UNIVERSITY OF HANOVER

DEPARTMENT OF HORTICULTURE

INSTITUTE OF PLANT DISEASES AND PLANT PROTECTION

Efficacy of entomopathogenic nematodes (EPNs) for the management of western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) with particular reference to persistence and potential for combining EPNs with the predatory mite *Hypoaspis aculeifer* Canestrini (Acarina: Laelapidae) in soil

Thesis submitted in partial fulfilment of the requirement for the award of degree of Master of Science in Horticulture (Phytopathology and Entomology)

W.T.S. DAMMINI PREMACHANDRA

Supervisors:

Prof. Hans-Michael POEHLING

PD Dr. Christian BORGEMEISTER

Prof. Manfred SCHENK

September 2001

Declaration

I, W.T.S. Dammini Premachandra hereby do declare, that the research work presented in this thesis is my own and has not been submitted for a degree in any other University.

Dammini Premachandra

Dammini Premachandra

Abstract

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabtiditae have been considered as promising biological control agents against various soil-dwelling insect pests. Laboratory and semi-field experiments recently conducted in the Institute of Plant Diseases and Plant Protection (IPP), University of Hanover, showed the potential of EPNs for control of soil-dwelling life stages of western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), one of the most destructive pests associated with economically important crops in the field and greenhouses. In the present study, efficacy of six selected EPN strains, i.e. *Heterorhabditis bacteriophora* Poinar (Hdo1 strain [HBHDO1]), *H. bacteriophora* hybrid (the commercial formulation Nematop® [HBN]), *Steinernema arenarium* (Artyukhovsky) (Anomali strain [SAA]), *S. carpocapsae* (Weiser) (Agriotos strain [SCA]), *S. feltiae* (Filipjev) hybrid (the commercial formulation Nemapuls® [SFN]) and *Steinernema* spp. Travassos (Marocco strain [SM]) was evaluated against prepupae and pupae (pupal stages) of WFT in Petri dish bioassay. In these bioassays late second instar larvae (L2), i.e. nine days after hatching, were introduced on to the surface of the soil to promote penetration into the soil and subsequently the further development into prepupae and pupae. This methodological set-up enabled us to closer mimic field conditions. Among the six EPN strains assessed, SFN and HBHDO1 resulted in 65% and 59% mortality, respectively. SCA and SAA showed intermediate efficacy (40-45% mortality), whereas applications of SM and HBN had little effect on WFT pupal stages. Susceptibility of late L2 to three EPN strains, i.e. HBHDO1, SFN and *H. bacteriophora* (Hk3 strain [HBHK3]), was evaluated using the same bioassay protocol. We observed that late L2 stage was more susceptible to EPNs than pupal stages. In addition, we conducted a dose

response study to identify the minimum dose rate of EPNs causing highest mortality in WFT. In these experiments concentrations of 100, 400 and 800 infective juveniles (IJs) per cm^2 soil of HBHK3, HBHDO1 and SFN were compared. Results indicated that a dose rate of 400 IJs/cm^2 was the most effective concentrations against pupal stages of WFT with 94%, 84% and 58% for HBHK3, HBHDO1 and SFN, respectively. Further dose rate increase did not yield any significantly higher mortality of pupal stages. Post application short-term persistence of HBHK3 and SFN was assessed three and six days after the initial inoculation against late L2. Results showed that infection rates of WFT did not differ substantially between the two post application periods. This implies that under laboratory conditions the two strains can persist at least up to six days after inoculation in the soil without loosing virulence (expressed in terms of mortality).

The influence of the combined releases of HBHK3 and SFN and *Hypoaspis aculeifer* Canestrini (Acarina: Laelapidae) on a mixture of soil-dwelling life stages of WFT was investigated under semi-field conditions, i.e. in pots, using beans as the model plant species. In comparison to the untreated control HBHK3 and SFN reduced WFT adult emergence by 46% and 61%, respectively. The reduction of the WFT adult emergence by *H. aculeifer* alone was 46%. The combined releases of EPNs and *H. aculeifer* impaired adult emergence in WFT by 71% and 82% for combinations of SFN and HBHK3 with *H. aculeifer*, respectively. The combined application of SFN and *H. aculeifer* significantly reduced the number of emerged adult WFT compared to individual releases of SFN and *H. aculeifer* only. The combined releases of *H. aculeifer* and HBHK3 significantly reduced the number of emerged adult WFT compared to all individual treatments but not the combination of SFN and *H. aculeifer*. No antagonistic reactions between EPNs and *H. aculeifer* were observed in this investigation, and, hence, no sign of intra guild predation was apparent between these two groups of natural enemies.

