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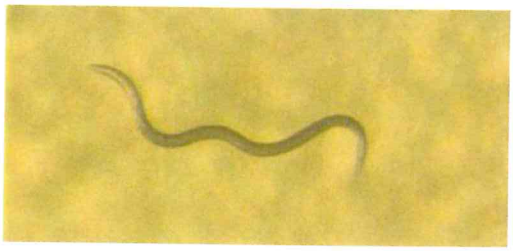
Persistence, self-perpetuation, time of application and foraging strategy affecting the efficacy of entomopathogenic nematodes against soil-dwelling life stages of *Frankliniella occidentalis*



By

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Thesis submitted in partial fulfilment of the requirements of Master of Science (M.Sc.) degree in Horticulture (major in Entomology)

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Abstract

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most important destructive pests attacking economically important crops of the world both under field and greenhouses conditions. Chemical control of WFT is difficult because of its cryptic feeding behaviour and because of pupation in the soil building a "reservoir" which cannot be reached by foliar application of pesticides calling for a repeated application. Short generation time favours the rapid selection of individuals with lower sensitivity and hence has led to the development of resistance to commonly used insecticides. Moreover, use of natural enemies that attack WFT on the foliage alone does not provide adequate control since the thrips spend one-third of its life cycle in the soil as prepupae and pupae. Nowadays, entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae have shown promising results in attacking the soil-dwelling life stages of several species of insect pests including WFT. Previous studies in the Institute of Plant Diseases and Plant Protection, University of Hannover, Germany indicated that all soil-dwelling life stages of WFT are susceptible to EPNs. However, it is important to know conditions that could help to improve impact of these natural enemies on WFT. Therefore, in the present study, the short-term post application persistence of two EPN strains, i.e. *Heterorhabditis bacteriophora* strain HK3 and *Steinernema carpocapsae* strain DD136 was studied under semi-field conditions (in a pot experiment) against soil-dwelling late second instar larvae (L2) of WFT. The nematodes were applied at 200 and 400 infective juveniles (IJs) cm^{-2} and the L2 were introduced at 0 (same day), 3, 6, 9, or 12 days after nematode application (DANA). Results of this experiment showed that all EPN treatments caused higher WFT mortality compared to the untreated control. For HK3, higher thrips mortality was obtained from the higher concentration and persisted at least for 12 and 9 days at 400 and 200 IJs cm^{-2}, respectively. However, in the case of DD136, higher WFT mortality was

recorded at 200 than at 400 IJs cm^{-2} and it persisted at least for 12 DANA. Moreover, we have investigated the possibility for self-perpetuation of one EPN strain (HK3) in WFT and we found that IJs were not able to develop in to adult or even to the next juvenile stage (J_4). We have also studied different times and frequencies of application of two EPN strains, HK3 and *Steinernema feltiae* strain Sylt, with the presence of host plants (*Phaseolus vulgaris* L.) to optimise application in term of efficiency. The nematodes were applied at a concentration of 200 and 400 IJs cm^{-2} once (10, 15, or 20) or twice (10 and 15, 10 and 20, or 15 and 20) days after introduction of ten female and two male WFT adults onto the bean plants. All EPN applied treatments caused significantly higher WFT mortality compared to the untreated control treatment. All the repeated applications of HK3 at 400 IJs cm^{-2}, and double treatments at 10 and 15, and 15 and 20 days with 200 IJs cm^{-2} caused significantly higher and similar level of WFT mortally than single applications both at higher and lower concentrations except for 10 days at the higher concentration. For Sylt, higher WFT mortality was record from the repeated applications of 10 and 15, and 15 and 20 days at 400 IJs cm^{-2}. For this strain, a splitted application at lower concentration did not result in higher thrips mortality than single applications at higher concentration. From our experiment on population dynamics of WFT we found that at 10^{th} day after adult introduction, the majority of the population was larvae and found on the plant. However, after 20 days the majority was found in the soil as prepupae/pupae. More than 96% of WFT population pupated in the soil. We have also investigated possibilities for combined application of two EPN strains with different foraging strategy, on a commercial plant growing substrate under laboratory condition. HK3 (a "cruise" forager) and DD136 (an "ambusher") were applied in combination or alone at 400 and 200 IJs cm^{-2}. From the EPN applied treatments, higher WFT mortality was obtained from HK3 applied alone at both concentrations and combined applications at 400 IJs cm^{-2}. Application of DD136 alone resulted in a significantly lower WFT mortality than HK3.

Dedication

This M.Sc. thesis work is dedicated to my beloved wife, my parents, brothers and sisters.

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Abbreviations used in the text

Abbreviation	Meaning of the abbreviation
∅	Diameter
ANOVA	Analysis of variance
ca	About
cm^{-2}	Per square centimetre
CO_2	Carbon dioxide
DD136	*Steinernema carpocapsae* strain DD136
DJ	Dauer juvenile
DNA	Days after nematode application
e.g.	Example
EPN(s)	Entomopathogenic nematode(s)
F	Statistical F-value
GLM	General linear model
h	Hour(s)
HK3	*Heterorhabditis bacteriophora* strain HK3
i.e.	That is
IJ(s)	Infective juvenile(s)
J4	Fourth stage juvenile
L:D	Light: dark (in relation to photoperiod)
L2	Second instar larvae of WFT
LSD	Least significant difference
m^{-2}	Per square meter
P	Statistical probability value

RH	Relative humidity
SAS	Statistical analysis system
SE	Standard error
UV	Ultraviolet radiation
WFT	Western flower thrips

1 General introduction

1.1 Western flower thrips

Thrips are minute, slender insects having band-like wings delicately fringed with long cilia from which the name of the order, Thysanoptera, is derived. Of the about 500 known species, only few species, mainly in the family Thripidae are serious crop pests (Lewis, 1997).

The order Thysanoptera is subdivided into two sub-orders, i.e. Tubulifera and Terebrantia. The western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) is included in the sub-order Terebrantia (Thripidae) in which most of the economically important thrips species are found (Brødsgaard, 1989; Tommasini and Maini, 1995). Below the species level strains of biotypes of WFT are described, e.g. with different abilities to colonize plant species or with different susceptibilities to pesticides (Palmer, 1989).

1.1.1 Origin and distribution

Thrips are mainly phytophagous, mycophagous or predatory insects that inhabit a wide range of habitats, generally in the tropical, subtropical and temperate regions (Ananthakrishanan, 1993). Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a native insect of the western part of North America where it was first reported in 1895 (Brødsgaard, 1989). In the 1970s and early 1980s, WFT was distributed throughout North America (Beshear, 1983). Soon thereafter, it was found in Dutch greenhouses in 1983, and has since become an exotic but after short time well established pest of greenhouse production in many countries throughout the world (Tommasini and Maini, 1995). Shortly after its invasion into Europe, WFT became a more severe pest of greenhouse crops than the onion thrips, *Thrips tabaci* Lindeman which was the dominating greenhouse thrips before

(Van Rijn *et al.*, 1995). The rapid spread of WFT is mainly facilitated by international trade in planting materials (Brødsgaard, 1993).

1.1.2 Description and biology

Western flower thrips are tiny insects and adults are less than 2 mm in length. Male adult WFT are light yellow and have narrow abdomens while female adults are larger than the males and vary in colour, ranging from light yellow, yellow with brown splotches to dark brown (McDonough *et al.*, 1999; Tommasini and Maini, 1995).

It is important to understand the life cycle and behaviour of thrips in order to develop an effective control strategy. The female WFT has an external ovipositor with two opposable serrated blades that are used to cut through the plant epidermis and deposit eggs in the plant tissue. Depending on environmental conditions and nutrient level, female WFT lay 150 – 300 eggs during their lifetime (Moritz, 1997; Jensen, 2000a). Eggs are deposited in leaves or petals and hatch in two to four days depending on temperature. The eggs of WFT are fairly protected in the plant tissue and only few pesticides can affect them. Study by de Kogel *et al.* (1997b) on oviposition preference of WFT on cucumber leaves showed that thrips reproduction was highest on apical leaves and lowest on basal leaves, and female WFT preferred younger leaves to older leaves for oviposition.

As mentioned in Pearsall and Myers (2000), Bailey (1938) indicated that WFT are believed to overwinter as sexually mature females in soil, curled leaves, evergreen plants and protected places such as under bark. Under field condition, the life cycle of WFT begins with emergence of adult female thrips in the early spring from overwintering locations (Pearsall and Myers, 2000).

The life cycle of WFT consists of four stages namely adults, eggs, two feeding larval instars (first and second instar larvae) followed by two immobile non-feeding stages (the prepupa and pupa) that both occur predominantly in the soil. Eggs are about 0.2 mm long, kidney-shaped, opaque and are produced either sexually or parthenogenetically (Brødsgaard, 1989). Fertilised eggs with a full diploid chromosome number develop into females and unfertilised eggs develop into males. Therefore, the female thrips are diploid whereas the males are haploid (Moritz, 1997). Oviposition of females normally starts 72 hours after emergence and continues intermittently throughout the adult life.

The small, white, first instar larvae (L1) starts moving around and feeding as soon as it hatched. The yellow second instar larvae (L2) feed voraciously. The young instars have nearly the same appearance with the adults though they are wingless, the eyes are reddish and the antennas have fewer segments. Fully developed second instar larvae stop feeding, empty their alimentary canal and descend to the soil for pupation or remain on the leaf before changing into pale non-feeding and non moving prepupae. Within the family Thripidae pupation in the soil is common ((Tomasini and Maini, 1995; Lewis, 1973). However, it remains unclear whether entering into the soil for pupation is obligatory for most species belonging to Thripidae (Berndt, 2003). If the plant architecture or phenology is more complex, a lot of alternative sites of pupation may be directly available on the plant (Lewis, 1973; Kirk, 1996). Berndt (2003) hypothesized that since WFT has a broad host range, the proportion of population that enters into the soil is reduced on a plant with more complex architecture (e.g. flowers of Asteraceae). According to Kirk (1996) soil passage in flower-dwelling thrips is an adaptation to the short period of presence of preferred flower habitat. Moreover, the adaptation value of the soil passage within the life cycle of thrips might be related to protection against unfavourable abiotic conditions (e.g. desiccation of thrips due to

low humidity) and against natural enemies, especially for the prepupal and pupal stages, which are less mobile and almost defenceless (Lewis, 1973; Kirk, 1996). For these reasons WFT would prefer to pupate in the soil rather than on the plant even if complex plant structures were available (Berndt, 2003). Hypothesis of Berndt was verified by recent experiments Berndt (2003) that showed that at least 91% of the thrips population completed its life cycle in the soil even with the use of flowering ornamentals as a host plants. The majority of thrips (78%) pupate at a depth of 1 – 5 mm, 20% between 25 and 35 mm and the reminder between 6 and 24 mm (Helyer *et al.*, 1995). Ebssa (2003, personal communication) also observed that WFT could pupate up to a depth of 50 mm in a commercial substrate.

Morphologically prepupae differ from larvae with two external wing buds which are short and the antennae are not completely folded backwards over the head. During the pupal stage the wing buds are long and the antennae are completely folded backwards over the head (Gaum *et al.*, 1994). The prepupa and pupa are immobile and do not feed, move only when they are disturbed.

The developmental time required from egg to adult varies depending on temperature, relative humidity (RH) and host plant. At a temperature of 25°C, the total developmental time for all immature stages was 14.71 days on cucumber (Gaum *et al.*, 1994) and 13.8 days on peanut (Lawry *et al.*, 1992). The average total developmental time from egg to adult at a temperature of 15, 20 and 30°C was 44.1, 22.4 and 15 days respectively (Lublinkhof and Foster, 1977). The optimal conditions for reproduction and development are around 25°C and 80% RH (Lublinkhof and Foster, 1977). Longevity of adult female varies depending on temperature and it was 70.8 and 27.5 days at 15 and 30°C, respectively (Lublinkhof and Foster, 1977), and males can survive only half as long as females (Hulshof and Vanninen, 2003).

Depending on life cycle length, under greenhouse conditions six or seven generations per year may occur and highest population densities are present in the hottest months of the year (Lublinkhof and Foster, 1977; Gaum *et al.*, 1994; Soria and Mollema, 1995; Kirk, 1997).

1.1.3 Damage and economic importance

Over the last decade, a lot of herbivorous thrips species have been recognized as important pests of a wide range of crops worldwide (Tommasini and Maini, 1995; Lewis, 1997). Plant feeding thrips in general, including WFT, are damaging and difficult to control in protected crops (Tommasini and Maini, 1995). WFT itself is a very serious pest on a wide range of economically important crops throughout the world and causes substantial economic losses. It is a highly polyphagous insect pest, which can cause both cosmetic and economic damage on a wide range of host plants. There are at least 250 plant species from more than 65 families (Anonymous, 1989) as hosts of WFT.

WFT is a damaging pest and vectoring virus on a variety of outdoor crops such as peanuts, tomatoes, peas, onions, etc., and in greenhouses, attacking vegetables and ornamentals including tomatoes, cucumber, roses and others (Yudin *et al.*, 1986; Daughtrey, 1996). Direct damage is through feeding and egg laying leads to e.g. curling and stunting symptoms, silver spots and/or reduced photosynthetic capacities of the host plants. The piercing-sucking multi-purpose mouthparts are used to pierce leaves, flowers, seeds, pollen grains, and fruits as well as to drink open liquids on the plant. Thus, damage by WFT can be on fruit, flowers, flower buds, leaves and leaf buds, depending on the type of host, (Childers and Anchor, 1995). Feeding by WFT leads to abortion of flowers and leaf buds and distortion of emerging leaves. Feeding always does not result in immediately visible damage because many flowering species do not show injury until flower buds open (McDonough *et al.*, 1999). The spread of

pollen over petals and presence of brownish faecal drops are indications of thrips infestation (Tomasini and Maini, 1995). The degree of damage depends on the plant tissue that is infected, the developmental stage of the plant, susceptibility of the cultivars or species attacked and salivary toxicity (Bournier, 1983).

Thrips can cause different types of feeding damage to plants. When they feed on developing tissues, affected tissues are unable to expand, so that leaves and petals become distorted upon subsequent growth. Feeding on expanded tissues cause cells to become filled with air, which results in a silvery appearance of the affected tissues (De Jager *et al.*, 1993).

Equally important to the direct damage, WFT is a vector for tospo viruses such as tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV), which may result in total crop failure (Allen and Broadbent, 1986). Only -the first instar larvae can acquire the virus, which is transmitted in a persistent manner by adults during their feeding (Ullman *et al.*, 1997). When the larvae feed on virus-infected plants, they absorb the virus, which will go into the haemocoelic cavity through the digestive tube, and finally into the salivary glands from where they are reinjected into healthy plants. The virus is transmitted within a period of 3 – 4 weeks after acquiring it (Tommasini and Maini, 1995). If the development of the first instar larvae into second instar is slowed by temperature factors, the second instar larvae can transmit the virus (Bournier, 1983). The virus is retained by the infected thrips throughout lifetime. However it is not transmitted to subsequent generations through egg production (Drees and Cole, 1988). In a recent survey in the Midwest part of the US, greenhouse operators identified WFT as the most difficult greenhouse pest to manage (McDonough *et al.*, 1999).

Floriculture crops are an excellent example of crops, which cannot tolerate thrips damage (De Jager *et al.*, 1993). While feeding may not reduce production, the resulting scaring and deformation drastically reduce the aesthetic value of these products. Damaged flowers are less valuable or even unmarketable (Parrella and Jones, 1987). In ornamental plants in general, control should be almost complete because of the zero-damage demand for export quality (van Lenteren and Woets, 1998). Economic injury level of WFT depends on seasonal stage and market price of the host plants. A density of 8 adult thrips per 200 cm^{-2} leaf area is sufficient to reach economic damage level on cucumbers (Rosenheim *et al.*, 1990). Shipp *et al.* (1998) also reported that a mean density of 15-20 adult WFT per sticky trap per day or 8 adult thrips per plant tapping would result in the occurrence of unmarketable sweet pepper fruits.

The increasing evidence of thrips infestation in various cropping systems, their ability to migrate from weed reservoirs to crops and vice versa, and their intensive intercrop movement and related adaptive strategies are basic aspects underlying the economic importance of thrips in general and WFT in particular (Lewis, 1970).

1.1.4 Control

Infestations of crops with WFT can be difficult to control. Multiple tactics are often necessary to obtain an acceptable level of control. Different strategies are possible and the applicability depends on the crop system. Integrated pest management strategies for control of thrips in the field, on trees (e.g. orchards) and greenhouses are highly needed. Management options to control WFT infestations in greenhouses include cultural control (such as disposal of plant residues, elimination of weeds), host plant resistance, physical control (such as prevention of

thrips from entering the greenhouse), biological control (use of predators, parasitoids, and pathogens) and chemical control (Jensen, 2000a).

Chemical control

WFT is difficult to control with insecticides. Thrips usually concentrate on rapidly growing tissues such as young leaves, flowers and terminal buds, and eggs are inserted into the plant tissue. This affinity for tight places makes thorough coverage with pesticides difficult. Just prior to pupation, the majority of larvae move down from the plant to pupate in the soil or leaf litter. Hence chemicals applied on the foliage cannot reach these life stages. Yet, just after hatching and before pupation WFT are most vulnerable to insecticides (Greer and Steve, 2000). Because of their short life cycle and rapid multiplication that calls for frequent application of insecticides, WFT has developed resistance to major groups of insecticides (Immaraju *et al.*, 1992). Moreover, the haploid breeding system in WFT in which the resistance genes in the haploid males are directly exposed to selection following insecticide treatment can accelerate the development of resistance in WFT (Jensen, 2000a). The occurrence of cross-resistance to insecticides makes the problem more serious. For instance, resistance to methiocarb exists in populations of WFT never exposed to methiocarb before testing (Brødsgaard, 1994; Jensen, 2000b). But chemical control is still a major option in practical horticulture and some efficient ingredients are available (e.g. Spinosad, Methiocarb, Neem, etc.)

