (1994) reported that WFT larvae are very active in moving. Moreover, the disturbance from the IJs, i.e. their attempts to penetrate the immature stages, could make the larvae restless and stimulate them to leave the filter paper and search for other media for pupation. Prepupae and pupae, which move only upon disturbance, also could not stay at the place where they have been placed, i.e. the top of the filter paper. Like the L2, the disturbance from IJs might force prepupae and pupae to leave the filter paper and crawl on the wall or the lid of the Petri dish, reducing the chances of coming into contact with the IJs. That is probably the reason why the corrected mortality values obtained in the preliminary filter paper assays were very low. The size of WFT contributes

also to the low efficiency of EPNs on filter paper. Unlike *G. mellonella*, which is 1.5–2.0 cm in length (Woodring and Kaya, 1988), from the tip of the antennae to the tip of the abdomen WFT are only 1.0–1.5 mm long (Tommasini and Maini, 1995). Thus, they may have less chance to come into contact with the IJs compared to *G. mellonella*. Hence, WFT immatures can easily pass many IJs when they move on and leave the filter paper. Moreover, in wider arenas (Petri dish with Ø 100 mm) filled with soil, the disturbed prepupae and pupae could move into the soil of the arena or to the upper part (wall or lid) of the arena leaving the soil surface. If the prepupae and pupae choose to leave the soil and stay on the lid or the upper remaining part of the arena, they would avoid any contact with the IJs, thereby greatly reducing the efficiency of the nematodes.

In our experimental set-up, the primary objective was to enable a successful selection of the most effective EPN species/strains against soil-dwelling life stages of WFT. Therefore, the assay arena was prepared in such a way that both the host insect and the EPNs could be in the same area. Larvae that were in the soil could come into contact with nematodes as the IJs were percolating into the soil with the water used to suspend the IJs. Prepupae and pupae that stayed on the top of the soil, inside the soil or moved out of the soil and stayed on the wall of the assay arena were not out of the reach of the IJs. Prepupae and pupae leaving the soil could not escape the experimental set-up since the lid was painted with insect glue. The only 'safe' niche for the WFT was the wall of the Petri dish just above the soil surface. However, the arena was small and filled with soil, and the IJs could even encounter and subsequently attack WFT on the wall of the Petri dish. However, these problems only relate to experimental laboratory studies because under natural conditions, most of the late L2, prepupa and pupa are in the soil. Under such conditions, application of EPNs have to be accompanied by irrigation to assure that the IJs on the top of the soil can be washed down to the depth where the insect pupae.

The soil (Fruhstorfer Erde Type P) used as media in our experiments is commercially available and used in many greenhouses in Germany, hence assuring that the investigations were carried out in a practical manner. Since we only used fresh batches of soil, there was no need to sterilise the substrate. Moreover, in series of pre-experiments we could show, that the efficacy of EPNs against WFT was more pronounced in soil than on filter paper and/or on sand.

6.2. Screening

In the experimental set-up used in this study the late L2 WFT readily descended into the soil, thus reflecting natural conditions. Consequently the IJs mainly attacked their hosts in the soil, and most of the EPN strains tested showed high virulence against WFT, indicating that the IJs percolated into the soil with the suspension applied and successfully infected the thrips. However, the soil depth in our bioassays was only approximately 0.8 cm, whereas under field conditions WFT pupation occurs at a depth of 1.5–2.0 cm (Tommasini and Maini, 1995; Moritz, 1997). In ongoing studies we are investigating the effect of varying soil depth on the efficacy of EPNs against WFT. Contrary to the late L2, the majority of the prepupae and pupae introduced into the bioassay arena were on the top of the soil at the time of EPN application. A direct application of IJs to the prepupae and pupae resulted in a comparatively higher mortality than in late L2. In additional experiment, the position of the WFT in the arena (i.e., bottom, middle, and top) as indicated by S.f S, did not affect the efficacy of EPNs against WFT. This might be attributed to the small depth of the soil in our assay arena enabling the IJs to distribute throughout the arena and infecting the host. This agrees with the findings of Georgis and Poinar (1983) where nematodes introduced 14 cm below the soil surface (silica sand) were able to infect wax moth (*G. mellonella*) pupae located 10 cm above and 10 cm below the point of application. Thus, in present study, the lower mortality of L2 was most likely not due to the position of the larvae in the soil, but reflects a lower

susceptibility of WFT larvae compared to prepupae and pupae.