Acknowledgements

At first, I extend my deep thanks to Prof. Dr. Hans-Michael Poehling for his guidance and advice. I am most indebted to PD Dr. Christian Borgemeister for his continuous advice and assistance extended to me throughout this research work and also for correcting the thesis.

I am highly grateful to Dr. R.-U. Ehlers, Christian-Albrechts–University Kiel, Germany, for kindly providing me all the entomopathogenic nematode strains used in this research project.

I extend my sincere thanks to the colleagues: Oliver Berndt, for providing me the specimens of *Hypoaspis aculeifer* for the experiments and for his advice and technical assistance throughout the whole project, especially relating to rearing of western flower thrips and to Lemma Ebssa for his valuable advice and support regarding this research work.

I take this opportunity to express my deepest gratitude to my husband, Chandima Fonseka for his immense help extended to me especially through taking care of our son Dilina Fonseka.

I also like to thank most sincerely the German Academic Exchange Service (DAAD) who sponsored me during this study.

Table of Contents

List of Figures

List of Tables

Abbreviations used in the text

Abbreviation	Meaning of the abbreviation
~	Approximately
ANOVA	Analysis of variance
df	Degree of freedom
DJ(s)	Dauer juvenile(s)
EPN(s)	Entomopathogenic nematodes
F	Statistical F-value
F1	First generation
F2	Second generation
H	Sole release of *Hypoaspis aculeifer* in the pot experiment
HBHDO1	*Heterorhabditis bacteriophora* Hdo1 strain
HBHK3	*Heterorhabditis bacteriophora* Hk3 strain
HBN	*Heterorhabditis bacteriophora* hybrid (commercial product Nematop®)
H-HBHK3	Combined releases of HBHK3 and *H. aculeifer* in the pot experiment
H-SFN	Combined releases of SFN and *H. aculeifer* in the pot experiment
IGP	Intra guild predation
IJ(s)	Infective juvenile(s)
IPM	Integrated pest management
IPP	Institute of Plant Diseases and Plant Protection
L: D	Light: dark (in relation to photoperiod)

L2	First instar larvae of WFT
LSD	Least significant difference
MC	Moisture content
Ø	Diameter
P	Statistical probability value
RH	Relative humidity
SAA	*Steinernema arenarium* Anomali strain
SAS	Statistical analysis system
SCA	*Steinernema carpocapsae* Agriotos strain
SCD	*Steinernema carpocapsae* Dd136 strain
SDW	Sterile distilled water
SE	Standard error
SFN	*Steinernema feltiae* hybrid (commercial product Nemaplus®)
SFS	*Steinernema feltiae* Sylt strain
SM	*Steinernema* spp. Marocco strain
TSWV	Tomato spotted wilt virus
w/w	Weight by weight
WFT	*Frankliniella occidentalis*

1 General introduction

1.1 Western flower thrips (WFT)

Thrips belong to the insect order Thysanoptera (thusanos, a fringe; pteron, a wing). 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 (family Thripidae) in which most of the economically important thrips species are found (Brødsgaard, 1989; Tommasini and Maini, 1995).

1.1.1 Origin of WFT

WFT has only recently, i.e. in 1980ies, become a worldwide pest (Jensen, 2000). The first record of it dates back to 1895 in California (Brødsgaard, 1989). In Europe, it was first found in 1983 in *Sainpaulia* nurseries in the Netherlands. By the end of the 1980ies it had been found in most European countries. The serendipitous introduction of WFT to Europe in 1983 was most likely caused through importation of infested plant material from California (Tommasini and Maini, 1995). The rapid spread of WFT is mainly facilitated due to international trade in planting material (Brødsgaard, 1993). In addition, greenhouses provide favourable conditions for WFT even in regions with colder climates.