Cultural control

One option for cultural control is to have a fallow period in summer. The grower should first remove all plants and weeds followed by heating of the greenhouse artificially or naturally until the soil temperature reaches 16°C. The temperature in the greenhouse should be

maintained at this level for three weeks. During this time, thrips eggs will hatch and the nymphs will starve and die due to lack of food (Greer and Steve, 2000). Such strategy was found to be effective against WFT. Research findings showed that an air temperature of 40°C with a relative humidity of 10% provides an environment, which is fatal to plants in four days and was sufficient to kill WFT. However, such a strategy is costly and difficult to practice for larger plots.

Weeds can serve as reservoirs for pests and allow continuity of infestation by providing a bridge between crops. Therefore, removal of weeds in and around greenhouses can greatly reduce the likely hood of pest invasion (Jacobson, 1997). Because WFT have hundreds of hosts, control of weeds in most crops helps to lessen infestation.

Trap crops have shown potential as part of "push" strategy to concentrate pest insects in particular area where control agents can be applied. The use of such strategy under glasshouse would reduce application times and cost of biocontrol agents against pest insects. In glasshouse trials, flowering Verbena X hybrida Voss plants attracted WFT from iuy geraniums and chrysanthemums, and are therefore potential trap plants (Hooper *et al.*, 1999).

Host plant resistance

Host plant resistance could be another option for the control of WFT. De Jager *et al.* (1993), from their study on resistance of chrysanthemum cultivars to WFT reported that after three weeks of introduction of WFT larvae to chrysanthemum leaves, only 14% of the thrips survived on most susceptible cultivars and over the entire period only 16% became adult. On the most resistant cultivars all larvae died before becoming adult. Under greenhouse conditions, strong reduction in thrips damage was observed in resistant cucumber (*Cucumis*

sativus L.) accessions (de Kogel *et al.*, 1997a). However, often strong variation in adaptability to resistant plants among populations of phytophagous insects is a potential threat to the durability of host plant resistance. Aggressive biotypes may overcome the defence mechanism of formerly resistant cultivars through adaptation to resistance factors of the plant (de Kogel *et al.*, 1998).

Biological control

In the recent years the emphasis of control measures of WFT has shifted to the use of natural agents such as predators and entomopathogens, because WFT has developed resistance to many chemical pesticides (Vestergarrd *et al.*, 1995).

Biological control of greenhouse thrips can be achieved through release of biocontrol agents such as predatory mites, lady beetles and anthocorid predatory bugs. Most commonly several *Amblyseius* species (Acari: Phytoseildae) and *Orius* species (Hetroptera: Anthocoridae) are used to control foliar-feeding life stages of WFT (i.e. larval instars and adults) (Sabelis and van Rijn, 1997; Ramakers, 1995). Lower population densities of WFT combined with a more rapid decline in thrips numbers were observed when a predatory mite, *Neoseiulus cucumeris* (Oudemans) was applied on cyclamen at 200 and 300 mites m^{-2} (Williams, 2001). Berndt (2003) studied efficacy of two soil-dwelling predatory mites, *Hypoaspis miles* (Berlese) and *H. aculeifer* (Canestrini) against WFT and he found that a release of five *H. miles* per plant caused a corrected thrips mortality of 44.9% and increasing the number of predatory mites to 20 per plant resulted in 75.7% WFT mortality. A release of five *H. aculeifer* per plant caused 58% thrips mortality and increasing the mite density to 20 per plant resulted in 80.5% mortality of the thrips. In general he found that *H. aculeifer* was more efficient against the soil-dwelling life stages of WFT than *H. miles.* . However, augmentative releases of predatory

mites and bugs do not provide sufficient control of WFT, particularly in high value crops like ornamentals (Gillespie and Ramy, 1998; Bakker and Sabelis, 1989). Common reasons are that thrips larvae often not the preferred host of the predator as shown with predatory bugs (Chyzik *et al.*, 1995) that only first instar larvae of WFT are attacked as with Amblyseius (Higgns, 1992) or that common plant dwelling predators cannot kill (or encounter) the soil stages of WFT. This results in low efficiency of beneficials and failure of biocontrol. Thus, alternative biological control strategies with more efficient predators on the plant or those targeting also at the soil-dwelling stages are needed for a successful management of WFT. Focusing on the soil stages two major options seem to be interesting: Soil-dwelling predacious mites (*Hypoaspis spp.*) that attack thrips in their prepupal and pupal stages when they inhabit the soil or growing medium (Greer and Steve, 2000).

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are currently used as biological control agents for soil inhabiting insect pests. (Georgis and Manweiler (1994). They are obligate lethal parasites that mutually associated with symbiotic bacteria and the third stage infective juveniles of these nematodes locate and infect suitable hosts killing their hosts within 24 – 48h (Kelein, 1990; Boemare *et al.*, 1996). Previous studies indicated that soil-dwelling life stages of WFT are susceptible to different EPN species/strains (Ebssa *et al.*, 2001 a & b, 2003; Premachandra *et al.*, 2003 a & b; Tomalak, 1994; Chyzic *et al.*, 1996).

According to Premachandra *et al.* (2003a), combined releases of EPNs, i.e. *S. feltiae* (Filipjev) and *H. bacteriophora* strain HK3 (Poinar) with *H. aculeifer* reduced WFT adult emergence by 71% and 82% respectively, compared to the untreated control. When the biocontrol agents were applied alone, their effect was significantly lower than the combined

releases. The potential of EPNs to control soil-dwelling life stages of WFT and other insect pests is discussed in the following section.

1.2 Entomopathogenic nematodes

1.2.1 Description, biodiversity and distribution

Nematodes are simple and non-segmented worms that 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 insects. Nematodes that attack insects are called EPNs and comprise of three major families, i.e. Steinernematidae, Heterorhabditidae and Mermithidae.

Steinernematidae and Heterorhabditidae are the most important families for control of soil-dwelling insects. These two families are phylogenetically not closely related but they share similar life histories through convergent evolution (Poinar, 1993). EPNs have a wide spread distribution and the only continent where they have not been found is Antarctica (Griffin *et al.*, 1994; Poinar, 1990). Steinernematids have probably greater distribution than that of heterorhabditids. Within steinernematids, *S. feltiae* (Filipjev) and *S. carpocapsae* (Weiser) have a global distribution. Among the Heterorhabditids species, *H. indicus* (Poinar) is widely distributed and found throughout the tropics. Another species, *H. bacteriophora* (Poinar) has been isolated from all continents, although so far not recorded from India and South-East Asia. *H. megidis* (Poinar) is found widely distributed in temperate regions (Hominick *et al.*, 1999). EPNs can also exist as strains that differ in their biology and ecology (Hominick and Reid, 1990).

1.2.2 Biology of EPNs

Steinernema and *Heterorhabditis* spp. nematodes, which belong to the families Steinernematidae and Heterorhabditidae, respectively (Rhabditida: Nematoda), have similar life cycles. They have a simple life cycle that includes egg, four juvenile stages (separated by moults) and adult. A special development stage within the life cycle of rhabditid nematodes is the dauer juvenile (DJ), also called infective juvenile (IJ), formed as a response to depleting food resources, high population density and adverse environmental conditions. The DJ is a free-living third juvenile stage that is physiologically and morphologically well adapted to long-term survival in the soil without feeding while searching for a new food source. It is the infective stage that carries cells of its symbiont in the anterior part of its intestine (Endo and Nickle, 1994). The third stage IJ of steinernematids and heterorhabditids has been linked to a guided missile because it carries the "warheads" of the mutualistic bacteria cells in its intestine (Akhurst, 1993). This stage is equipped with two layers of membrane, i.e. the cuticle of the second moult remains attached to provide additional protection under unfavourable environmental conditions.

The non-feeding IJ seeks out insect hosts, especially in the soil environment. When a host has been located, the nematodes penetrate into the insect body, usually through natural body openings (mouth, anus, spiracles) or areas of thin cuticle. Once they entered in the body cavity, a symbiotic bacterium (*Xenorhabdus* spp. for steinernematids, *Photorhabdus* spp. for heterorhabditids) is released from the nematode, which multiplies rapidly and causes rapid insect death (Boemare *et al.*, 1996; Spiridonov *et el.*, 1991). The nematodes feed upon the bacteria, thereby liquefying the insect, and mature into adults. Nematode growth and reproduction depend upon conditions established in the host cadaver by the bacterium. The nematodes provide protection and transportation for their bacterial symbionts. The bacterial

symbionts contribute to the mutualistic relationship by killing insect hosts, by establishing and maintaining suitable conditions for nematode reproduction, and by providing nutrients and antimicrobial substances that inhibit growth of a wide range of microorganisms. Interaction among EPNs, symbiotic bacteria and insect host may be one of the principal forces driving diversification and specialization in these organisms (Liu *et al.*, 2000).

Steinernematid IJs may become males or females, whereas heterorhabditid IJs develop into self-fertilizing hermaphrodites although subsequent generations within a host produce males and females as well (Strauch *et al.*, 1994). Thus, steinernematids require both sexes to be present within the host but in heterorhabditids potentially only one individual is required for initiation of the life cycle (Baur and Kaya, 2003). The life cycle is completed in a few weeks, and hundreds of thousands of new IJs emerge in search of new insect hosts.

1.2.3 Behaviour of EPNs and host finding strategies

Dispersal of IJs is a behavioural mechanism that EPNs use to locate new habitats for survival and infection. Movement in EPNs can be active or passive. In the former, dispersal of nematodes is through their own locomotion and in the later dispersal is through action of other agents like water, human activity and through infected hosts or external phorosis (Kaya, 1990).

Chemoreception is the main sensory mode used by nematodes in orienting to their hosts during their active dispersal (Kaya and Gaugler, 1993). Carbon dioxide (CO_2) has been shown to elicit host-seeking behaviour in EPNs (Gaugler *et al.*, 1991). Infective juveniles found aggregating around and invading host spiracles suggest that these nematodes might locate their host by CO_2 attraction (Triiggiani and Poinar, 1976). Moreover, IJs have been shown to

respond to cues other than CO_2 and their response is affected by the order of cues presented during host finding process (Lewis *et al.*, 1995b). Some species like *S. carpocapsae* and *S. scapterisci* Nguyen and Smart use an "ambush" strategy to parasitise the host, while *S. glaseri* (Steiner) and *H. bacteriophora* adopt cruising approaches (Grewal *et al.,* 1994; Campbell and Gaugler, 1993). Ambush foragers tend to remain stationary and nictate by standing on their tail elevating more than 75% of their body (Campbell and Gaugler, 1993) to attach a nearby passing host and are therefore most effective against highly mobile insects near the soil surface. However, cruisers search constantly and are typically more effective at finding relatively sedentary prey species within the soil profile (Kaya and Gaugler, 1993). Ambushers respond poorly to distal volatile cues released by hosts, where as cruise foragers are highly responsive to long-range host volatiles (Gaugler *et al.*, 1997).

Nematode infected hosts, which live about 48 hours before they die, may serve as means to disperse nematodes in the soil. Infected larvae may move laterally and downward in the soil and infected adults may fly several meters before dying and establishing new foci of infection (Kaya, 1990). Other reports indicated that, adult mole crickets can live and fly for 10 days or longer after infection with *S. scapterisci* (Parkman *et al.*, 1993).

The entomopathogenic Steinernematid and Heterorhabditid nematode species possess many attributes of parasitoids and pathogens. They are analogous to parasitoids because they have chemoreceptors and can actively search for their hosts (Kaya and Gaugler, 1993) and they need caused (or induced) mortality of the host to reproduce (Hundson and Norman, 1995). They are similar to pathogens because of their association with mutualistic bacteria, in which the nematode-bacteria complex is highly virulent, killing its host within 48 hours through the action of the mutualistic bacteria (Lacey *et al.*, 2001).

1.2.4 Importance of EPNs in biological control of insect pests

EPNs are excellent biological control agents. Utilization of these nematodes is developing rapidly almost with a doubling of newly described species in the past few years (Liu *et al.*, 2000). Advances in molecular biology and phylogenic reconstruction have revolutionized understanding of population structure, identification, genetic improvement, systematics and the symbiosis between EPNs and their symbiotic bacteria (Liu *et al.*, 2000). This importance of nematodes has led to an increase in the number of surveys for the isolation of EPNs worldwide and considerable exchange of nematode materials between various laboratories (Ehlers and Hokkanen, 1996).

Among the heterogeneous groups of EPNs, the genera *Steinernema* and *Heterorhabditis* contain several important EPN species used in biological control and are often referred as beneficial or insecticidal nematodes (Kaya, 1993; Smart, 1995). They can be distinguished by their symbiotic relationship with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively. It is mainly due to this symbiotic association that these two EPN genera are the only ones currently used in a significant extent in biological control (Smits, 1996). Most significantly, because the associated bacteria kills hosts so quickly, these nematodes have not to adapt to a specific host life cycle and are able to parasitise hundreds of insect pest species once they locate and penetrate in to the homcoel of their hosts (Gaugler, 1988). Moreover, they are environmentally compared to pesticides if specific nematode strains are selected to the target insect even though they do not need to adapt to specific host since symbiotic bacteria plays the major role in host killing process, and the IJs can be applied with conventional spraying equipment and are compatible with most pesticides (Rovest and Deseo, 1990, 1991). They find their hosts either actively or passively in cryptic habitats and in the soil.

The entomopathogenic activity of *Heterorhabditis* and *Steinernema* spp. has been documented against a broad range of insect pests in a variety of habitats (Kaya and Gaugler, 1993). They are especially efficacious against insects in the soil and cryptic habitats. For example the citrus root weevil *(Otiorhynchus sulcatus)* in nurseries and cranberries, the black cutworm, *Agrotis ipsilon*, and mole crickets *Scapterricus* spp. in turf grass, the peach borer moth, *Carposina niponensis* Walsingham, in apple have been successfully controlled (*Long et al.*, 2000; Kaya and Koppenöfer, 1996).

Results from different experiments showed that that they can effectively control root weevils, fire ants, mole crickets, Colorado potato beetles, root maggots, cutworms, Japanese beetle and other important insect pests (Gaugler, 1988). In a pot experiment, Belair and Boivin (1995) showed that application of *S. carpocapsae* at the rate of 400 IJs cm^{-2} significantly reduced the survival rate of carrot weevil, *Listronotus oregonesis*, by 75 – 94% compared to the untreated control.

Gazit *et al.* (2000), reported that S. riobrave Texas (Sr TX) and *Heterorhabditis* sp. IS-5 showed high activity and induced greater than 85% mortality in Mediterranean fruit fly, *C. capitata. S. carpocapsae* have been shown to cause significant codling moth mortality when applied on apple trees (Unruh and Lacey, 2001). In a laboratory bioassays, *H. bacteriophora* Strain GPS11, and *H. zealandica* strain SX1 resulted in larval mortality over 85% of grape root borer, *Vitacae polistiformis* (Harris) (Williams *et al.*, 2002). EPNs have been also considered as potential control agents for leaf miners in the recent years (Olthof and Broadbent, 1990).

The role of EPNs against soil-dwelling stages of WFT

Control of thrips in the soil at prepupae/pupae stage offers a chance of utilizing pathogens not suitable for use in integrated control program at adult and larval stages (Helyer *et al.*, 1995). Ebssa *et al.* (2001a, b, 2003) studied the virulence of different strains of EPNs and assessed their impact on mortality of soil-dwelling stages of WFT under laboratory and semi-field conditions. Results from the laboratory experiment showed that all EPN strains tested caused significantly higher larval mortality compared to the control and the highest mortality was recorded from *S. feltiae* strain Sylt, *S. feltiae* strain OBSIII and *H. bacteriophora* strain HK3. In the semi-field experiment, *S. feltiae* strain Sylt, *S. carpocapsae* strain DD136 and *H. bacteriophora* strain HK3 at the concentration of 400 and 1000 IJs cm^{-2} significantly reduced the WFT populations. Under semi-field conditions up to 70% reduction of the WFT population was recorded at the higher EPN concentration. In another study Premachandra *et al.* (2003b) showed that among the six different strains of EPNs tested against WFT, *S. feltiae* strain Filipjeve hybrid (the commercial formulation Nemapuls®) and *H. bacteriophora* Poinar (Hd01 strain) resulted in 65% and 59% mortality on pupal stages of WFT, respectively.

1.2.5 Post application survival of EPNs and factors affecting their survival

A biological pest control system based on the use of EPNs requires not only an effective strain of nematode, but also one that can maintain its infectivity after application in the soil under wide range of environmental conditions that the pest inhabits (Gazit *et al.*, 2000). Hence, biological control of WFT using EPNs requires the presence of IJs in the soil that are ready to encounter the incoming larvae, prepupae /pupae and it is important to determine post application persistence of EPNs in the soil.

The natural habitat for EPNs, the soil, is a difficult environment for persistence of any organism considering its complexity of physical, chemical and biological components (Glazer, 2002). EPNs are mainly used as inundative agents, with the aim of obtaining immediate pest suppression and with expected persistence at high numbers at least for 2 – 3 weeks (Glazer, 1996). However, like other biological control agents, EPNs require specific conditions to be effective.