The experimental set-up in set A for L2 and prepupae on one side and pupae on the other side was different. Thus, unlike in Set B where a uniform experimental set-up was used, for Set A mortality of late L2 and prepupae caused by different EPN strains cannot be directly compared with mortality in pupae. For the comparison of the mortality of all the stages by EPN the later experiments, i.e., Set B, C, and D can be used.

At low soil moisture content EPNs are less infective because they lack a sufficient water film for effective movement to their hosts (Gaugler, 1988). Movement is considered an optimum when soils are near their field capacity at which IJs are able to initiate host seeking and can cause high rates of infection. The soil used in the present study has a high content of peat, which is accountable for the higher field capacity of the soil. Thus, the 45% MC of the soil used in the experiment with pupae in Set A is considerably lower than its field capacity. Hence, the quantity of water in which IJs were suspended might not have been sufficient to assure a uniform distribution of the IJs throughout the soil in the arena. Moreover, the soil could get dry relatively soon and such a low MC would interrupt the activity of IJs. All these factors explain why pupal mortality in the experiment in Set A was comparatively lower than at 67% MC used in the other experiments.

6.2.1. EPN Strain Comparison

In the present study, six different EPN strains were tested. Variability in virulence even among EPN strains within the same species is common (e.g. Bracken, 1990). Considerable differences in virulence were also observed in this study, particularly among the four *Steinernema* spp. strains tested. The two *S. feltiae* strains S.f S and S.f O caused similar levels of mortality in late L2 and prepupae, but under low soil moisture conditions the S.f O strain was significantly less virulent against WFT pupae. Hence, in the subsequent dose rate

experiments, the S.f O strain was tested only against late L2. Moreover, the high virulence of the S.f O strain under high soil moisture content conditions against late L2 could not be attributed to a high host searching ability of the IJs, as indicated by the comparatively low numbers of IJs recorded on the sticky traps. Of all *Steinernema* spp. strains tested, the S.f C strain showed the lowest virulence against the three immature stages of WFT.

Compared to the two *S. feltiae* strains S.f S and S.f O, S.c D caused a lower though not significant mortality in WFT larvae during the screening experiments of Set A. However, in the subsequent dose rates experiments in Set B, applying various concentrations of S.c D IJs resulted in significantly lower mortality in WFT L2 and prepupae, but not in pupae compared to S.f S and S.f O (for L2) and S.f S (for prepupae and pupae). IJs of *S. carpocapsae,* a nictating species, remain near the soil surface and are less effective in the soil than non-nictating species like *H. bacteriophora* and *S. feltiae* (Grewal *et al.*, 1995), possibly explaining the lower infectivity of S.c D against WFT larvae in our experiments.

According to Grewal *et al.* (1995) IJs of *S. carpocapsae*, unlike that of *H. bacteriophora* and *S. feltiae,* do not respond to host volatiles and thus in our study, their distribution within the arena was at random. Compared to the *H. bacteriophora* strain H.b H we recorded significantly lower numbers of S.c D IJs on the bottom of the Petri dishes. Moreover, the significantly higher IJs numbers of S.c D on the sticky traps stress the ambushing ('sit-and-wait') behaviour of *S. carpocapsae* where the IJs stay on the top surface of the soil searching for hosts (Lewis *et al.* 1992).

We recorded significantly higher IJs numbers of the *H. bacteriophora* strain H.b H from the bottom of the Petri dish, which reflects the cruiser behaviour in foraging of *H. bacteriophora* (i.e. actively searching for its host) (Campbell and Gaugler, 1993). However, the IJs of H.b H caused significantly lower mortality in WFT L2 compared to the two *S. feltiae* strains S.f S

and S.f O. IJs of *S. feltiae* posses some characteristics of ambushing and some of cruisers foraging behaviour. They are equally effective at finding mobile and non-mobile hosts on a two-dimensional nictation surface substrate (Grewal *et al.*, 1995). Our results indicate, that IJs of the *S. feltiae* strain S.f S used this foraging advantage on soil-dwelling stages of WFT that are mobile upon disturbance. If some of the IJs search and disturb the resting prepupae and pupae, the other juveniles, which are sitting and waiting for bypassing hosts, could attach themselves to the moving immatures of WFT. Thus, in most of the experiments, IJs of S.f S were constantly superior to that of the other EPN strains tested for control of WFT.