1.1.2 Biology of WFT

The life cycle of WFT includes an egg stage, two larval instars, a prepupal and pupal stage and the adult. Adult WFT are about 1 mm long, possess two pairs of hairy wings and mainly inhabit flower heads. The colour of the females can vary from pale yellow to dark brown or black. The males are often yellowish, slightly smaller than the females and have a narrow abdomen (Tommasini and Maini, 1995). Adult females lay eggs inside the

young leaves, buds or fruits. Eggs are about 0.2 mm long, kidney-shaped, opaque and are produced either sexually or parthenogenetically (Brødsgaard, 1989). Fertilised eggs develop into females and unfertilised eggs develop into males, i.e. arrhenotoky. The female thrips are diploid and the male thrips are haploid (Moritz, 1997). Oviposition of females normally starts 72 hours after emergence and continues intermittently throughout the adult life. Female fecundity can range 150-300 eggs.

Eggs hatch into larvae, which usually remain protected in flower buds or terminal foliage. The insect passes through two larval stages, both of which feed in the protected areas until they are ready to pupate. The 1st larval instar is small and glassy white in colour while the 2nd larval instar is waxy yellowish in colour. The 2nd larval instar is more active than the 1st (Tommasini and Maini, 1995). The young instars have nearly the same appearance as the adults though they are wingless and the eyes are reddish and the antenna have fewer segments. Towards the end of the 2nd larval stage, the insects stop feeding and either move down the plant into the soil (at a depth of 1.5-2.0 cm) and/or the leaf litter to pupate, or stay in the flower petals or the leaf axils where they subsequently pupate (Moritz, 1997; Jensen, 2000). The prepupae and pupae usually do not feed and move only when being disturbed. The pupal stages resemble the larvae in shape and colour but possess short (prepupa) to long (pupa) wing pads. Antennae of pupa are long and faced backwards. Pupae acquire adult characteristics at the end of the developmental stage.

The duration of development from egg to adult depends on the environmental conditions, especially on temperature. At moderate temperatures, i.e. 20-25°C, it usually takes about 2-3 weeks to develop from egg to adult. At 15°C the development from egg to adults may takes more than a month, but at 30°C and above it may take less than 10 days (Tommasini

and Maini, 1995). The fecundity of WFT may also depend on temperatures but it is probably more affected by the host plant and especially the availability of pollen as a high quality food source (Brødsgaard, 1989). The optimal development conditions are >20°C and >80% relative humidity (RH). Longevity of adult female is about 40 days under laboratory conditions but they can survive as long as 90 days. Males can survive only half as long as females.

Under greenhouse conditions six or seven generations per year may occur and highest population densities are present in the hottest months of the year. However, in temperate regions, WFT can diapause during the winter months within a temperature range 5-6°C in the field, e.g. on the wild host plants (Kirk, 1997a).

1.1.3 WFT as a crop pest

WFT is a polyphagous insect and thus has a wide range of host plants. Two hundred and forty–four plant species belonging to 62 different families have been registered as host plants for WFT in the US (Jensen, 2000). Host plants include open-field ornamental, fruit, garden and agricultural crops (Tommasini and Maini, 1995). Due to the high value of the crops, thrips damage on ornamentals is more important than on vegetables. A density of eight thrips per 200 cm^2 leaf area is sufficient to reach the economic damage level on cucumbers (Rosenheim *et al.*, 1990).

The main factors that are responsible for the pest status of WFT are their small size, the ability of females to lay large number of eggs which can lead to rapid population increases, their broad host range, resistant to insecticides, cryptic feeding behaviour, transmission of viral diseases and the ability to be carried over long distances by wind.

Nature of the damage caused by WFT

Larval instars and adults which feed on leaves, buds and fruits cause direct physical damage to the plants through feeding punctures. The feeding behaviour of this insect is characterised by rasping, puncturing and sucking. The feeding punctures result in necrosis of the leaves and subsequent reduction of the photosynthetic capacity of the damaged tissue. Damaged leaves may also become papery, distorted and drop leaves prematurely. In addition, feeding by adult or larval instars causes tiny scars on leaves or fruits, called 'stipping' and can stunt growth. Black varnish-like specks of excrements are distinct features of thrips activities. Dead spots or blotches on flowers and malformations of fruits can also be caused by feeding of thrips. When WFT populations are very high, flower buds may become deformed and fail to open (Tommasini and Maini, 1995).