Persistence of EPNs added to the soil vary widely depending on experimental conditions in which the nematodes are exposed including temperature, humidity and moisture supply. For instance, *S. carpocapsae* persisted *in vitro* for 30 days at levels >50% in sandy soil (Shroeder and Beavers, 1987), whereas survival was reported to be <10% after 7 days in soils of similar texture *in vitro* and in field (Duncan and McCoy, 1996). Recently Premachandra *et al.* (2003b) showed that under laboratory conditions EPNs could persist at least for six days without losing their virulence against WFT. Brown and Gaugler (1997) reported that IJs could survive adverse environmental conditions by remaining in the cadaver of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) for up to 50 days. Survival varies among the species and is dependent up on the environmental conditions to which the cadaver is exposed. Simsons and Poinar (1973) showed that *S. carpocapsae* Agriotis strain survived approximately 28 days at 79.5% RH and 28°C outside the cadaver.

Parkman *et al.* (1993) have discussed the establishment and dispersal of *S. scapterisci*, and have shown that this nematode have a suppressing effect on mole crickets of the genus *Scaptriscus* for up to several years. Klein and Georgis (1992) have also reported that *H. bacteriophora* strain NC was suppressing Japanese beetle populations 8 months after application.

Duncan and McCoy (1996) have shown that, after 7 days of field application, *S. riobravis* surviving at the surface of the soil has decreased to 1% of those at day zero and at a depth of 3 cm in the soil survivors decreased by 69%. However at a depth of 15 cm there was little or no decrease in survivors. On exposed surfaces, steinernematids and heterorhabditids can survive no longer than several hours, depending on species, temperature and relative humidity (Glazer, 1992). On the other hand Kung and Gaugler (1990) reported that EPNs can persist 2-3 weeks in dry soils.

Factors affecting post application persistence and mortality of nematodes applied for the control of soil-born pests include abiotic factors (e.g. exposure to UV radiation and dehydration after application, oxygen deficiency, etc.) biotic factors (e.g. predation and infection by antagonists), depletion of energy reserves after application to the soil and pre-application factors associated with production, storage and transport condition (Smits, 1996).

Abiotic factors affecting survival and persistence of EPNs

IJs in the soil may encounter many potential hazards including predators, desiccation, freezing, UV radiation and disease (Smits, 1996). Understanding the environmental parameters affecting short and long-term persistence of EPNs is important for predicting the fate of nematodes released as biological control agents (Georgis and Gaugler, 1991).

Sensitivity of IJs to desiccation and other environmental constraints reduces their efficacy in the field (Kaya and Gaugler, 1993). However, desiccation did not affect all nematode species equally and "ambushers" like S. carpocapsae are less susceptible than "cruisers" (e.g. H. bacteriophora) (Smits, 1996).

The most critical periods for EPN survival are a few minutes to hours after application (Smits, 1996). According to this author, up to 80% losses of EPNs occur within the first day of application under field conditions, mainly due to UV radiation and dehydration. Soil desiccation and low humidity are the major environmental factors for EPN dehydration. The remaining nematodes settle in the soil and their number gradually decrease at levels of 5 – 10% per day and in most cases only <1% of the applied population are alive after 2 – 6 weeks post application. Humidity is also important factor affecting survival and efficacy of EPNs. High relative humidity (>80%) appears to increase the efficacy of *S. carpocapsae* under controlled conditions, and its survival was shown to decrease rapidly with relative humidity (Kung *et al.*, 1991; Glazer, 1992). When it was applied onto the soil surface, *S. carpocapsae* All strain survived for 16 days at 97% RH, but for only 2 days at 50% RH (Kung *et al.*, 1991). These results suggest the importance of preferably applying nematodes at dawn or during the evening when low evaporation rate and high RH are recorded. To overcome the effect of dry soil and UV light on dauer persistence, adjuvants designed to reduce or prevent the drying of dauers have been incorporated in to soil application (Schroeder, 1990) and protective adjuvants should be used against the UV (Georgis and Gaugler, 1991).

EPNs are sensitive oxygen deficiency and lower oxygen content in organic soils decreases dauer persistence (Kaya, 1990). Clay soils or excess moisture impede dauer movement and infectivity in addition to affecting dauer survival (Baur and Kaya, 2003).

Biotic factors influencing survival and persistence of EPNs

Biotic factors in the soil environment affect persistence of EPNs either directly by parasitism or predation or indirectly by competition (Kaya and Koppenöfer, 1996). Fungal parasites and invertebrate predators have more direct effect on nematode persistence because they kill the

IJs. For instance, Van Sloun *et al.* (1990) found that the trapping fungi *Arthrobotrys superba* and *A. robusta* trap more than 90 percent of *S. carpocapsae*, *S. feltiae* and *Heterorhabditis* sp. on agar plates after 48 h. Moreover, intra- and interspecific competitions occur among dauers for insect hosts and for nutrients within insect hosts (Kaya and Koppenöfer 1996). Such competition will affect the persistence of entomopathogenic nematodes in the soil profile by limiting their access to suitable microsites and hosts.

Mechanisms of Survival used by EPNs

When the environment is not favourable, some nematode populations may avoid stress by moving into protective niches. For instance, Glazer *et al.* (1996) isolated higher number of heterorhabditid nematodes in deep soil layers (35 – 40 cm) during summer seasons as compared with upper soil layer (5 – 10 cm depth).

Some free-living stages of animal and plant parasitic nematodes can survive exposure to dry conditions for many years (Cooper and van Gundy, 1971). These nematodes are capable of anhydrobiosis in which following slow water loss, levels of trehalose and glycerol increases markedly while those of glycogen and lipids decrease (Womersley *et al.*, 1982). Anhydrobiosis is a general term for a reversible physiologically arrested state of dormancy. Under the condition of true anhydrobiosis, nematodes can lose up to 95 – 98% of their body water, and while in anhydrobiosis, they have virtually no metabolism thereby conserving energy (Barrett, 1991). Solomon *et al.* (1999) reported that a slow dehydration regime (pre-conditioning at 97% RH for 3 days at 23°C) induced a quiescent anhydrobiosis state in three strains (IS-6, IS-15 and N8) of *S. feltiae*, which enable them to survive at lower humidities (75% and 85% RH).

In many parasitic nematodes, which spend part of their life cycle in the host, only free-living stages have the capability to survive the harsh environment outside the host (Glazer, 2002). IJs do not feed and are so dependent on initial sources of energy that enable them to survive until a host is located. Nematode infectivity declines as energy reserve are depleted in storage. For example, a decline in neutral lipids was closely correlated with the observed decline in infectivity and survivorship of *S. riobrave*, *S. feltiae* and *S. glaseri* (Patel *et al.*, 1997).

1.2.6 Advantages of EPNs as a biocontrol agent

The increasing importance of biological control and the advantage of EPNs have resulted in rapid development of *Steinernema* and *Heterorhabditids* spp. as biological control agents (Peters, 1996). EPNs possess many attributes of an excellent biological control agent. They are relatively environmentally safe and acceptable, can be produced in a large quantity using artificial media, and are easily applied with standard spraying equipment or irrigation systems (Kaya and Gaugler, 1993; Smart, 1995). Several nematode species are readily mass-produced, stored, shipped, and are commercially available.

Most species have a broad host range and are highly virulent, killing their host rapidly. The broad host range and high host virulence of EPNs make them amendable for inundative insect pest control (Gaugler, 1988). This broad host range can be explained by the efficient pathogenic properties of the bacterium-nematode complexes, in which both partners are involved in the symbiosis and act together (Boemare *et al.*, 1996). These combinations of attributes have generated intense interest in the development of EPNs for use against insect pests (Berry *et al.*, 1997; Kaya and Gaugler, 1993). More than 30 EPN species exist and hundreds of isolates have been collected from every continent (Nguyen and Smart, 1996).

Other advantages of use of EPNs as well as other microbial insect pathogens like virus, fungi or bacteria include safety to farmers involved in pest control, reduction of pesticide residue in food reducing toxic risks to food consumers and preservation of other natural enemies leading often to increased biodiversity and natural pest control in managed ecosystems (Lacey *et al.*, 2001).

IJs tolerate short exposure (2 – 6 hours) to most agrochemicals including herbicides, fungicides, acaricides and insecticides (Rovesti and Deseo, 1990) and therefore can be often tank mixed with other inputs to save time and money. The future for nematode based commercial products is excellent. More efficient methods of production, formulation, etc, will lower the cost of nematode products and make them more competitive economically (Smart, 1995). There are many commercial EPN products available and they are used against different pest species. *H. bacteriophora*, *H. megidis*, *S. carpocapsae* and *S. feltiae* are some EPNs currently formulated as commercial products. Fully developing biological control methods using EPNs will improve their virulence, host range and environmental safety.

1.3 Statement of the research problem

Western flower thrips is one of the dominant thrips species in the world attacking a wide range of economically important crops in the field and greenhouses. Control of this pest is very difficult since it spends one-third of its life cycle in the soil. Moreover, its fast development of resistance to insecticides coupled with its cryptic feeding behaviour, make chemical control more difficult (Yudin *et al.*, 1986; Brødsgaard, 1989; Tommasini and Maini, 1995).

Attempts were made to identify highly virulent strains of EPNs with their effective concentration (Ebssa *et al.*, 2001a, 2003; Premachandra *et al.*, 2003b) and all soil-dwelling life stages of WFT were susceptible to the tested EPN strains. Moreover, Premachandra *et al.* (2003b) studied the short term persistence of EPNs under laboratory conditions in a petri dish bioassay. However, EPNs can provide best control of WFT, i) if they are applied when the majority of susceptible stages of WFT population are in the soil for pupation, and ii) if high population densities persist for a sufficiently long period. However, until now there is no sufficient information about the relationship between WFT population in the soil and on the plant, and persistence of EPNs under semi-field conditions. Moreover, there is no information whether EPNs can self-perpetuate in WFT or not.

EPNs differ in their foraging strategy in which some are "ambushers" while others are "cruisers". But there is no information if combined application of EPNs with different foraging strategies can provide better control of WFT than application of EPNs with the same foraging strategy alone.

Therefore, the present study is important in order to investigate necessary frequency of EPN application, post application short-term persistence, and as well as to determine appropriate time of EPN application with respect to population records of the pest on the plant (i.e. sticky trap catches). Moreover, it will enable us to answer the question of self-perpetuation of EPNs in WFT, which is important for persistence of EPNs, and also it will enable us to simulate the field (greenhouse) condition about the relationship between WFT population on the plant and in the soil.

1.4 Research objectives and hypotheses

1.4.1Objectives

The general aim of the present study was to investigate conditions that could help to improve efficacy of EPNs against WFT. The specific objectives of the study were:

1.To asses the persistence of EPNs for the control of WFT under semi-field conditions.

2.To determine whether EPNs can self-perpetuate in WFT under laboratory conditions.

3.To determine the time and frequency of EPN application with respect to population dynamics of WFT.

4.To study population dynamics of WFT and the relationship between soil-dwelling and foliage life stages of the pest.

5.To investigated possibilities for combined applications of EPNs with different foraging strategies for the control of WFT.

1.4.2 Research hypotheses

EPNs have a short period of post application persistence if exposed on plant or soil surface mainly due to UV radiation and dehydration. On the other hand, the IJs can migrate into the soil to protect themselves from the effect of UV and dehydration. Thus if EPNs are applied at higher concentrations some of them may persist for longer time.

In this study we hypothesised that the efficiency of EPNs in parasitising WFT declines as we proceed starting from the date of application due to the limited persistence of EPNs in the soil and also EPNs may not be able to perpetuate in WFT due to small size of the host. Therefore, high concentrations or repeated application of EPNs may be required to provide sufficient control of WFT. Moreover, we expect to elucidate the best time and frequency of EPN

application with respect to population densities on the plants as measured by sticky trap catches. In addition, better control of WFT can be achieved through combined application of EPNs with different foraging strategise than applying alone at same concentration, since WFT that wonder on the surface of the substrate can be easily parasitised by EPNs with ambush foraging strategy and those entered into the substrate by curies foragers.

2 Post application short-term persistence and ability to self-perpetuate affecting efficacy of entomopathogenic nematodes against soil-dwelling life stages of western flower thrips *Frankliniella occidentalis* (Pergande)

2.1 Abstract

The short-term post application persistence of two entomopathogenic nematode (EPN) strains, i.e. *Heterorhabditis bacteriophora* strain HK3 and *Steinernema carpocapsae* strain DD136 was studied under semi-field conditions (in a pot experiment) against soil-dwelling life stage, late second instar larvae (L2) of WFT. The nematodes were applied at two concentrations, 200 and 400 infective juveniles (IJs) cm^{-2}, and the L2 were introduced at 0 (same day), 3, 6, 9, or 12 days after nematode application (DANA). Results of this experiment showed that all EPN applied treatments caused higher WFT mortality compared to the control. For HK3, higher thrips mortality was obtained from the higher concentration and persisted at least for 12 days at 400 IJs cm^{-2} and for 9 days at 200 IJs cm^{-2}. However, in the case of DD136, higher WFT mortality was recorded from the lower concentration than the higher concentration. For this strain, there was no significant difference in thrips mortality between same day and 12 DANA at both concentrations. Moreover, we have investigated the possibility for self-perpetuation of one EPN strain (HK3) in WFT and we found that IJs were not able to develop into adult or even to the next juvenile stage (J_4). Pupa of WFT was seen to be totally disintegrated within 48 – 72 h after infection and not able to support the development of IJs that committed infection.

Key words: Entomopathogenic nematodes; *Heterorhabditis bacteriophora*; *Steinernema carpocapsae* concentration; persistence; self-perpetuation; western flower thrips; *Frankliniella occidentalis*; soil-dwelling life stages.

2.2 Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the predominant thrips species in the world attacking a wide range of economically important crops in the field and greenhouses (Yudin *et al.*, 1986; Brødsgaard 1989; Tommasini and Maini, 1995). It causes damage to plants directly by feeding on leaves, leaf buds, flowers, flower buds and fruits (Rosenheim *et al.*, 1990; Lewis, 1997, Tommasini and Maini; 1995, Childers and Achor, 1995) and indirectly by serving as a vector to tospoviruses such as tomato spotted wilt virus and impatiens necrotic spot virus (Allen and Broadbent, 1986). The life cycle of WFT consists of four stages namely, adult, egg, two feeding larval instars (first and second instar larvae) followed by two immobile non-feeding stages (the prepupa and pupa) that both occur predominantly in the soil. Eggs are deposited in the plant tissue, and both the adults, first and early second instar larvae feed on the plant. The late second instar larvae enter into the soil for pupation to a depth of 1.5 – 2.0 cm.

Chemical control of western flower thrips is very difficult due to its cryptic nature of feeding and life strategy (i.e., L2 enter into the soil for pupation). Short generation time and high fecundity of WFT (Robb and Parrella, 1987) coupled with excessive use of insecticides (Zhao *et al.*, 1995) has led the development of resistance against insecticides by WFT (Broadbent and Pree, 1997). A limited range of natural enemies including several species of predatory bugs of the genus *Orius* (Hetroptera: Anthocoridae) and predatory mites such as *Amblyseius barkeri* (Hughes) and *A. cucumeris* (Oudemans) (Acari: Phytoseiliidae) control the foliage life stages of WFT (Smart 1995; Tommasini and Maini, 1995; Loomans and van Lenteren, 1995). However, the available natural enemies do not give sufficient control of WFT, especially the soil-dwelling prepupae and pupae due to the nature of their habitat (Loomans and van Lenteren 1995; Ruidavets, 1995).

In recent years, Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae (Rhabditida) are acquiring a great attention as potential biological control agents against a large number of insect pest species in the soil and cryptic habitats (Klein, 1990; Kaya and Gaugler, 1993). The free living (third juvenile) stage, the so called "infective Juvenile" (IJ) of these nematodes carry and transmit symbiotic bacteria that are lethal to their host and this characteristic makes them suitable for control of insects (Boemare *et al.*, 1996; Spiridonov *et al.*, 1991). Previous studies showed that soil-dwelling life stages of WFT are proved to be susceptible to different EPN species /strains and also effective concentration of the nematodes was studied (Ebssa *et al.*, 2001b; Premachandra *et al.*, 2003b). Tomalak (1994) and Chyzik *et al* (1996) also reported the susceptibility of WFT to EPNs.

In addition to virulence, nematode persistence could be an important factor in determining which nematode to apply (Sheilds *et. al*, 1999). Recently Premachandra *et al.* (2003b) showed that under laboratory condition EPNs can persist at least for six days without loosing their virulence against WFT. However, there is no information about the post application persistence of EPNs under more practical semi-field conditions for the control of WFT. There are reports on recycling of EPNs and provision of sustained pest control after initial application (Parkman and Smart, 1996; Parkman *et al.*, 1993; Shanks et al 1990; Grewal and Richardson, 1993; Gouge and Hauge, 1995). However, due to the small body size of WFT, self-perpetuation of EPNs in WFT seems rather unlikely. Hence, in a greenhouse situation repeated applications of EPNs are most likely required for a sufficient control of WFT.

Therefore, the objectives of the present study were: ii) to assess the short-term post application persistence of EPNs under semi-field condition against soil-dwelling life stages of

WFT, and ii) to investigate whether EPNs can self perpetuate in WFT or not i.e. to see if IJs can develop in to adults and subsequently give next generation.

2.3 Materials and methods

2.3.1 Nematode and WFT cultures

Two EPN strains, *Heterorhabditis bacteriophora* (Poinar) Strain HK3 and *Steinernema carpocapsae* (Weiser) strain DD136 that have shown high efficacy in the previous experiments against WFT (Ebssa *et al.*, 2001a; Premachandra *et al.*, 2003b) were obtained from Dr. R. -U. Ehlers, Institute of Phytopathology, Christian Albrechts University of Kiel, Germany. In vivo rearing of the nematodes was done on last instar larvae of greater wax moth *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), following the modified production system originally developed by Woodring and Kaya (1988), at a temperature of 23 $\pm$ 2°C.