6.2.2. Dose Comparison

Results from the dose rate experiments show that irrespective of the tested EPN strains, a comparatively high concentration of 400 IJs/cm^2 was needed for a high control of the soil-dwelling stages of WFT. In the experimental set-up used in our study, such a dose rate resulted in approximately 60% mortality of late L2 in the soil and around 80% mortality of prepupae and pupae on the soil surface. Lower concentrations of IJs yielded a significantly lower mortality in WFT. Most likely, the small body size of WFT immatures is the reason why such high concentrations of IJs are required to obtain high mortality. In a similar study, Chyzik *et al.* (1996) also recorded highest mortality of WFT prepupae and pupae at a dose rate of 400 IJs/cm^2. Contrary to these results, Helyer *et al.* (1995) observed low mortality in WFT pupae in compost irrespective of the high dose rate of EPN used. However, other small-bodied insect pests like fungus gnats, *Bradysia* spp. (Diptera: Sciaridae), can be efficiently controlled by EPNs at comparatively lower concentrations (Harris *et al.*, 1995; Gouge and Hague, 1995). Our results also indicate that lower dose rates i.e., 100–200 IJs/cm^2 already caused 30–50% mortality in soil-dwelling life stages of WFT. At present the efficacy of the biological control strategies used against WFT is considered rather low (Parrella *et al.*, 1999).

Hence, any biological alternatives are potentially of great interest for WFT control. We believe that EPNs can possibly become part of a rather elaborated biological control approach, attacking WFT from several angles, by using predatory bugs and predatory mites against the foliar feeding life stages of the thrips, and EPNs against the soil-dwelling immatures. Moreover, recent findings show that soil-dwelling life stages of WFT can also be efficiently controlled through releases of predatory mites of the genus *Hypoaspis* (Acari: Laelapidae) (Berndt and Poehling, 1999). Several studies indicate that EPNs can be successfully combined with other biological control agents (e.g. Choo *et al.*, 1996). In ongoing experiments we are evaluating the combined impact of EPNs and inundative releases of *Hypoaspis* spp. for control of soil-dwelling life stages of WFT.

6.3. Different Population Structures of WFT

6.3.1. Laboratory study

In the laboratory experiments, like the previous experiments, late L2 descended into the soil in the arena while the majority of the prepupae and pupae remained on the top of the soil. However, data from the experiment on spatial distribution of WFT pupae already showed that the position of the pupae in the arena did not affect mortality induced by S.f S. Moreover, since mortality by S.f S in prepupae and pupae did not differ significantly, the lower mortality level in treatments with high proportions of late L2 in the population structure was most likely not influenced by the position of the immatures in the arena but reflects a higher susceptibility of WFT prepupae and pupae to EPNs.

Differences in susceptibility to EPNs exist among different developmental stages of insects. Early L1 of *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) are the most susceptible stages (Glazer and Navon, 1990). In contrast, later larval stages of *Maladera martida*

Argaman (Coleoptera: Scarabaeidae) were reported to be the most susceptible stage (Glazer and Gol'berg, 1989). In *Tipula paludosa* Meigen (Diptera: Tipulidae) and *T. oleracea* L. (Diptera: Tipulidae) highest mortality was recorded for the L1 approaching the first moult while young L1 were less susceptible (Peters and Ehlers, 1994).

Under natural conditions, all soil-dwelling life stages of WFT occur at a given moment. However, our results suggest that the highest efficacy of an EPN treatment with up to 75% population reduction will be achieved when the majority of the soil-dwelling life stages of WFT will be either prepupae and/or pupae. Hence, EPNs should be applied when the majority of the thrips population reaches the prepupal and/or pupal stage.