Physical damage is also caused by oviposition of female WFT (Tommasini and Maini, 1995). They oviposit in plant leaf tissues and around the oviposition sites a 'haloing' occurs when the oviposition site is surrounded by whitish tissue. 'Haloing' is more common in tomatoes than in cucumbers (Salguero Navas *et al.*, 1991).

Besides the direct damage, WFT may also cause severe indirect damage through transmission of viral diseases. The most serious indirectly induced damage of WFT is transmitting plant viruses of the genus *Tospovirus* i.e. Tomato spotted wilt virus (TSWS) (Allen and Broadbent, 1986; Tommasini and Maini, 1995). It is the only virus species that is transmitted by thrips in a persistent manner (Cho *et al.*, 1989). Larval and adult WFT thrips feed on epidermal and sub-epidermal mesophyll cells and can thereby acquire TSWS from infected plants. Transmission occurs via the salivary fluid. Adults may also acquire the virus, but fail to transmit it due to the long latent period. The virus is retained by the infected thrips throughout their lifetime. However, it is not transmitted to

subsequent generations of the thrips through egg production (Drees and Cole, 1988). Symptoms of TSMV are variable. Infections on the same host species vary according to the plant age, plant nutrition and environmental conditions such as temperatures (Yudin *et al.*, 1986).

1.1.4 Management strategies of WFT

Chemical control

WFT is difficult to control with insecticides. It shows a secluded behaviour in all the life cycle stages. Eggs are inserted into the plant tissue, the larvae feed in tight, protected areas such as flower buds or foliage terminals, the pupal stages are passed in the soil or leaf litter, and the adult thrips also feed in protected areas. Larvae and the adult show thigmokinetic behaviour, occupying narrow crevices within or between plant parts. This behaviour makes infestations of WFT difficult to restrain with insecticides (Jensen, 2000).

WFT may eventually come into contact with the insecticide when using the right spray equipment and spray application intervals. However, chemical control may not be effective because of presence or development of insecticide resistance in the thrips populations. Resistance to the three major classes of insecticides, i.e. organophosphates, carbamates and synthetic pyrethroids, has been reported in different populations of WFT (Immaraju *et al.*, 1992). Particularly the short generation time coupled with the high fecundity as well as the frequent application of insecticides favour the development of resistant strains in WFT.

Biological control

In commercial biological control programs of WFT, predatory mites of the *Amblyseius* genus (Acarina: Phytoseiidae) which attack immature larval instars, and anthocorid bugs *Orius* spp. (Heteroptera: Anthocoridae), which attack both larval and adult thrips, are most commonly used (Chambers *et al.*, 1993; Chyzik *et al.*, 1996; Williams, 2001).

In *Amblyseius spp.* the adults and two nymphal stages feed on immature stages of thrips. *Amblyseius barkeri* Hughes and *A. cucumeris* Oudemans have been used extensively in greenhouses in Europe to control WFT on sweet peppers and cucumbers (Jacobson, 1997). Recently, there has been considerable interest in applying these predators to ornamental crops (Wardlow *et al.*, 1992; Murphy and Broadbent, 1996).

Anthocorids have many characteristics of ideal biological control agents, i.e. high searching efficiency, an ability to increase more rapidly when prey is abundant, a density-dependent decrease in fecundity resulting from interference, and the ability to aggregate in regions of high prey density. Adult *Orius* spp. consume all thrips stages, while nymph *Orius* spp. feed only on thrips larvae. *Orius insidiosus* Say, *O. tristocolor* (White), *O. insidiosus* Say, *O. laevigatus* (Fieber), *O. albidipennis* (Reuter) and *O. majusculus* (Reuter) are effectively used against WFT (Chambers *et al.*, 1993). Anthocorids in general, and *Orius* spp. in particular, are not only polyphagously preying on different arthropods, but their younger nymphal instars are also omnivorous, feeding on plant juices and pollen (Kiman and Yeargan, 1985).