Two types of sieves, with 30 and 15 μm pore-size prepared from nylon tissue and acryl-glass cylinder (∅ 84 mm) was used for harvesting the IJs. The 30 μm sieve was used to separate adult and dead juveniles from the live ones. It allows the passage of live juveniles only, which were finally collected using the 15 μm sieve.

Harvested nematodes were stored at a temperature of 5°C in 0.1% ringer solution in tissue culture flasks until they are used. Viability of nematodes was checked microscopically by taking 0.25 ml of samples from the stock suspension. Quantification of EPNs was done using the standard procedure by Kaya and Stock (1997), and the rate of application of EPNs was adjusted for viability. The nematodes were acclimatised at room temperature at least for 12 hours before application.

In order to obtain uniform aged thrips, WFT was reared in a climate chamber with a temperature of 23 ± 2°C, 50 – 60% relative humidity (RH) and 18:6 L: D photoperiod following the rearing protocol developed by Ullman *et al.* (1997) on pods of green beans (*Phaseolus vulgaris* L.). Rearing of WFT was continued until the required population with the required stage was obtained for the experiments.

2.3.2 Persistence study

Plastic pots with a diameter of 7.5 cm (at their base) and 8.5 cm height, and perforated base were used to simulate the normal conditions where the growing substrate loses moisture over time through percolation. The pots were filled with Fruhstorfer Erde, a commercially available plant growing substrate (Archut GmbH, Luterbach-Wallenrod, Germany). It is composed of humus, clay and peat at a proportion of 15:35:50% respectively. Bean plants (*Phaseolus vulgaris* L.) were raised in pots filled with the above standard growing substrate and caged at the two-leaf stage using an acryl-cylinder (∅ 84 mm). Plants were required so that the soil in the pot can lose water over time through transpiration, thereby mimicking field conditions. To prevent escaping of WFT from the cage, the gap between the pot and the acryl-cylinder was filled with modelling clay. In the same way the open end of the acryl-cylinder (at the top) was glued with a nylon tissue (64 µm ∅ pore size). To provide additional ventilation, two side holes (∅ 35 mm) were drilled in the upper part of the cylinder and covered with the same nylon tissue. Two similar side holes in the lower portion of the cylinder were used as windows for releasing the WFT larvae and for pipetting EPN suspension.

The two EPN strains, *H. bacteriophora* strain HK3 and *S. carpocapsae* strain DD136 were applied at a concentration of 200 and 400 IJs cm^{-2} onto the soil in the pots (6 ml of EPN

suspension/ pot). Thereafter, the pots were irrigated with 40 ml of tap water to rinse the nematodes down to levels in the soil where WFT pupates. Normal agronomic practices, like watering, were done to maintain the seedlings. Twenty late L2 (8 – 9 days old) were transferred to the pot onto the surface of the soil on the following day after nematode application (DANA): on 0 (same day), 3, 6, 9, or 12 days. Each treatment was replicated five times that was conducted two times, two replication during the first time and the rest three replications during the second time. Each treatment (i.e. DANA) has its own control treatment where instead of nematode solution 6 ml distilled water were applied per pot. The treatments were factorially arranged in a completely randomised design.

The cylinder and shoot parts of the plant were removed 3 days after WFT introduction and the shoots and cages were examined for the possible presence of WFT. Then the pots were covered with a Petri dish (∅ 100 mm) with two ventilation holes (∅ 25 mm) that were covered with nylon tissue to prevent escaping of adults emerging from the soil. The inner part of the petri dish except the holes was painted with insect glue that served as a "sticky trap" for emerging adults from the substrate. Starting one day after removal of the cylinder the emerging adults in all treatments were counted on daily bases from the sticky trap as well as from the top of the substrate on daily basis for ten consecutive days, until no further adult emergence. Persistence of EPNs was assessed by comparing corrected mortality (CM) data from the different DANA treatments for EPN applied treatments.

2.3.3 Self-perpetuation study

The experiment was conducted in a petri dish bioassay (on filter paper) in a climate chamber at a temperature of 23 ± 2°C and 65% relative humidity. A filter paper (∅ 45 mm) was placed in a small petri dish (∅ 45 mm and 25 mm height) with a ventilation hole (∅ 12 mm) on its

lid that was covered with a nylon tissue (∅ 64 μm). Twelve days old 10 pupae of WFT were introduced onto the filter paper. *H. bacteriophora* strain HK3 was applied at concentration of 400 IJs cm^{-2} (400 μl of the nematode suspension/ petri dish). Equal amount of distilled water was pipetted to the control treatments. In order to prevent drying of the filter paper, 150 μl of distilled water was added to the petri dishes every 3 days.

All dead cadavers of WFT per arena were dissected under the binocular for the presence of adult EPN on the first, second, third, fifth, seventh, and tenth day after EPN application. Moreover, using destructive sampling techniques, both the filter paper and the petri dish were washed using distilled water and examined under binocular on the respective days for the possible presence of adult EPNs. In addition to this, the number of dead and live WFT were recorded on each sampling day to see what percent of the population was killed after inoculation on each day. There were eight petri dishes per treatment that were completely randomised.

2.3.4 Statistical analysis

Mortality values were corrected for natural mortality, or the number of adults emerged from the control according to Abbott's (1925) formula as:

$$CM = \frac{C_A - T_A}{C_A} * 100$$

Where: CM = Corrected mortality

C_A = Control alive

T_A = Treatment alive

Percent mortality data were transformed for variance stabilisation using the arcsine transformation. For data analysis, analysis of variance (ANOVA) was performed using the general linear model (Proc GLM; SAS Institute, 1999). Whenever two factors (e.g. strain and concentration) significantly interacted, means of the level of one factor were compared at each level of the other factor. In the absence of significant interaction, means of the level of one factor were compared irrespective of the levels of another factor (Sokal and Rohlf, 1995). The corrected mortality means were compared to zero (corrected mortality of control) using Dunnett's two-sided test (SAS Institute, 1999). When ANOVA showed significant treatment effects, individual means were compared using the least significant difference (LSD) multiple range test procedure (SAS Institute, 1999). Regression analysis was performed using the proc REG procedure of SAS (SAS Institute, 1999) to investigate the relationship between time (DANA) and thrips mortality caused by EPNs in the persistence study and to see the relationship between percent WFT mortality and time after nematode application in the self-perpetuation study. A significant level of $\alpha = 0.05$ was used in all the analyses.

2.4 Results

2.4.1 Persistence study

In general, both HK3 and DD136 resulted in higher thrips mortality compared to the control treatment ($P< 0.05$). HK3 gave significantly higher mortality compared to the control treatment at both concentrations for all DANA. Similarly mortality values caused by DD136 significantly differed from the control except for 6 DANA at 200 IJs cm^{-2} and for 6 and 9 DANA at 400 IJs cm^{-2}. Here 9 days old L2 were introduced in 3 out of 5 replications instead of 8 days old in all replications for the rest of DANA for both strains. The highest mean corrected mortality (76.6%) was recorded from HK3 at 400 IJs cm^{-2} at 3 DANA followed by same day and 6 DANA, 66.6% and 61.6% respectively, for the same strain and concentration.

Of all the nematode applied treatments, loweest thrips mortality was recorded from DD136 at 6 and 9 DANA at 400 IJs cm^{-2} and on 9 DANA at 200 IJs cm^{-2}.

Since there was no significant interaction between time, strain and concentration ($F_{8, 106}$= 0.71, P = 0.6798), time and strain ($F_{4, 106}$ = 2.04, P = 0.0935), and time and concentration ($F_{8, 106}$ = 1.42, P = 0.1949), both concentrations and strains were compared irrespective of time (DANA). Concentration and strain interacted significantly ($F_{2, 106}$ = 31.30, P = < .0001), so that the two strains were compared at given concentration. The results of this comparison showed with HK3, significant difference between the two concentrations ($F_{1, 30}$ = 17.73, P = 0002). Even if it was not significant in all the cases, a concentration of 400 IJs cm^{-2} always caused higher thrips mortality than a concentration of 200 IJs cm^{-2} (Figure 1a). At 400 IJs cm^{-2} there was no significant difference between same day and 12 DANA. At the lower concentration, there was no significant difference in thrips mortality from same day to 9 DANA. However, significantly lower WFT mortality was recorded at 12 DANA compared to same day at this concentration.

Like that of *H. bacteriophora*, in DD136, concentration significantly affected WFT mortality ($F_{1, 32}$ = 4.37, P = 0.0447). However, unlike in HK3, WFT mortality was always higher at the lower concentration than the higher (Figure 1b). For this strain, lower thrips mortality was recorded from 6 and 9 DANA at both concentrations. There was no significant difference in WFT mortality between same day and 12 DANA at l oncentrations.

Results from the comparison of the two strains at the same concentration showed that, at 200 IJs cm^{-2} the two strains significantly differed only at 3 and 9 DANA in which HK3 caused

higher mortality than DD136 (Figure 2a). At 400 IJs cm^{-2}, HK3 caused significantly higher WFT mortality than DD136 for all DANA (Figure 2b).

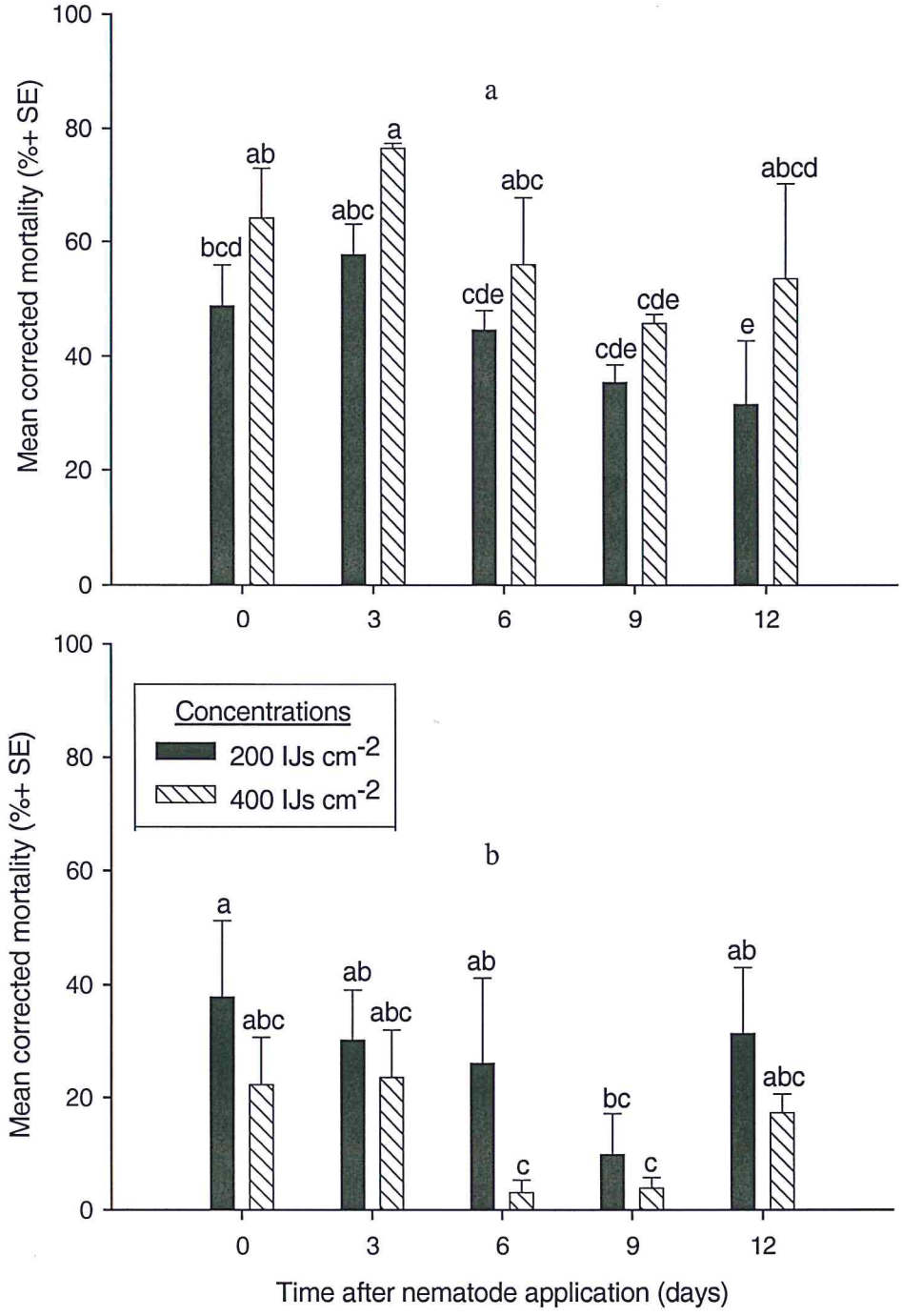

Figure 1. Mean corrected mortality (% + SE) of western flower thrips late second instar larvae introduced at 0 (same day), 3, 6, 9 and 12 days after nematode application (DANA) caused by *Heterorhabditis bacteriophora* HK3 (a) and *Steinernema carpocapsae* DD136 (b) applied at 200 and 400 IJs cm^{-2}. Means followed by the same letters do not differ significantly (LSD, $\alpha = 0.05$).

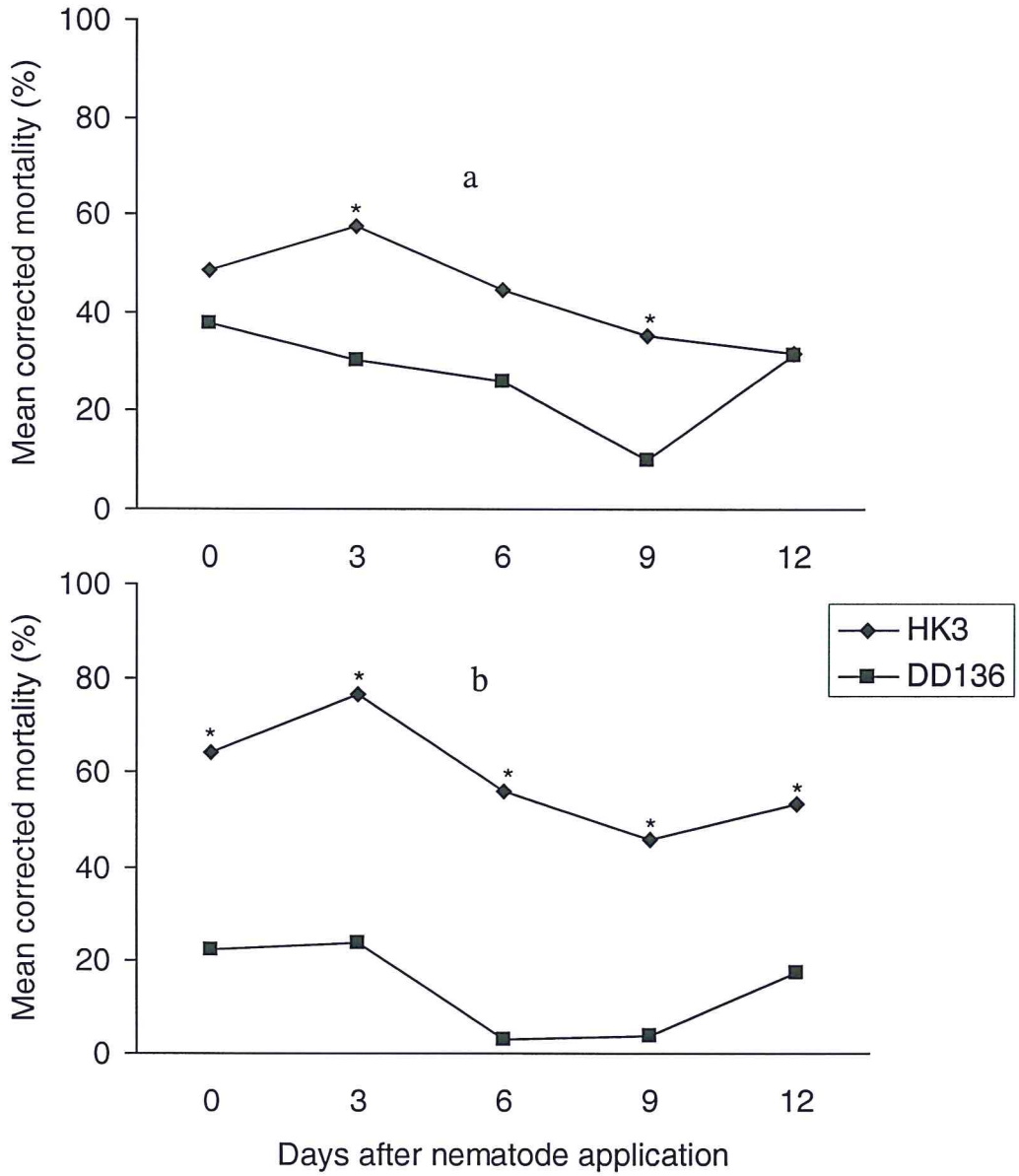

Figure 2. Mean corrected mortality (%) of western flower thrips late second instar larvae introduced at 0 (same day), 3, 6, 9 and 12 days after nematode application (DANA) caused by *Heterorhabditis bacteriophora* HK3 and *Steinernema carpocapsae* DD136 applied at 200 (a) and 400 (b) IJs cm^{-2}. * Indicates significant difference at α = 0.05.