6.3.2. Semi-field experiment

Four weeks after releasing 20 adult female WFT to different chrysanthemum cultivars, Jager *et al.* (1993) reported that 148.9 to 390 WFT were found on a susceptible cultivar with flowers. Higgins (1992) reported that first and second instar larvae of WFT formed the major part of the population (> 85%) of the insect found on the foliage of bell peppers and English cucumbers. These data corroborate our findings on number of offspring and population structure in the semi-field experiments, indicating that the effect of EPN on the WFT can be well studied in such a methodological set-up.

In our semi-field experiments, no host plants were provided as a food source for the emerging adults, and about 30% of the adults that emerged from pupae did not leave the soil but stayed on the top of the surface. However, the remaining 70% of the F1 showed negative geotaxis and consequently were caught in the sticky traps. Ten days after first adult emergence, most of the adults that were counted from the top of the soil were dead, indicating that they might have been the first F1 adults, which emerged from the pupae and died presumably due to starvation. IJs might have been still active at the time of adult emergence, as it was observed

for S.c D even at the end of the experiment. However, the proportion of adults on the soil and on the sticky traps did not differ between control and EPN treatments presumably adult WFT did not recognise presence of IJs

We did not record significant differences between corrected mortality values calculated using total number of WFT in the arena and using only the adults that got stuck in the sticky traps. Hence, for future experiments it seems possible to depend only on the less time-consuming adult counts from the sticky traps. However, for data uniformity, collecting all WFT in the experimental set-up might be required especially if few numbers of replications per treatment are used.

Very often EPN-induced mortality in laboratory assays cannot be reproduced in the field (Georgis and Gaugler, 1991). However, correct choice of the bioassay method in the laboratory can substantially enhance the likelihood of later success in the field (Griffin, 1993). In previous laboratory assays of dose rate study, up to 97% mortality of WFT pupae could be attained using a dose rate of 1,000 IJs/cm^2. In the laboratory, EPNs were directly applied to the pupa and prepupa, or reached L2 within 0.8 cm soil depth. However, in the semi-field experiments, nematodes reached the soil-dwelling life stages of thrips mainly by passive movement with percolating water. This means that under field conditions, the concentration of IJs that reaches the resting WFT in the soil is lower than the applied concentration. Therefore, the amount of post EPN release irrigation water is super crucial with respect to the depth of WFT pupation if too much the majority of IJs go down with the drain if too little the IJs remain on the surface only. Hence, in ongoing studies we are investigating the effect of varying amount of irrigation water on the efficacy of EPNs against WFT.

In a study by Chyzik *et al.* (1996) all EPN strains tested, except for *H. bacteriophora* HP88, failed to control WFT in pots at a concentration of 400 IJs/cm^2. In our experiments, the water

used to rinse nematodes down helped most of the IJs to reach the resting WFT. Gaugler *et al.* (1997) suggested that a pre-EPN-application irrigation is required. Prior to the EPN applications the soil in our semi-field experiments was moist enough and hence, for all strains EPN-induced mortality in WFT even at a concentration of 400 IJs/cm^2 was significantly higher than control mortality.

In the semi-field experiments EPN-induced mortality in WFT significantly increased with increasing the dose rate from 400 to 1,000 IJs/cm^2. Hence, in greenhouses higher EPN concentrations can result in enhanced WFT control. However, at present, a dose rate of 1,000 IJs/cm^2 is very high compared to EPN concentrations used for control of other insects. Sulistyanto *et al.* (1995) reported that *H. bacteriophora* (strain EN0043) applied at 1.5 million IJs/m^2 (i.e. 150 IJs/cm^2) successfully controlled grubs *Phyllopertha horticola* (L.) (Coleoptera: Scarababiae) in a golf course with population reductions of up to 83%. WFT is economically a very important pest and already developed resistance to many insecticides (e.g. Broadbent and Peer, 1997). Moreover, at present there is no single control technique available that assures a high control level of WFT (Parrella *et al.*, 1999). Our results indicate, that EPNs could become an important and promising component in future biocontrol strategies against WFT, targeting the pest from different angles through combined releases of predators against life stages on the foliage and EPN applications against soil-dwelling life stages. In ongoing studies we are studying the combined effects of predator releases and EPN applications on the population dynamics of WFT under greenhouse conditions.