Efforts have also been made to use soil-inhabiting predatory mites of the *Hypoaspis* genus (Acarina: Laelaptidae) to combat WFT. *Hypoaspis* spp. feed on springtail larvae, nematodes, fly larvae as well as on thrips pupae. However, sole releases of *Hypoaspis*

spp. do not provide full control of WFT (Anonymous, 2000). *Hypoaspis aculeifer* Canestrini and *H. miles* (Berlese) are the most widely investigated biocontrol agents in the *Hypoaspis* genus. Both nymphs and adults feed on soil-inhabiting arthropods, consuming up to 5 prey items per day. They can survive on algae and/or plant debris when arthropod prey is not available. Both females and males are present, but males are smaller than females and rarely seen. They inhabit the top 1.3 cm layer of the soil.

The parasitic wasp *Ceranisus americensis* Girault (Hymenoptera: Eulophidae) has also shown some success in controlling WFT (Drees and Cole, 1988). Recently, attempts have been made to control WFT in protected crops by using the entomopathogenic fungi *Verticillium lecanii* (Zimmerman) Viegas (Deuteromycotina: Moniliales) and *Metarhizium anisopliae* (Metachnikoff) Sorokin (Deuteromycotina: Hyphomycetes) (Vestergaard *et al.*, 1995).

Studies conducted by Tomalak (1994) and Ebssa *et al.* (2001a and b) have indicated the potential of entomopathogenic nematodes (EPNs) for WFT control (refer to the following chapter for more details on EPNs).

Other control measures

Breeding for resistance could help to reduce the population growth of WFT and other thrips species below damaging thresholds. Thrips resistant cucumber and chrysanthemum varieties have been developed and their negative impact on the reproductive fitness of WFT population could be demonstrated (Mollema *et al.*, 1995; Soria and Mollema, 1995).

Moreover, cultural methods like control of weeds to avoid virus reservoirs and thrips harbourage areas, avoidance of continuous cropping systems, elimination of thrips

susceptible plant species in the vicinity of the crops etc. can be used in integrated pest management (IPM) strategies for WFT control (Drees and Cole, 1988). Soaps and organic products, such as extracts of the neem have also been reported to control WFT (Lindquist and Casey, 1990).

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. IPM strategies for control of thrips in the field, tree and greenhouses are urgently needed. Management options to control WFT infestations in greenhouses include cultural control (such as disposal of plant residues, elimination of weeds and 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, 2000).

1.2 Entomopathogenic nematodes (EPNs)

EPNs 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). The two genera *Steinernema* Travassos (previously known as *Neoaplectana*) (Steinernematidae) and *Heterorhabditis* Poinar (Heterorhabditidae) contain the most important EPN species.

1.2.1 Distribution and biodiversity of EPNs

EPNs are widespread and have been isolated in almost all inhabited continents and on many islands, but not in Antarctica (Poinar, 1990). EPNs have a ubiquitous distribution

that includes all major climatic zones and they occur in all main natural and modified terrestrial ecosystems, i.e. forests and agricultural fields, meadows, but have not been recorded from water. However, populations appear to be extremely patchy within and among sites and may be prone to frequent extinction and colonisation events (Hominick and Reid, 1990; Stuart and Gaugler, 1994).

Many species of *Steinernema* and comparatively few of *Heterorhabditis* spp. have been identified so far. Two species of Steinernematidae, namely *S. feltiae* Filipjev and *S. carpocapsae* (Weiser) appear to have a global distribution, while others like *S. affine* (Bovien) and *S. riobrave* Cabanillas, Poinar and Raulston, appear to be more restricted in distribution and recorded at continental or national levels (Hominick *et al.*, 1999).

One widely distributed *Heterorhabditis* species is *H. indicus* Poinar, Karunakar and Kaya, which appears to be found throughout the tropics. *Heterorhabditis bacteriophora* Poinar has been isolated from all continents, although so far not recorded from India and South-East Asia. *H. megidis* Poinar, Jackson and Klein, have showed wide distribution in temperate regions (Hominick *et al.*, 1999).