Regression analysis was performed to see effect of time on persistence of EPNs, which is inferred from the corrected mortality. As can be seen from table 1, even though it was not statistically significant, there is a decreasing trend in thrips mortality with increasing time

after nematode application, indicated by the negative slope for both strains at all concentrations.

Table 1. Regression analysis for effect of time on infectivity of *Heterorhabditis bacteriophora* HK3 and *Steinernema carpocapsae* DD136 against late second instar larvae of western flower thrips introduced at 0 (same day), 3,6,9, and 12 days after nematode application for the regression equation CM (%) = a – b*t, where, CM = corrected mortality, t = time (DANA).

Strain	Concentration (IJs cm^{-2})	*a*	*b*	*p*	R^2
DD136	200	36.79	–3.02	0.40	0.2503
DD136	400	23.06	–3.24	0.13	0.223
HK3	200	57.86	–3.82	0.09	0.733
HK3	400	76.28	–5.44	0.77	0.494

a, Intercept
b, Slop of the regression equation: CM% = a – b*t
p, Probability that the slop is different from zero ($P = 0.05$).
R^2, Regression coefficient for the model.

2.4.2 Self-perpetuation study

During the whole experimental period we did not find any adult EPN or fourth juvenile stage (J_4) inside or outside the cadaver in the petri dishes. Most of the dead pupae were disintegrated 3 days after nematode application and only the head and part of skin were seen on the filter paper and the colour of these parts has turned to red (Figure 3a). Moreover, 7 days after EPN application, adult WFT were eaten were disintegrated (Figure 3b). In the control treatment natural mortality was low and it was in the range of 8.0 – 17%. All EPN applied treatments caused significantly higher WFT mortality ($P < 0.05$) in all sampling days. In EPN treatments, percent mortality has increased linearly with time ($F_{5, 37} = 52.11$, $P < 0.0001$) from 25.7% at day 1 to 90% at day 5 after EPN application (Figure 4). There was

100% mortality after 7 days of EPN application and during this time not only the pupae but also dead adults were eaten and disintegrated.

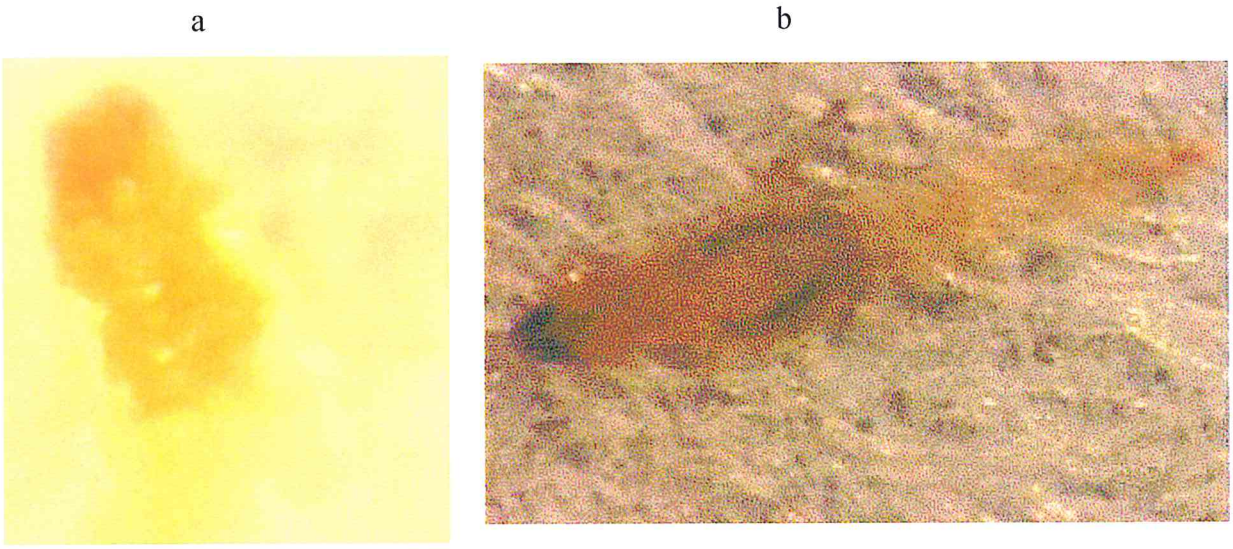

Figure 1. Disintegrated pupa (a) and adult (b) of western flower thrips 3 and 7 days, respectively, after application of *Heterorhabditis bacteriophora* HK3 on the pupae at 400 IJs cm^{-2}.

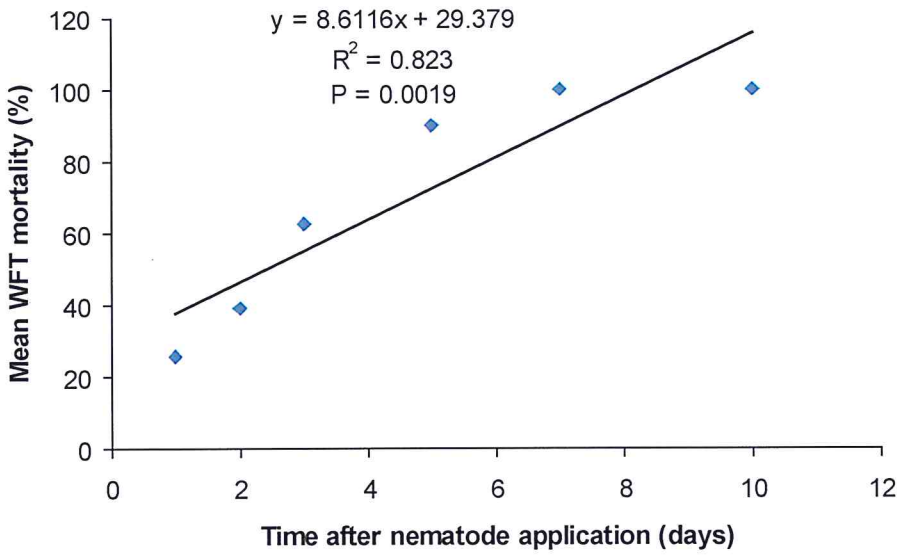

Figure 2. Percent mortality (cumulative) of pupae of western flower thrips over time after initial application of *Heterorhabditis bacteriophora strain* HK3 applied at 400 IJs cm^{-2}.

2.5 Discussion

2.5.1 EPNs short-term persistence

From our preliminary experiment (data not presented here), we have observed that some of the larvae tend to remain and pupate on the leaf instead of descending to the soil when late L2 were applied on the leaf of the bean plants. . This may result in biased information concerning the number of larvae that developed into adults from the different EPN treatments. Moreover, if the larvae were applied on the leaf, we cannot be sure that all the larvae will enter into the soil at the same time and be exposed to IJs on the time of our interest (DANA) as the plant will grow and become taller after 12 days as compared to at two leave stage. Therefore, the methodology we used to apply the late L2 onto the soil helped us to reduce variations that could result from a more complex environment and to guarantee that all introduced late L2 enter into the soil for pupation.

All EPN treatments resulted in higher thrips mortality compared to the control except DD136 on 6 DANA at 200 IJs cm^{-2}, and on 6 and 9 DANA at 400 IJs cm^{-2}. Since it was necessary to fit our experiment (thrips application schedule) into the main thrips rearing culture, for both strains we have introduced 9 days old L2 on 6 and 9 DANA instead of 8 days old L2 for the rest of DANA. The most probable reason for low thrips mortality of DD136 at 6 and 9 DANA could be that, after application onto the soil surface the 9 days old L2 enter into the soil very soon and changed in to prepupa/pupa and become immobile. This reduces the chance that the thrips become into contact and parasitised with the IJs of DD136 that have a wait and sit strategy (ambushing behaviour) thus most of the applied L2 will develop in to adults. On the other hand, HK3 that has a cruise foraging strategy, which actively search for potential hosts by moving through the soil profile, can come into contact and parasitise both sedentary and mobile hosts. Our findings corroborate with a number of reports on host searching behaviour

of these EPN strains. For instance, Lewis *et al.* (1993), Campbell and Gaugler (1997) and Choo *et al.* (1989) mentioned that, foraging behaviour of EPNs can affect their efficacy, in which some species are "ambushers" (e.g. *S. carpocapsae* and *S. scapterisci*) that tend to remain near the soil surface and attach to and infect mobile hosts at the soil-litter interface. Other species (e.g. *S. glaseri* and *H. bacteriophora*) are "cruisers" that have an active searching strategy and are more effective against less mobile insects in the soil than "ambushers" like *S. carpocapsae*. This difference in search strategy was seen in the superiority of *H. bacteriophora* and *S. glaseri* over *S. carpocapsae* in Japanese beetle control (Georgis and Gaugler, 1991).

For most of the cases, HK3 caused higher mortality at 400 IJs cm^{-2} than at 200 IJs cm^{-2}. Our results are in agreement with the findings of Ebssa *et al.* (2001b), in which from their concentration study they have observed that mortality of WFT larvae significantly increased with increasing concentrations up to a concentration of 400 IJs cm^{-2} for the nematode strains they tested including HK3. Chyzic *et al.* (1996) also found that with an increase in the concentration of *H. bacteriophora* strain HP88, there was an increase in WFT mortality.

Unlike in HK3, in the case of DD136, higher thrips mortality was obtained at the lower concentration than with the higher concentration. This difference between the two EPN strains regarding the effect of concentration at the same host density may be attributed to the difference between EPNs in their response to host cues and foraging strategy. IJs of ambushing nematodes such as DD136 respond to host cues only when they come into contact with a cuticle of a passing host whereas IJs of cruise foraging nematodes like HK3 can respond to host cues from a distance (Lewis *et al.*, 1993; Lewis *et al.*, 1995b). This indicates that if the number of IJs is very high in relation to the host density, the IJs of ambushers will

encounter not only passing hosts but also other IJs making repositioning moves that can take the form of crawling or jumping (Campbell and Gaugler, 1993). These IJs frequently came into contact with other nictating conspecific nematodes and it may be possible that nictating IJs encountering individuals from the same species several times, were negatively influenced concerning their response abilities to real hosts resulting in less efficient recognition and parasitisation of hosts. There are reports that are in agreement with our findings concerning the effect of dose and host density in S. *carpocapsae* on host mortality. For example, when wooden fruit bins containing codling moth (*Cydia pomonella*) larvae were treated with *S. carpocapsae* containing 0, 90, 150 (first trial) or 0, 50, 90 (second trial) IJs mL^{-1}, codling moth mortality was significantly higher at the lower concentration than the higher concentration in both trials (Cossentine *et al.*, 2002). In another study Lewis *et al.* (1996) found that at a concentration of 200 IJs per host, there was a positive relationship between host recognition and host mortality. However, at a concentration of 500 IJs per host, this relationship was decreased. This finding suggests that for some EPN strains /spp. exposing hosts to very high concentrations of nematodes can confuse the natural nematode-host interaction and intraspecific competition may occur. In contrary to our results, Ebssa *et al.* (2001b) reported that increasing the concentration of this nematode from 100 to 400 IJs cm^{-2} significantly increased WFT mortality. This may be due to the fact that they have used a higher host density of 1 L2 cm^{-2} of soil in their studies compared to 0.35 L2 cm^{-2} in our case. This was also true in our experiment on combined application of EPNs in which higher WFT mortality was obtained with the higher concentration compared to the lower one at relatively higher host density (Chapter 2).

When we compare the two strains, in general HK3 caused higher WFT mortality than DD136 at both concentrations. This could be due to the fact that in addition to the difference between

EPN strains/ species in their virulence to insect pests (Bracken, 1990) the time in which the thrips and nematodes stay together is shorter for DD136 than HK3 as the larvae enters into the soil for pupation and the prepupal and pupal stages occurred in the soil and are immobile unless disturbed so that there is less chance to be infected by DD136. On the other hand, IJs of HK3 with a cruise foraging strategy can actively search and parasitise both mobile and non-mobile stages of WFT in the soil resulting in thrips high mortality.Supporting our findings, previous studies proved that HK3 is superior to DD136 in WFT control even at higher host density (Ebssa *et al.*, 2001a).

When we assess the short-term persistence of the two EPNs, HK3 did not show significant difference in WFT mortality from same day to 12 DANA at 400 IJs cm^{-2} and from same day to 9 DANA at 200 IJs cm^{-2}. This suggests that this strain has persisted at least for 12 and 9 days at 400 and 200 IJs cm^{-2} respectively without loosing its virulence after application in the soil. In the case of DD136, there was no significant difference in thrips mortality between same day and 12 DANA at both concentrations indicating that IJs of DD136 had persisted at least for 12 days (the maximum time of the experimental period).

Since HK3 persisted for 12 days at 400 IJs cm^{-2} and only for 9 days at 200 IJs cm^{-2}, where as DD136 persisted at least for 12 days at both lower and higher concentrations, we can conclude that DD136 can persist longer than HK3. Moreover, if we see the percent decrease in WFT mortality from same day to 12 DANA, for HK3 WFT mortality decreased by 30.1% and 45.4% at 400 and 200 IJs cm^{-2} respectively. For DD136 the percentage decrease in thrips mortality was 22.2% and 17% at 400 and 200 IJs cm^{-2} respectively. The higher percent decrease in WFT mortality for HK3 at both concentrations than DD136 may indicate that HK3 has less persistence compared to DD136. In addition, the shorter persistence of HK3

than DD136 is evident from the higher negative slopes of the regression analysis for HK3 than DD136 at both concentrations with increasing time (DANA).

This difference in persistence between the two EPN strains could be attributed to their physiological and behavioural differences as well as their response to abiotic factors that can affect nematode survival or persistence. Lewis *et al.* (1995a) mentioned that *S. carpocapsae* has a lower metabolic rate compared to *H. bacteriophora* and there were significant differences in survivorship that correspond to differences in metabolic. For example products based on S. *carpocapsae* can be stored longer without decreasing efficacy than H. *bacteriophora* based ones. Adaptation of *S. carpocapsae* to its preferred niche, staying and nictating on the soil surface, could explain its relatively high resistance to desiccation (Kung *et al*, 1991; Peters, 1996) and UV radiation (Gaugler *et al.*, 1992).

Aguilar *et al.* (1999) discussed that the survival of *H. bacteriophora* and *S. carpocapsae* varied in relation to the bulk density of sandy loam soil. Survival of *H. bacteriophora* decrease quadratically with increasing bulk density while that of *S. carpocapsae* was not affected by bulk density. *H. bacteriophora* probably moved considerably through the soil profile, thus survival was reduced with increasing bulk density. The lack of significant bulk density effects on *S. carpocapsae*'s survival may reflect the tendency of this species to move over the soil surface rather than the soil profile and to remain immobile until a host comes in to contact (Molyneux, 1985).

Even though we have argued that DD136 has showed greater persistence than HK3, we suggest that it seems advantageous to use HK3 than DD136 in WFT control since HK3 has

already caused 53.5% mortality in WFT 12 days after initial application compared to 17.35% for DD136 at 400 IJs cm^{-2}.

2.5.2 Self-perpetuation of EPNs

There are reports on long-term persistence and recycling of some species of nematodes in the host (target insect) leading to long term establishment of efficient population densities and a sustained suppression of some insect pests (Kaya, 1990) as shown with in pests like mole crickets (Parkman and Smart, 1996). However, EPNs do not reproduce in small insects including WFT because of their small size in relative to EPNs and thus are not self-sustaining (English-Loeb et al., 1999). This statement supports our finding in which IJs of EPNs were not able to develop in to next stage (J4) or adult in pupae of WFT even after 10 days of initial inoculation. This could be due to the small size of the insect, which cannot provide enough food for IJs to develop into the next stage. Corroborating this idea we have observed that once infected, WFT is disintegrated with 48 – 72 h and only the head and part of the skin was observed on the filter paper. In our experiment adults emerged from EPN treated dishes were not removed or sticky trap was not used to see whether IJs could infect adult WFT or not. From this we have observed that IJs do not infect and kill adults while they are active. This could be due to fast movement of adults and avoidance of contact with IJs. However, once they become weak and less mobile due to lack of food, they were killed and disintegrated after 7 days of EPN application on the pupae. This suggests that it is important to collect data on time and remove emerging adults while evaluating EPN treatments against WFT. Because, if experiments on EPNS and WFT is conducted without host plant, some of the adults emerged from the EPN treated substrate (soil) will stay on the surface on the substrate (which actually could have gone to the host plant if it was there) and others stuck onto the "stick trap". Those adults, which remain on the surface of the substrate, can be infected and

disintegrated by EPNs and become difficult to count (recognize) if data collection id delayed. If IJs of EPNs once commence infection, they resume development and will die unless they are able to develop in to the next stage and give another generation inside the infected host (Lewis, 2002). This indicates that IJs applied for the control of WFT will die after infecting the first host and further infection will be possible only by the remaining IJs until they lose their infectivity and persistence. In our experiment, the increased mortality of WFT with time most probably could be due to increased contact of WFT pupae with IJs of HK3 that are still alive and actively search for potential hosts (Campbell and Gaugler, 1997) and yet do not commence infection. Therefore, repeated application of EPNs may be necessary to get sufficient control of WFT under greenhouse or field condition. This was also true from our semi-field experiment on time and frequency of EPN application in which repeated application of EPNs gave higher WFT mortality than single applications, even at a higher concentration.