6.4. Pupation and Distribution of WFT in the Assay Arena

Western flower thrips used in the study had maximum age difference of 48 h, as egg laying in WFT rearing was interrupted after this period of time. However, according to this finding, some of the individuals in the later stages (i.e. L2, prepupa, pupa, and adult) differ with more

than 48 h developmental duration. Seven days after application of late L2 to the arena, prepupae (that have to finish this stage of development, change to pupae, and finally develop to adults) and dead adults (that might be emerged some days before data collection) were found. The youngest prepupa and the oldest adult would have developmental duration difference of more than 48 h. Van Rijn *et al.* (1995) reported that at 25°C WFT stays for 2.64 – 2.79 days on cucumber leaf disc at pupa stage indicating that the youngest prepupa and the oldest adult can have more than at least 2.6 days of developmental duration difference as the prepupa must undergo pupa stage. In present study, this difference was minimum for the experiments with older (pupa) than younger (L2) WFT. One can anticipate that the difference would be more pronounced if the experimental insect were started with L1. Such developmental duration difference found in WFT pupated in the sand was similar to those pupated in the soil (Fruhstorfer Erde Type P) and likewise, it was similar for WFT pipetted with EPN or distilled water. This was confirmed by the same rate of adult emergence under these different conditions (i.e. in sand or in soil, and treated with EPN or water). This indicates that the presence of EPN in the media did not influence the rate of development of WFT and thus WFT do not escape from the pathogenicity of EPNs by faster development to adult.

Seven days after application of late L2 or pupae, the prepupae, pupae, and adults were found distributed to the different parts of the experimental set-up. The mortality of WFT by EPN is calculated from living WFT in the arena based on Abbott's formula (1925). It is common that, for small insects like thrips, corrected mortality is calculated by comparing only the number of emerged adults that stayed at accessible parts of the experimental set-up in control and in main treatment (e.g., Chyzik, *et al.* 1996; Helyer *et al.* 1995). But WFT that are not yet developed to adult and those adults that did not leave the media of pupation (thus, are not accessible for collection/counting on the final date of data collection) may affect the actual

corrected mortality calculation. In present study, all other proportions of WFT in different positions of the arena were significantly lower than the total proportion of the WFT in the arena (i.e., 100%). These differences were consistent in EPN-applied and control treatments. Thus, the corrected mortality of WFT by the use of EPNs did not differ whether it was calculated using the total WFT in the arena or by using only the WFT counted at accessible portion of the arena, e.g. in the sticky trap. However, for data uniformity, considering all the WFT in the arena might be required than considering only the WFT at one or two portions of the arena in the calculation of the corrected mortality of WFT by EPNs for the similar assay arena.

7. CONCLUSIONS AND RECOMMENDATIONS

Experimental set-ups under both laboratory and semi-field conditions for testing EPNs against soil-dwelling stages of WFT were developed and can be used for future studies. Moreover, these techniques can form the base for future improvement of the assay arena when carrying out similar experiments.

All soil-dwelling life stages of WFT, (i.e. late L2, prepupae and pupae), were susceptible to the tested EPN strains. Higher dose rates of IJs were required for sufficient control of WFT. Generally, at 1,000 IJs/cm^2 i.e. 10 million IJs/m^2, *Steinernema feltiae* Sylt, *S. carpocapsae* DD136 and *Heterorhabditis bacteriophora* Brecon resulted in up to 90 and 60% corrected mortality of soil-dwelling stages of WFT under laboratory and semi-field conditions, respectively. In future studies, additional EPN strains should be screened for higher mortality at lower dose rates.

In addition to the type of the soil-dwelling developmental stages, where L2 proved to be less susceptible than prepupae and pupae, efficacy of EPNs against WFT depended on additional factors like type and moisture content of the media of pupation. Thus, in future studies, the influences of external factors (both biotic and abiotic) on the efficacy of EPNs against WFT need to be investigated.

However, the efficient strains selected in this study should be utilised for further investigations on the potential EPNs for control of WFT. These strains could thus become candidates for new integrated control techniques in which predators and EPNs are jointly used to combat foliar-feeding and soil-dwelling stages of WFT, respectively.

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Declaration

I hereby declare that this study is an original research finding conducted by myself. I have used only the cited materials. Moreover, this thesis has never been submitted elsewhere.

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