Much of the world remains to be surveyed for EPNs and understanding the biogeography is in its infancy (Hominick *et al.*, 1996). It is expected that natural populations of nematodes are adapted to their particular local environments. EPNs can also exist as strains, and these strains can differ in their biology and ecology (Hominick and Reid, 1990).

1.2.2 Life cycle and biology of EPNs

The life cycle of EPNs includes the egg, four juvenile stages and the adult. The third-stage juvenile is the infective stage, which is called infective juvenile (IJ) or dauer larva.

The IJs are non-feeding and the only free-living (outside the host) form of EPNs. IJs are physiologically and morphologically adapted for long-term survival in the soil environment (Glazer, 1996). IJs consist of two layers of external membrane. The cuticle of the second moult remains attached to provide additional protection, i.e. which protects the IJs from desiccation (Timper and Kaya, 1989; Campbell and Gaugler, 1991). Their natural openings, mouth and anus are closed (Mracek *et al.*, 1981; Endo and Nickle, 1994) and thus protected from penetration of microbial antagonists and from toxic chemicals. Furthermore, IJs contain carbohydrate energy reserves and are thus under favourable conditions able to survive for long periods in the soil until a susceptible host is encountered (Woodring and Kaya, 1988).

Another superficial characteristic of IJs includes that part of the intestine is modified as a bacterial chamber in which they carry between 0 and 250 cells of symbiotic bacteria belonging to *Xenorhabdus* spp. and *Photorhabudus* spp. (Enterobacteriaceae) in Steinernematidae and Heterorhabditidae, respectively (Boemare *et al.*, 1996; Spiridonov *et al.*, 1991).

An EPN infection consists of a sequences of events initiated by host location, host attachment, host penetration by IJs, bacterial release, bacterial proliferation and death of the host.

Host location strategies and associated behaviour

In order to optimise the survival in the soil, the IJs combine minimal movement with maximal host encounter success. EPNs employ a targeted host finding strategy rather than random movement. EPNs orientate themselves in two ways towards a suitable host.

Some species like *S. carpocapsae* and *S. scapterisci* Nguyen and Smart use an 'ambush strategy' to parasitize 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 for passing insect prey and are therefore most effective against highly mobile insects near the soil surface, whereas cruisers search constantly and are typically more effective at finding relatively sedentary prey species within the soil profile (Kaya and Gaugler, 1993). *Steinernema* spp. accomplishes the ambush strategy through nictation behaviour, which enables nematodes to attach themselves to passing insects. In nictation the IJs stand on their tail elevating more than 75% of their body (Campbell and Gaugler, 1993). This tactic is believed to reduce the surface tension forces, which allows the nematode to contact with mobile insects easily. When a suitable host comes in close proximity to an IJ the nematode will use chemical cues to locate the host. CO_2 has been shown to elicit host-seeking behaviour in EPNs (Gaugler *et al.*, 1980). CO_2 is a non-specific, non-contact, volatile metabolite that is produced by many organisms, including plant roots and soil microbes, as well as by insect hosts. IJs found aggregating around and invading host spiracles suggest that these nematodes might locate their hosts by CO_2 attraction (Triggiani and Poinar, 1976). IJs have been demonstrated to move toward the roots of germinating tomato seedlings (Kanagy and Kaya, 1996), ball cabbage and radish plants (Lei *et al.*, 1992). However, roots of certain plant species release substances that are repulsive to EPNs (Kanagy and Kaya, 1996). There are reports that EPNs move toward or away from host excretory products (Schmidt and All, 1979; Kondo and Ishibashi, 1986). Ambushers respond poorly to distal volatile cues released by hosts. By contrast cruise foragers are highly responsive to long-range host volatiles (Gaugler *et al.*, 1997).

Passive movement of EPNs can also occur as internal infection or as external phoresis. Many adult insect hosts are capable of flying after infection at least for one or two days post inoculation and in some cases even for longer. For instance adult mole crickets live for ten days or longer after infection with *S. sacpterisci* and can fly flowing infection (Parkman *et al.*, 1993). This type of passive movement usually operates over relatively short distances, however wind-transported insects are capable of travelling 2,000 miles (Downes and Griffin, 1996).