3 Time and frequency of application of entomopathogenic nematodes with respect to population dynamics of western flower thrips Frankliniella *occidentalis* (Pergande) and possibilities for combined application of entomopathogenic nematodes with different foraging strategies

3.1 Abstract

Time and frequency of application of two entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora* strain HK3 and *Steinernema feltiae* strain Sylt, was studied under semi-field conditions with the presence of host plants (*Phaseolus vulgaris* L.) against soil-dwelling life stages of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande). The nematodes were applied at a concentration of 200 and 400 infective juveniles (IJs) cm^{-2}, once (10, 15 or 20) or twice (10 and 15, 10 and 20, or 15 and 20) days after introduction of ten female and two male WFT adults onto the bean plants. All EPN treatments caused significantly higher WFT mortality compared to the control. Timing of nematode application was found to be important in determining efficacy of EPNs against WFT. All the repeated applications of HK3 at 400 IJs cm^{-2}, and 10 and 15, and 15 and 20 days at 200 IJs cm^{-2} caused significantly higher or similar level of WFT mortality than single applications both at higher and lower concentrations except for 10 days at the higher concentration. For Sylt, higher WFT mortality was record from the repeated applications of 10 and 15, and 15 and 20 days at 400 IJs cm^{-2}. For this strain, a splitted application at lower concentration does not result in higher thrips mortality than single applications at higher concentration. From our experiment on population dynamics of WFT we found that at the10^{th} and 15^{th} day after adult introduction, the majority of the population was larvae and found on the plant. However after 20 days, the majority was found in the soil as prepupae/pupae. More than 96% of WFT population pupated in the soil. We have also investigated possibilities for combined

application of two EPN strains with different foraging strategy, on non-sterilized commercial plant growing substrate under laboratory condition. HK3 (a cruise forager) and *S. carpocapsae* strain DD136 ("ambusher") were applied in combination or alone at 400 and 200 IJs cm^{-2}. Higher WFT mortality (73 – 79.4%) was obtained from HK3 applied alone at both concentrations and combined applications at 400 IJs cm^{-2} than. application of DD136 alone at both concentrations. In DD136, relatively higher mortality was obtained from the higher concentration than the lower concentration. Possibilities for splitted application of EPNs at lower concentration and timing of nematode applications with respect to population dynamics of the pest are discussed here.

Key words: Entomopathogenic nematodes; *Heterorhabditis bacteriophora*; *Steinernema carpocapsae*; *Steinernema feltiae*; split application; concentration; persistence; western flower thrips; *Frankliniella occidentalis*; soil-dwelling life stages.

3.2 Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important pest of a wide range of crops of economic importance both under greenhouse and field condition and causes substantial economic looses to growers (Yudin *et al.*, 1986; Lewis, 1998; Tommasini and Maini, 1995). It causes damage directly through feeding on or ovipositing in leaves, flowers and fruits (Rosenheim *et al.*, 1990; Tommasini and Maini, 1995; Childers and Achor, 1995) and indirectly through transmission of plant viruses such as tomato spotted wilt virus (TSWV) (Marchoux *et al.*, 1991; De Angelis *et al.*, 1994; Allen and Broadbent, 1986). Damage to plants is caused by the larvae (first and second instars) and adults that both occur on the plant. The majority (at least 91%) of WFT leave the host plant and enter into the soil for pupation (Berndt, 2003).

Generally, WFT is difficult to control. Chemical control is difficult because eggs are inserted into plant tissues, the larvae feed in tight and protected places such as flower buds or foliage terminals, the pupal stages are passed in the soil or leaf litter, and adult thrips also feed in protected places. Thus, insecticides are not effective against the prepupal and pupal stages making it difficult to control WFT with chemicals alone. Chemical control is also hampered by the rapid development of resistance to insecticides due to high fecundity and short generation time of WFT that calls for repeated chemical application (Immaraju *et al*, 1992; Brødsgaard, 1994; Zhao *et al*, 1995; Kontsedalov *et al*, 1998). Furthermore, the use of insecticides to control WFT limits biological control methods against other pests.

Biological control agents, such as predatory mites, *Amblyseius barkeri* (Hughes) and *A. cucumeris* (Oudemans) (Acari: Phytoseiliidae), and predatory anthocorid *Orius* spp. (Hetroptera: Anthocoridae) are used to control the foliage life stages of WFT, but are not successful in all crops and under all situations (Jacobson, 1997; Jarosi *et al*, 1997).

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae (Rhabditida) are lethal obligate parasites of a large number of insect species (Kaya and Gaugler, 1993) and are established biocontrol agents for several soil-inhabiting insect pests (Klein, 1990). The free living (third juvenile) stage, the so called "infective Juvenile" (IJ) of these nematodes carry and transmit symbiotic bacteria that are lethal to their host (Boemare *et al.*, 1996; Spiridonov *et al.*, 1991). Previous studies have indicated that EPNs can effectively control soil-dwelling life stages of WFT (Ebssa *et al*, 2001a, 2001b; Premachandra, 2003b; Chyzic *et al.*, 1996; Tomalak, 1994). However, to maximise the impact of inundative release of natural enemies like EPNs, they should be applied at the appropriate time and frequency when the maximum proportion of susceptible stage of WFT population is in the soil. Time of

nematode application is critical as the age of the insect has been important for nematode efficacy (William and Walters, 1994). But in WFT, little is known about the relationship between foliage and soil-dwelling life stages, i.e. when the maximum proportion susceptible soil-dwelling stages will be found in the soil after the first infestation of the plant by the adults.

EPNs differ in their foraging strategy in which some are "ambushers" (e.g. *Steinernema carpocapsae*) that tend to remain stationary and nictate by standing on their tail elevating more than 75% of their body (Campbell and Gaugler, 1993) to attach a nearby passing host and are therefore more effective against highly mobile insects near the soil surface while others are "cruisers" (e.g. *Heterorhabditis bacteriophora*) that search constantly and are typically more effective at finding relatively sedentary hosts within the soil profile (Kaya and Gaugler, 1993). Hence, Combined application of EPNs with different foraging strategies may improve the efficacy of the nematodes. Thus, the objectives of the present study were: 1) To identify appropriate time and frequency of EPN application once WFT threshold (as recorded on the sticky traps) are reached, 2) To study population dynamics of WFT (to the relationship between soil and foliage dwelling life stages of the pest), and 3) To investigate possibilities for combined application of EPNs with different foraging strategies against the soil-dwelling life stages of WFT.

3.3 Materials and methods

3.3.1 Nematode and thrips cultures

The three nematode strains used in this study, *Heterorhabditis bacteriophora* strain HK3, *Steinernema feltiae* strain Sylt and *Steinernema carpocapsae* strain DD136 were obtained from the Institute of Phytopathology, Christian Albrechts University of Kiel, Germany. The

strains were reared on last instar larvae of greater wax moth larvae, *Galleria mellonella* (L.) Lepidoptera: Pyralidae) following the rearing protocol of Woodring and Kaya (1988) at a temperature of 23 ± 2°C. Infective juveniles were stored at a temperature of 5°C until they were used. The nematodes were acclimatized at room temperature for about 12 hours before they were applied.

WFT was reared on Pods of green beans (*Phaseolus vulgaris* L.) following the protocol developed by Ullman *et al.* (1997). The insect was reared in a climate chamber at a temperature of 23 ± 2°C, 50 – 60% relative humidity (RH) and 18:6 L: D photoperiod and only uniform aged insects were used in the experiments.

3.3.2 Population dynamics study of WFT

To determine population dynamics of WFT, i.e. to see what proportion of the total population will be found in the soil on the day of nematode application and to determine the relationship between soil and foliage dwelling life stages, experiment on population dynamics of WFT was conducted before our experiment on time and frequency of EPN application. For this experiment, bean plants *Phaseolus Vulgaris* L. were grown in plastic pots on commercially available growing substrate (Fruhstorfer Erde) and caged at two- leave stage with an acryl-cylinder (∅ 84 mm). Twenty days old ten female and two male adult WFT were introduced after caging. The seedlings were watered whenever necessary with equal amounts of water. Both the cage and shoot part of the plants were removed on the 10^{th}, 15^{th} and 20^{th} day after adult introduction and the pots were covered with a petri dish that served as "sticky trap". The removed shoot was washed with soap and water solution to collect all WFT on the plant and those stuck on the inside wall of the cage were collected using a fine camelhair brush. The soap solution containing WFT was first filtered with a sieve (30 μm ∅) and then using a

lined filter (185 mm ∅) that was kept on a funnel. Seventy percent ethyl alcohol was added on the filter paper to remove the foam of the soap, and to kill and preserve the thrips until they were counted under the binocular. WFT at each day of cage removal were counted separately into larvae, prepupae/pupae, and adults. Emerging adults from the soil were counted both from the sticky trap and surface of the substrate in the pot on daily basis starting from one day after cage removal until no further adult emergence from the soil. The population in the soil at the time of cage removal was considered as pupae /prepupae, which were inferred from the number of adults emerged from the soil. There were six pots for each time of cage removal that were completely randomised.

3.3.3 Time and frequency of EPN application

Seedlings of green beans (*Phaseolus vulgaris* L.) were raised in plastic pots with a diameter of 11 cm (at their base) and perforated base using a commercially available plant growing substrate (Fruhstorfer Erde) and caged at two- leave stage with an acryl-cylinder (∅ 84 mm). Twenty days old ten female and two male WFT adults (assumed as a threshold adult density per plant) were released into the caged seedlings. Adult WFT were counted after anaesthetization with CO_2 and collected with aspirator. Two EPN strains, *H. bacteriophora* Strain HK3 and *S. feltiae* strain Sylt, were applied to the pots at 200 and 400 IJs cm^{-2} once (10, 15, or 20) or twice (10 and 15, 10 and 20, or 15 and 20) days after adult WFT introduction. Plants were not watered within the last 3 days before nematode application to avoid over wetting of the substrate in the pot and 40 ml of water was used to rinse the nematodes after application. As a control treatment for a given application date per replication distilled water was applied instead of EPN. Each treatment was replicated six times. For all treatments, the seedlings were kept for 22 days after application of the EPNs. On this date both the shoot part of the plant and the cage were removed and thereafter the pots were

covered with "sticky trap" as described in chapter 2. Adult WFT on the shoot of the plant were collected as described in the population dynamics study and counted under binocular. Emerging adults were recorded both from the soil and sticky trap on daily basis until no further adult emergence. The treatments were replicated six times that were conducted over two times and factorial completely randomised design was used. Mean corrected mortality at different times of nematode application was compared to identify the appropriate time and frequency of application of EPNs.

3.3.4 Combined application of EPNs with different foraging strategies

Small plastic pots with a diameter of 5 cm at their base and 6.5 cm at the top were filled with 50 g of sieved non-sterilized commercially available plant growing substrate (Fruhstorfer Erde). Then the pots were irrigated with 40 ml of tap water (as determined in the preliminary experiment) from the top surface of the substrate. The pot were covered with a petri dish (100 mm ∅) that has a small ventilation hole (20 mm ∅) at its center that was covered with a nylon tissue (64 μm ∅ pore size) to prevent escaping of emerging adults but allowing passage of air. The inner part of the petri dish, except the holes, was painted with insect glue, which was used as "sticky trap". In order to have optimum soil moisture (about 70% moisture content) during nematode application, the pots were kept overnight in a climate chamber with a temperature of 23 ± 2°C and ca 70% RH where the experiment was conducted. Two EPN strains, *H. bacteriophora* strain HK3 and *S. carpocapsae* strain DD136 were applied according to the following treatment combinations (Table 1) and 5 ml of water was used for rinsing after nematode application.

Table 2. Treatments and concentrations used for the experiment on combined application of EPNs.

Treatment	Concentration (IJs cm^{-2})
1. HK3	400
2. DD136	400
3. HK3 + DD136	400
4. HK3	200
5. DD136	200
6. HK3 + DD136	200
7. Control (water)	–

The pots were kept for two additional days before thrips introduction in the same chamber to give enough time for the IJs to distribute in or on the soil surface according to their foraging strategy i.e. to give enough time for HK3 to enter into the soil. Twenty L2 (8 days old) were placed onto the surface of the substrate in the pot. Each treatment was replicated eight times that was conducted over two times, four replications during the first time and the rest during the second time. Adults emerged from the soil were counted both from the "sticky trap" and surface of the soil on daily basis until no further adult emergence.

3.3.5 Statistical analysis

Mortality values were corrected for natural mortality according to Abbott's formula (Abbott, 1925). Percent mortality data were transformed using the arcsine transformation. In the case of the population dynamic study non-transformed data were used. Effect of treatments was analysed using analysis of variance (ANOVA) in the general linear model (Proc GLM; SAS

Institute, 1999). Whenever two factors (e.g. strain and time) show significant interaction, means of the levels of one factor were compared at each levels of the other factor. In the absence of significant interaction, means of the level of one factor were compared regardless of the levels of another factor (Sokal and Rohlf, 1995). The corrected mortality means were compared to zero (corrected mortality of control) using Dunnett's two-sided test (SAS Institute, 1999). When ANOVA showed significant treatment effects, individual means were compared using the least significant difference (LSD) multiple range test procedure (SAS Institute, 1999). A significant level of $\alpha = 0.05$ was used in all the analyses.

3.4 Results

3.4.1 Population dynamics study

Significant difference was observed between the different sampling days after adult introduction in terms of percent population in the soil in relation to the total WFT population during each cage removal ($F_{2, 10} = 19.7$, $P = 0.0085$). Percent population in the soil at 15 and 20 days after adult WFT introduction was significantly higher than that of 10 days (Table 3).

Table 3. Mean percent WFT population in the soil at 10, 15 and 20 days after introduction of 10 female and 2 male WFT adults onto bean plants.

Days after adult introduction	*Mean Percent WFT in the soil (± SE)
10	11.7 ± 4.5b
15	40.5 ± 1.7a
20	54.3 ± 5.3a

Means within a column followed by the same letter are not significantly different at $\alpha = 0.05$.
* Number of WFT in the soil was inferred from the number of adults emerged from the soil in the treatments in which shoots parts of the plant was removed at 10, 15 and 20 days after adult introduction.

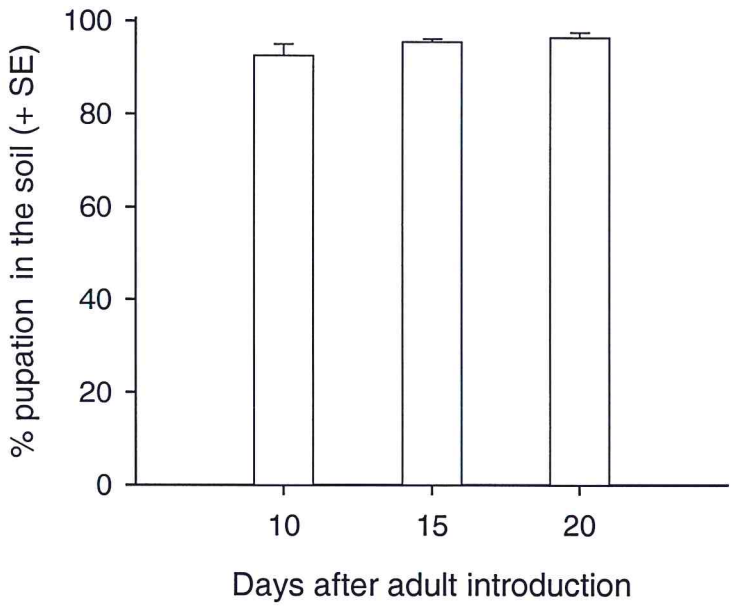

Figure 5. Mean percent (+ SE) WFT pupated in the soil on the 10th, 15th and 20th day after adult WFT introduction on the plant. The data were calculated from number of adults emerged from the soil (and considered as WFT pupated in the soil) and prepupae /pupae counted on the leaves on the respective days.

In this experiment the majority of WFT (92.6 – 96%) pupated in the soil and only few prepupae/pupae were recorded on the plant (Figure 5).

When we look at the composition of life stages of this pest considering all the population in the soil to be pupa/prepupae, until 15 days after initial release of 10 female and 2 male adults the majority of WFT was at larval stage which was significantly higher than at 20 days of the same stage. Generally the number of larvae showed a decreasing trend as we proceed from day 10 to 20 after adult introduction, but the reverse was true for number of pupae and it was significantly higher at day 20 than at 10 and 15 days after adult introduction. Moreover, 20 days after adult introduction, significant number of adults of the first generation emerged from the soil (Figure 6).

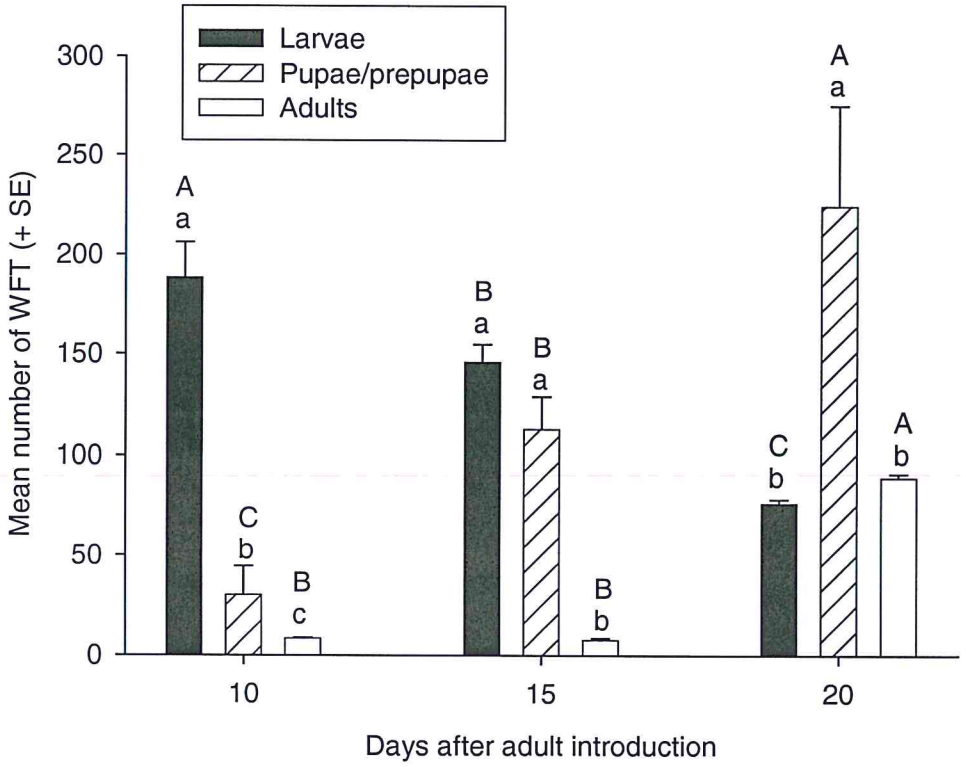

Figure 6. Mean number (+ SE) of larvae, pupae/prepupae and adults after 10, 15 and 20 days of 10 female and 2 male adult WFT introduction. Different developmental WFT stages on a given day and the same developmental stages across different days followed by different lower and upper cases, respectively are significantly different at $\alpha = 0.05$.