Host penetration and further development

When a host has been located, the nematode penetrates into the insect body. Usually, *Steinernema* spp. enter the insect through natural body openings like mouth, spiracles and anus, while *Heterorahbditis* spp. can additionally enter the host by piercing the body wall. Penetration process is supported by proteolytic factors produced by IJs (Poinar, 1979).

After penetration the IJs reach the haemocoel where they release the symbiotic bacteria, which subsequently excrete both a toxin and an inhibitor to block the insects humoral immune system (Burman, 1982). Moreover, the bacteria start to multiply rapidly and then destroy the host's haemocytes (Dunphy and Webster, 1984). Bacteria produce antibiotics, capable of inhibiting the growth of other bacteria and fungi and proteases, which could digest the insect tissue (Nealson *et al.*, 1990). Up to two days after infection the host death occurs. The nematodes then feed on the developing bacteria and the decaying host tissues (Kaya and Gaugler, 1993).

IJs of *Steinernema* spp. develop to amphimictic adults. Their offspring either develop to IJs or to an F1 adult generation. Another adult generation (F2) does not usually develop; instead, in response to depleting food resources, IJs are formed. Two to three weeks after

colonisation of the host insect the IJs leave the cadaver searching for new host in the soil (Ehlers, 1996). The development of *Heterorhabditis* spp. is different from that of *Steinernema* spp. The IJs develop to self-fertilising hermaphrodites. Their offspring can develop either to amphimictic females and males or to IJs. The decision of producing these stages is made early after the first juvenile stage hatches from the egg. High nematode population density and low concentrations of food induce the development to the IJs, whereas low nematode density and low concentrations of food induce the development to amphimictic adults. If the IJs-inducing low nutritional conditions continue to decline, then IJs further develop into IJs. If the conditions are stable, then the IJs develop to hermaphrodites. Hence, the development of either amphimictic or automatic adults is influenced by nutritional conditions during the first juvenile stage (Strauch *et al.*, 1994). Hundreds of thousands of new IJs emerge following a single infection.

1.2.3 Persistence of EPNs

Persistence is an important component for a potential control agent. Long-term persistence (normally several years) of EPNs depends on their ability to find and infect hosts and to produce offspring (self-sustaining). However, EPNs do not reproduce in small insects, including WFT, or insect stages because of their small size relative to the EPNs and thus not self-sustaining (English-Loeb *et al.*, 1999)

For the purpose of biological control of insects IJs are applied in high numbers. These high populations are often non-indigenous isolates. Not all IJs that are applied into the soil will immediately have the chance to enter a host. Hence, the remaining portion will continue to search. The question is now for how long, and whether after a given time they will be still able to successfully parasitise a host. Therefore, the short-term persistence

(several weeks or months) of IJs is important relating to the control of insect pests but also for their possible effects on non-target organisms.

Environmental conditions limit the survival, reproduction and growth of EPNs. IJs applied as a commercial product or emerged from infected cadavers are sensitive to many biotic (antibiosis, competition and natural enemies) and abiotic factors (temperature, soil texture, soil moisture and UV light) that affect their infectivity and persistence (Georgis and Gaugler, 1991; Kaya and Koppenhöfer, 1996). They have developed a wide range of mechanisms to overcome these unfavourable conditions. IJs do not feed but can survive for weeks on stored reserves and for months by entering a near-anhydrobiotic state (Smart, 1995), the latter being the most important survival strategy for EPNs.

Concerning the commercial products, the production technology can affect the persistence of EPNs applied to a site. A nematode behaviour can also be influenced by pre-application conditions, such as storage temperatures and storage periods. Storage at the production company, transport to the distributor, storage on the shelf and subsequent transport to the grower and standing a few hours at the farm or on the field lead to mortality and a reduction in quality of EPNs (Smits, 1996).

Factors operating at the time of application and over the following few hours are most critical for the establishment of EPNs. UV radiation can have major effects on EPNs (Gaugler *et al.*, 1992). Post application irrigation, i.e. to rinse the nematodes down, usually improves persistence (Selvan *et al.*, 1994) and pre-application irrigation may help under dry conditions.