3.4.2 Time and frequency of EPN application

Number of adults emerged from the control treatments in the different days of nematode application did not significantly differ ($F_{5, 25} = 0.43$, $P = 0.8202$). All the nematode applied treatments resulted in a significantly ($P < 0.0001$) higher WFT mortality compared to the control treatment. Higher WFT mortality was recorded from twice applications of HK3 at 400 IJs cm^{-2} (10 and 20 days 51.5%, 10 and 15 days 50.8% and 15 and 20 days 50.6%) and at 200 IJs cm^{-2} (10 and 15 days 49.7%, and 15 and 20 days 50.1%). Both HK3 and Sylt applied at 20 days after adult introduction at a concentration of 200 IJs cm^{-2} resulted in lower thrips mortality, 17.8% and 20.7% respectively, compared to other nematode applied treatments.

Concentration, time and strain did not interact significantly ($F_{5, 110}$ = 2.26, P = 0.0537), likewise concentration and strain ($F_{, 110}$ = 0.04, P = 0.8357), and concentration and time ($F_{5, 110}$ = 0.77, P = 0.5712). Time and strain interacted significantly ($F_{5, 110}$ = 3.33, P = 0.0072), therefore, strains were compared at each time of nematode application.

In HK3, at 400 IJs cm^{-2} from the single applications, early application (ten days after adult introduction) resulted in higher WFT mortality than the rest. At this concentrations, no significant difference between all the repeated applications and they caused higher WFT mortality than the single applications except at ten days. At 200 IJs cm^{-2}, in the single applications significant difference was observed only between 15 and 20 days in which the earlier application caused higher WFT mortality. From the repeated applications, significantly higher WFT mortality was observed on the 10^{th} and 15^{th}, and 15^{th} and 20^{th} days than on 10^{th} and 20^{th} day. Both concentrations of HK3 applied only once on the 20^{th} day after adult introduction resulted in lower thrips mortality compared to most of other nematode applied treatments for this strain. In HK3 increasing EPN concentration resulted in significantly higher WFT mortality only when the nematode was applied once at 10, or twice at 10 and 20 days after adult introduction (Figure7a).

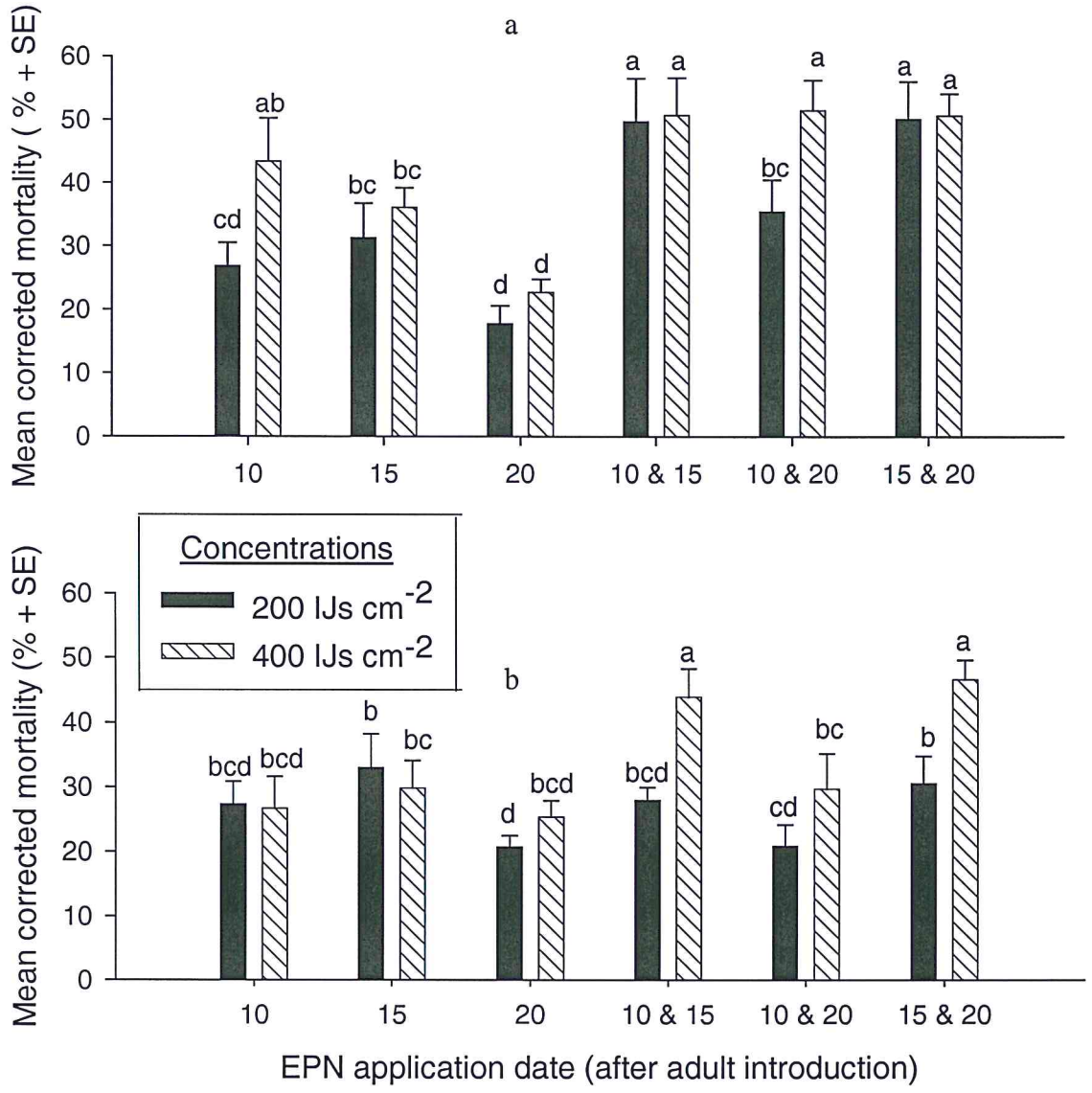

Figure 7. Mean corrected mortality (% + SE) of western flower thrips caused by *Heterorhabditis bacteriophora* HK3 (a) and *Steinernema feltiae* Sylt (b) at a concentration of 200 and 400 IJs cm^{-2} applied at 10, 15 or 20 (single application), 10 and 15, 10 and 20 or 15 and 20 (double application) days after adult WFT introduction. Means followed by the same letter do not differ significantly ($\alpha = 0.05$).

Repeated application of HK3 at a lower concentration on 10 and 15, and 15 and 20 days after adult introduction gave significantly higher WFT mortality than single applications of the higher concentration except at 10 days after adult introduction. Moreover, the repeated applications at 200 IJs cm^{-2} on 10 and 15, and 15 and 20 days after adult introduction gave similar level of WFT mortality as to all the repeat applications of 400 IJs cm^{-2}.

For Sylt, the two concentrations showed significant difference only at the repeated applications when IJs were applied 10 and 15, and 15 and 20 days after adult introduction in which higher WFT mortality was recorded from the higher concentration than the lower one (Figure 7b). At 200 IJs cm^{-2}, relatively higher mortality was obtained from 15 days (single application) and 15 and 20 days (double application). At 400 IJs cm^{-2}, the repeated applications, 10 and 15 days, and 15 and 20 days after adult introduction resulted in a higher WFT mortality than the rest of the treatments. At this concentration, WFT mortality at all single applications and the repeated application at 10 and 20 days did not differ significantly and were lower than other repeated applications. For this strain, repeated application at a lower concentration did not result in higher WFT mortality than a single application at a higher concentration. Results from the comparison of the two strains at same concentration at a given application date showed that at 200 IJs cm^{-2}, HK3 caused significantly higher WFT mortality than Sylt in all the repeated applications but not in the single applications (Figure 8a). At 400 IJs cm^{-2}, the two strains differed 10 days (one time application) and 10 and 20 days (repeated application) in which HK3 caused higher thrips mortality than Sylt (Figure 8b).

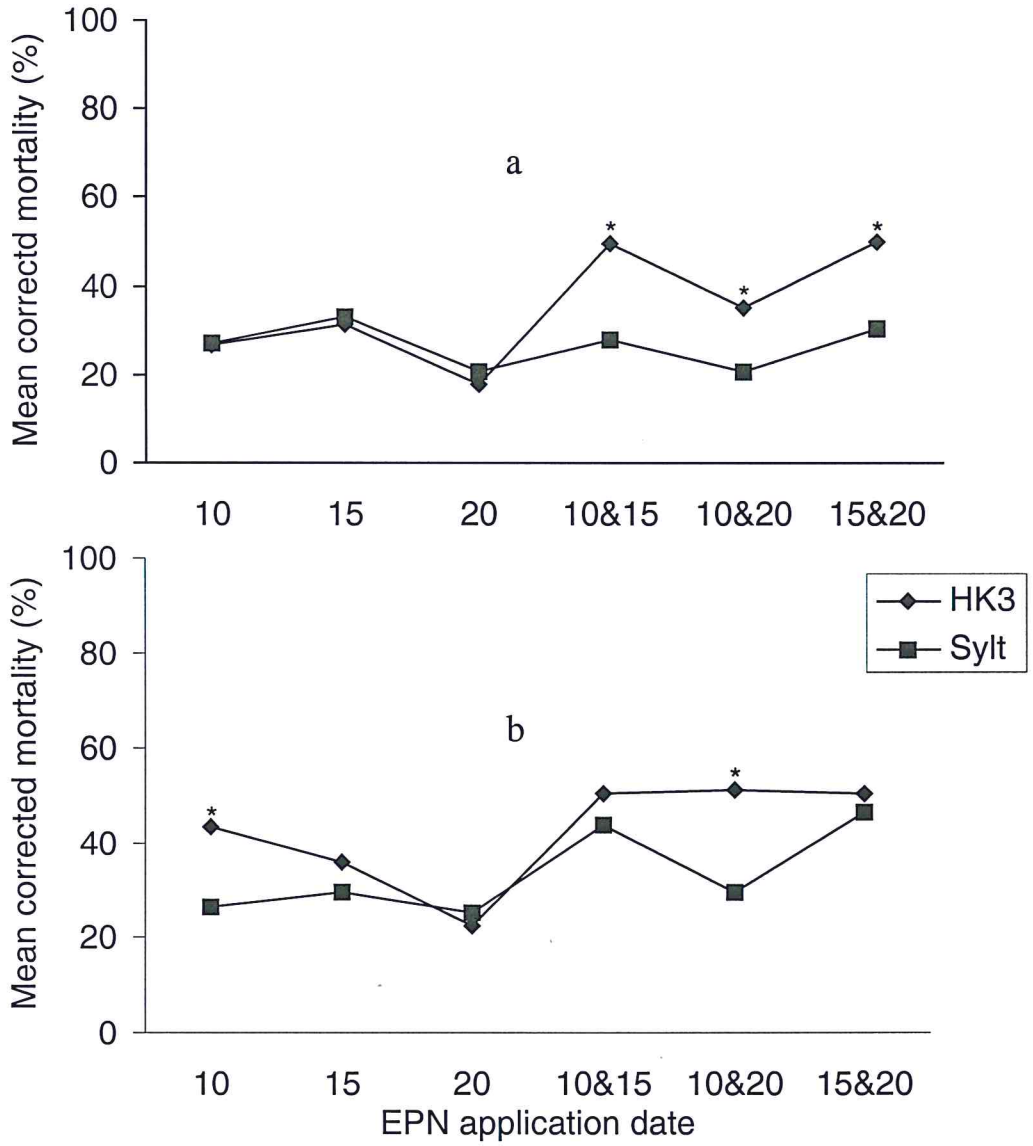

Figure 8. Mean corrected mortality (%) of western flower thrips caused by *Heterorhabditis bacteriophora* HK3 and *Steinernema feltiae* Sylt at 200 (a) and 400 (b) IJs cm^{-2} applied once (10, 15, and 20) or twice (10 and 15, 10 and 20 or 15 and 20) days after adult WFT introduction. *Indicates significant difference at $\alpha = 0.05$.

3.4.3Combined application of EPNs

All EPN treatments caused significantly higher WFT mortality compared to the control treatment, which was 12% ($P < 0.0001$). Moreover, significant differences were observed between EPN treatments in terms of WFT mortality ($F_{5, 32} = 8.77$, $P < 0.0001$). Application of HK3 both at 400 and 200 IJs cm^{-2}, and combined application of HK3 and DD136 at 400 IJs

cm^{-2} caused similar level of WFT mortality which was significantly higher than application of DD136 alone both at 400 and 200 IJs cm^{-2} (Figure 9). For DD136, there was a significant increase in WFT mortality with increasing nematode concentration from 200 to 400 IJs cm^{-2}. Combined application of HK3 and DD136 at a concentration of 200 IJs cm^{-2} gave similar levels of mortality as an application of DD136 alone at 400 IJs cm^{-2}, combined application at 400 IJs cm^{-2}, and HK3 alone at 200 IJs cm^{-2} but it was significantly higher than WFT

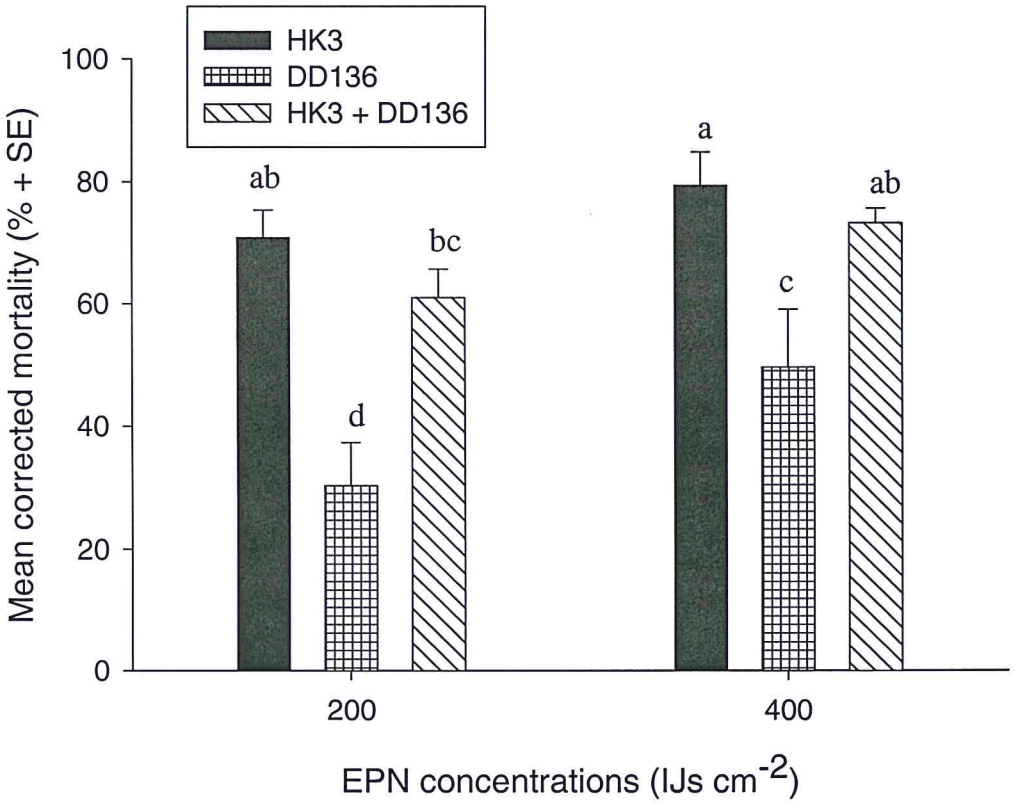

Figure 9. Mean corrected mortality (% + SE) of western flower thrips late second instar larvae caused by *Heterorhabditis bacteriophora* HK3 and *Steinernema carpocapsae* DD136 applied at 400 and 200 IJs cm^{-2} applied singly or in combination. Mortality values followed by the same letter are not significantly different at $\alpha = 0.05$.

mortality caused by DD136 alone at 200 IJs cm^{-2}. In this experiment, combined application of the two strains did not result in significantly higher WFT mortality than application of HK3 alone both at lower and higher concentrations.

3.5 Discussion

3.5.1Time and frequency of EPN application, and population dynamics of WFT

The absence of significant differences between the untreated controls for the different days of EPN application indicates that the different amounts of water applied to the control treatments during application of nematodes and other management practices (like watering) did not affect the emergence of WFT. Hence, the difference among the different EPN treatments in terms of WFT mortality could be attributed to the effect of the nematodes.

In our study an increasing concentration of HK3 from 200 to 400 IJs cm^{-2} caused higher WFT mortality only when it was applied once (10 days) or twice (10 and 20 days) after adult introduction. This could be due to the fact that 10 days after adult introduction the host density in the soil could be optimal to the higher concentration but high to the lower concentration so that large number of IJs at the higher concentration can encounter large number of hosts while they are searching for potential hosts (Campbell and Gaugler, 1997; Lewis *et al.*, 1993) and parasitise most of the hosts in the soil resulting in high WFT mortality compared to few number of IJs at 200 IJs cm^{-2}. Moreover, the difference between the two concentrations on the repeated application (10 and 20 days) may be from the effect of the first application which caused higher mortality and from our persistence study we have observed that IJs of this strain persisted for 9 days at 200 IJs cm^{-2} and at least for 12 days at 400 IJs cm^{-2}. From our population dynamics study of WFT, we have observed that between day 15 and 20 after adult introduction, significant number of adults emerged from the soil. This means that EPNs introduced with the second late application (in 10 days interval) have less chance to parasitised WFT that entered into the soil before 10 days.

In the case of Sylt, which is intermediate in its foraging strategy (Lewis, 2002), increasing concentrations resulted in higher WFT mortality only in the repeated applications, 10 and 15, and 15 and 20 days after adult introduction. For the single applications even 10 days after adult introduction, the host density could be high which may be beyond the capacity of the nematodes to cause high WFT mortality. Moreover, the second application after 20 days may contribute less to WFT mortality since the majority of WFT population is in the soil and others emerged as adults so that there is little chance to be parasitised with this nematode that has a partial wait and sit strategy. In other words, the time in which the nematodes and the thrips stay together will be shorter compared to HK3 as the larvae enter into the soil for pupation.

In our study we have observed that timing of EPN application has a profound effect on WFT mortality. In HK3 from one-time applications, early application at a higher concentration caused higher WFT mortality since the host density during this time could be optimal for this strain to locate and parasitise than later applications and also early applied IJs can persist at least for 10 days to cause continuous infection for the incoming larvae. Moreover, if the first application is delayed, WFT that are already in the soil may escape EPN application and emerge as adults. Timing of the second application seems more important in determining control success as the degree of control fluctuated when the timing of the second application varied. At 200 IJs cm^{-2}, repeated application within interval of 5 days, 10 and 15 or 15 and 20 days, resulted in a higher WFT mortality. But the control success declines as the interval for the second application increases (to 10 days) as large number of adults of the first generation emerge. Moreover, WFT of the first generation that entered into the soil earlier, 20 days after adult introduction could be at pupal stage, which is less susceptible to EPNs than the larval stage (Premachandra *et al.*, 2003b; Helyer *et al.*, 1995). From our EPNs self perpetuation

study we have also observed that Pupae of WFT on which HK3 was applied at a concentration of 400 IJs cm^{-2} on a petri dish and filter paper bioassay, were able to develop into adults. On the other hand Ebssa *et al* (2001b) reported that WFT mortality was higher when the proportion of pupae and prepupae in the population increased.

There is a benefit in splitting EPN concentration, if the second application is timed to coincide with the peak in numbers of susceptible stages of the host (Fenton *et al*, 2002). In our study repeated application of HK3 at 200 IJs cm^{-2} at 10 and 15, and 15 and 20 days after adult introduction resulted in a significantly higher WFT mortality than single application at 400 IJs cm^{-2}. Moreover, repeated applications on 10 and 15, and 15 and 20 days at the lower concentration gave the same level of mortality as the repeated applications of the higher concentration for the same time. Therefore, it could be possible to reduce the total concentration of nematodes to be applied for WFT control by half if the time of the second application coincides with the susceptible stages of WFT in the soil.

Concerning Sylt, splitted application at lower concentration does not result in higher WFT mortality than single application at a higher concentration. Therefore, for this strain repeated applications at higher concentration are necessary that coincides with the susceptible stages of the pest i.e. at 10 and 15, and 15 and 20 days after adult introduction as it can parasitise large number of larvae before they enter in to the soil. The need for higher concentration in this strain may be due to the low efficacy of the strain at low concentration against WFT compared to HK3. Our finding corroborate the findings of Ebssa *et al* (2003) in which WFT was found to be more susceptible to *Heterorhabditis* than to *Steinernema* spp./strains. The superiority of HK3 over Sylt was also observed in the repeated applications where WFT mortality was higher in HK3 than in Sylt.

3.5.2 Combined application of EPNs

Since EPNs differ in their foraging strategy in which some are ambushers, which have a sit and wait strategy and attach to a nearby passing host and are therefore most effective against highly mobile insects near the soil surface (Grewal *et al.,* 1994; Campbell and Gaugler, 1993), while others are cruisers that search constantly by moving through the soil profile and are more effective against sedentary hosts (Kaya and Gaugler, 1993), if they are applied in combination they may cooperate in attacking both mobile and sedentary stages of the pest. In this experiment our expectation was that WFT mortality could be higher from combined application of DD136, which has a wait and sit strategy and HK3 which is a cruise forager than application of the two strains alone at the same concentration. However, there was no significant difference in WFT mortality between combined application of the two strains and application of HK3 alone at the respective concentrations. Moreover, application of DD136 alone resulted in significantly lower thrips mortality than combined applications at the same concentration. This indicates that higher WFT mortality in combined application of the two strains is mainly caused by HK3 and contribution of DD136 is rather low. This finding is in agreement with previous reports in which HK3 is more effective against WFT than DD136 (Ebssa *et al.*, 2003, 2001a). In this experiment the host density was relatively high (0.6 L2 cm^{-2}) and increasing concentration of DD136 resulted in increased WFT mortality that corroborate previous findings of Ebssa *et al.* (2001a). In EPNs persistence study where host density was low (0.3 L2 cm^{-2}), for this strain higher thrips mortality was recorded from the lower concentration than the higher concentration which was in agreement with the findings of Cossentine *et al.* (2002) and Lewis *et al.* (1996).

From this study we can conclude that it is possible to reduce the total quantity of nematodes to be applied by half by adopting split application at lower concentrations, if the time of

application is carefully selected and coordinated with the population dynamics of the pest. Moreover, better control of WFT can be achieved through the use of Heterorhabditid nematodes like *H. bacteriophora* with cruise foraging strategy than Steinernematid nematodes like *S. carpocapsae* with wait and sit foraging strategy.

4 General discussion

The objective of the present studies was to assess conditions that could enhance the impact of EPNs against soil-dwelling life stages of WFT. Based on this idea, the post application persistence of two EPN strains was investigated under semi-field conditions at different intervals for about 12 days. Moreover. Time and frequency of EPN application with respect to population dynamics of WFT and ability of EPNs to self perpetuate in WFT was studied. In addition, we have examined the possibilities for combined application of EPNs with different foraging strategies. The results of these experiments revealed some important aspects to be considered to achieve better control of WFT using EPNs.

The results of the persistence study showed that, EPNs can persist at least for 12 days after their application in the soil at temperature of 23 ± 2°C and 60 – 65% RH without loosing their virulence against WFT. Premachandra *et al.* (2003b) also found that under laboratory condition EPNs can persist at least for 6 days without losing their virulence. The information gained from the present study can be used as a base for greenhouse and field experiments and also be helpful for growers to schedule their application to make economic use of EPNs against WFT. The self-perpetuation study makes clear that EPNs do not perpetuate in WFT. Since EPNs are obligate parasites (Boemare *et al.*, 1996) dauers require the presence of host insect to complete their development. However, WFT is small in size (less than 2 mm in length) and cannot provide sufficient food to support the development of dauers. Therefore, when we speak about persistence of EPNs against WFT, we are referring to dauer persistence rather than population persistence which could be possible through recycling of EPNs in the host. Nematodes with sit and wait strategy were shown to persist longer than those with cruise foraging strategy, which deplete their food reserve within short period of time due to their high mobility within the soil profile (Selvan *et al.*, 1993). Baur and Kaya (2003) also

mentioned that dauer persistence in the soil varies among nematode species and strains and those differences are related to physiological and behavioural adaptations. Curran (1993) reported that steinernematids persist longer than heterorhabditids. Moisture content of the soil is one of the most important factors for the survival and movement of EPNs (Gaugler, 1988). Movement of IJs in the soil is more important for "cruisers" than ambush foragers. Thus, from the continuous moisture supply in our experiment HK3 might have been benefited more than DD136 that could contribute to higher WFT mortality by HK3 in addition to the strain difference.

Persistence of EPNs added to the soil vary widely depending on experimental conditions. For instance, greater than 50% S. *carpocapsae* was shown to persist in vitro for 30 days in sandy soil (Shroeder and Beavers, 1987), whereas survival of the same species was less than 10% after 7 days in soils of similar texture in vitro and in the field (Duncan and McCoy, 1996).

Previous experiments on persistence of *S. carpocapsae* showed that it persisted as short as 8 days (Buhler and Gibb, 1994), as long as for 28 days (Wright *et al.*, 1993), and 3 months (Pye and Pye, 1995) after application. Other reports indicated that Heterorhabditid nematodes persisted for 10 months in Washington cranberry soils through recycling of the established nematode population (Shanks and Agudelosilva, 1990). Steinernematids and heterorhabditids can only survive for several hours on exposed surfaces (Glazer, 1992). Thus, the moderate moisture content and relative humidity in our experiment might have helped EPNs to persist longer than in the case of exposed surfaces.

Effect of tank mixing, exposure to UV radiation and dehydration after application, predation and infection by antagonists, depletion of energy reserves after application to the soil are

important factors that may affect post application persistence and mortality of nematodes applied to control soil born pests (Smits, 1996). Since our experiment was conducted in a controlled condition, it helped to minimize the negative impact of above factors and only the effect of food reserve was seen in longer persistency of DD136 than HK3.

In steinernematids and heterorhabditids the IJ is equipped with two layers of external membrane, the cuticle of the 3rd stage and of the 2nd moult, which is retained to provide additional protection (Campbell and Gaugler, 1991). EPNs use some other mechanisms like coiling and anhydrobiosis to resist extreme environmental conditions in the soil. In anhydrobiosis EPNs can loose up to 95 – 98% of their body water and they have no metabolism there by conserving energy (Barret, 1991) and by coiling they can reduce the surface area of their body exposed to the dry environment and thus to reduce the rate of water loss by evaporation (Patal *et al.* 1997). Irrigation during nematode application and continued moderate soil moisture is essential for nematode movement, persistence and virulence (Georgis and Gaugler, 1991). This idea supports our findings in which we have got longer persistence of EPNs against WFT through continuous supply of moderate moisture to the soil. Unrich and Lacey, (2001) showed that in the control of codling moth, *C. pomonella*, application of *S. carpocapsae* in the evening caused higher mortality than application in the morning when no supplemental wetting was used after treatments. Morning and evening applications caused equivalent larval mortality when a post wetting treatment was included.

In our population dynamics study of WFT, 10 days after adult introduction WFT have already entered in to the soil and prepupation occurred. Supporting our findings, Chyzik *et al.* (1996) reported that, when he introduced mated adults of WFT to a cotton plant at a temperature of 25 $\pm$ 2 °C, prepupation occurred 10 days after adult introduction. The majority of WFT

population of the first generation could be found in the soil between 15 and 20 days after establishment of adult WFT on the plant. However, 10 days after introduction of adult WFT onto the bean plant, about 11.7% of the total WFT population was in the soil suggesting that we have to apply soil acting biocontrol agents such as EPNs earlier than 10 days after detection of WFT adults on the plant. Ten days after releasing of 10 female and 2 male adult WFT per plant, on average, the total population was 226.6 individuals per plant (188.3 larvae, 30 pupae/prepupae and 8.3 adults) in the untreated control. Wang and Ship (2001) from their population dynamics study on WFT on cucumbers in greenhouse indicated that the population density of thrips increased from an initial density release of 5 adults per plant to a total of 350 larvae and 180 adult thrips per plant 49 days after release, and from the release of one adult per plant to a total of 102 larvae and 40 adult thrips. Temperature has a profound influence on thrips population dynamics. Within a range of 15 – 30 °C, higher temperature resulted in faster development, higher total production and higher daily rate of production (Van Rijn *et al.*, 1995). The information gained from this experiment could be useful to make effective use of biocontrol agents such as EPNs by coinciding susceptible stages of the pest and active stages of the biocontrol agents. The field efficacy of biological control agents depends upon coordinating application of the biocontrol agent with the susceptible host stages (Shapro-Ilan *et al.*, 2002). Our studies on time and frequency of EPN application make clear that timing of nematode application is important in determining control success.

This study showed that reducing the overall nematode concentration by half and applying in split has led to control level that was better than single applications at the larger concentration and as good as repeated applications of the higher concentration. Fenton *et al.* (2002) reported that in the control of sciarid flies (*Lycoriella* spp.) on mushroom (*Agaricus bisoprus*) using EPNs, reducing the total concentration and applying in split has resulted in a control success

that was at least as good as early single applications of the higher concentration. Williams and Macdonald (1995) also mentioned that in the control of leaf miner *Liriomyza bryoniae* using EPNs, applying a higher concentration (5000 IJs/ml) to first instar larvae did not significantly increase mortality compared to the repeated application at the lower concentration (1000 IJs/ml). This suggests that applying to the correct age in the life cycle is more important than applying nematode at a higher rate. Therefore, by selecting the appropriate time and adopting split application, it is possible to reduce the total EPN concentration by half and maintain high level of control while reducing costs. This could help to expand the marketing and use of EPNs, which is partially constrained by high costs of such agents compared to pesticides.

In general, percent mortality recorded in this experiment was lower, even at higher concentrations, compared to previous pot or petri dish experiments in which limited number of larvae or prepupae/pupae of WFT were introduced into the assay arena (Ebssa *et al*, 2001a, 2003; Premachandra *et al*, 2003b). Our results corroborate findings of Ebssa *et al.* (2003) in which increasing concentration at a very high host density did not result in higher mortality of WFT. This indicates that no linear relation exists between increases in EPN density and efficacy but that the relation is influenced by host density as well. Similar saturation like effects can be observed if density effects are studied with pathogen-host interactions in general. Helyer *et al.* (1995) also observed low mortality of WFT in compost irrespective of high concentration of EPN used.

Since EPNs differ in their host finding strategy, i.e., some are ambushers while others are cruisers, we made an attempt to see if there is a possibility of getting better control of WFT through combined application of cruise forager Heterorhabditid and ambusher Steinernematid nematodes. We introduced L2 of WFT onto the surface of the substrate in pots 2 days after

nematode application to give enough time for IJs of HK3 to enter in to the soil and during this time no IJs of HK3 were seen on the surface of the substrate under the binocular unlike DD136. This enabled us to mimic the natural condition in which the incoming larvae of WFT first encounter nictating IJs on the soil surface (e.g. DD136) and then "cruisers" (e.g. HK3) after they entered into the soil. However, the level of control obtained from the combined application was not better than application of cruise forager heterorhabditids alone both at higher and lower concentrations. The time in which the nematodes and thrips stay together may contribute for low efficacy of DD136 compared to HK3. The time to stay together is shorter for DD136 as it will encounter mostly only the late L2 for the short period before entering into the soil for pupation, while HK3 has the chance to parasitise all soil-dwelling developmental stages (larvae, prepupae and pupae).

Even though, the combined application of EPNs with different foraging strategy against WFT seems not attractive, it may be feasible to control inset pests that feed on the soil surface and pupate inside the soil (e.g. surface-feeding cutworms, *Agrotis spp.*) (Kaya and Koppenöfer, 1996). There are reports on intra- and interspecific competition among IJs of EPNs for insect hosts, which are mediated by the foraging strategies of the different nematode species. For instance, *S. carpocapsae* and *S. riobravis* are competitively superior for insect hosts near the soil surface (e.g. *Agrotis ipsilon*). *Steinernema glaseri* and *H. bacteriophora* are competitively superior for insect hosts that occur beneath the soil surface (e.g. white grubs such as *Popillia japonica* or *Cyclocephala hirta*) (Kaya and Koppenöfer, 1996). However, in our study even in the absence of interspscific competition, the performance of DD136 was significantly lower than that of HK3. Ebssa *et al.* (2003) also observed that Heterorhabditid nematodes were more effective than Steinernematids against soil-dwelling life stages of WFT. This suggests

that future research activities to improve efficacy of EPNs against WFT should focus on Heterorhabditids than Steinernematids.

To summarize, we have observed that EPNs can persist at least for 12 days under semi-field conditions without loosing their virulence and "ambushers" persisted longer than "cruisers". EPNs do not self-perpetuate in WFT. It is possible to reduce the overall nematode concentration needed by adopting splitted application at lower concentration if time of application is coordinated with population dynamics of the pest. In our study time and frequency of EPN application was determined based on population dynamics of WFT after introduction of adults on the host plant. However, we introduced a distinct number of nearly same aged adults to establish the population. Under field or greenhouse conditions much more heterogeneous immigration and population structures may occur. Therefore, it may be necessary to investigate the correlation between WFT stages on the plant, especially the adult stage which may be monitored using sticky traps in the crop canopy and soil dwelling life stages under natural infestation in a greenhouse condition more in detail. Even though the combined application of EPNs with "cruise" and "ambush" foraging strategies was not better than applying "cruisers" alone against WFT, which feed on the plant and pupate in the soil, this approach could be important against insect pests that feed near the soil surface and pupate in the soil.

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Declaration

I, Difabachew Belay Kondidie, hereby declare that the research work in this thesis is my own original work and has not been submitted for a degree in any other university. I have only used materials cited in the thesis.

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