1.2.4 Special attributes of EPNs as biological control agents of insects

Steinernematidae and Heterorhabditidae are the only nematodes, which have evolved the ability to carry and introduce symbiotic bacteria into the body cavity of insects.

They are the only insect pathogens with a broad host range, which includes the majority of all insect orders and families (Kaya and Gaugler, 1993). This broad host range can be explained by the efficient pathogenic properties of the bacterium – nematode complexes, in which both partners involved in the symbiosis to act together (Boemare *et al.*, 1996). Their increasing availability, their fast and high rate of kill (most often reaching a 100% mortality within 24-48 hours post application), their ability to search the susceptible hosts (actively or passively), and their limited effect on non-target organisms, have stimulated research (Kaya, 1993).

They can be cultured on a large scale on artificial solid media or in liquid media (Poinar, 1990), as well as inside the hosts, and these cultures can be stored for a long time without affecting the virulence of EPNs (Kaya, 1993; Surrey and Davis, 1996).

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. These products are recommended against black vine weevil and cranberry girdler in cranberries, mole crickets, cutworms, army worms on turf grass, sciariid flies on Mushrooms, and black wine weevil and fungal gnats on ornamentals (Gaugler *et al.*, 2000).

Moreover, IJs can be applied with conventional spraying equipment and are compatible with most pesticides (Kaya, 1993). EPNs have the potential to be used in a self-sustaining way, whereby they reproduce in the insect host, thereby providing new IJs to search for

new hosts. In many countries EPNs are excluded from government registration (Gaugler, 1981). Finally the rising public demand for alternatives to synthetic insecticides has also increased the interest in EPNs.

1.3 Research objectives and hypotheses

1.3.1 Objectives

1. Under laboratory conditions, investigations on the efficacy of selected EPN strains against different life stages, i.e. late L_2, prepupa and pupa, of WFT.

2. Under laboratory conditions, investigations on the persistence of the most effective EPN strains (based on the results of the here presented screening experiments and on previous research carried out at the Institute of Plant Diseases and Plant Protection), targeting on late L_2 of WFT.

3. Under semi-field conditions (i.e. in pot experiments), study the effectiveness of combined releases of EPNs and predatory mite, *Hypoaspis aculeifer* Canestrini against mixed population structures of WFT.

1.3.2 Research hypotheses

The activity of steinernematid and heterorhabditis species of EPNs has been recorded against a broad range of insects in a variety of habitats. However, the specific susceptibility of insects to different species and strains of EPNs vary considerably. Also, the degree of pathogenicity, i.e. the virulence, of EPNs differ against particular pest species as well as different developmental stages. Similarly, the rate of infectivity of EPNs has an inter-relationship with the concentration applied against a particular insect or

insect's developmental stage. Moreover, variations have been recorded among the different EPN strains relating to the short and long-term persistence.

Combined releases of EPNs and other natural enemies in biological control projects have shown that both synergistic and antagonistic effects may occur. The nature of such effects depends on the overlap of niches of the specific bio-control agents employed.

In this research we hypothesised that differences of virulence against WFT as well as different life stages of WFT relating to different EPN strains tested may exist. In addition, differential responses were expected in the infectivity against WFT with the different dosages of EPN strains investigated. Moreover, differences in short-term – persistence of EPNs were also expected. In the study of effectiveness of combined releases of EPNs and the predatory mite *Hypoaspis aculeifer* against soil-dwelling stages of WFT, we expected synergistic effect rather than antagonistic effect.

2 Efficacy and short-term persistence of entomopathogenic nematode strains against soil-inhabiting stages of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae): a laboratory evaluation[1]

2.1 Abstract

Efficacy of entomopathogenic nematode (EPNs) strains was evaluated in a Petri dish bioassay against soil-dwelling stages of western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Among the six EPN strains assessed against pupal stages of WFT, *Steinernema feltiae* (Filipjev) hybrid (commercial formulation Nemaplus® [SFN]) and *Heterorhabditis bacteriophora* Poinar (Hdo1 strain [HBHDO1]) caused 65% and 59% mortality, respectively. (Weiser) (Agriotos strain [SCA]), *S. arenarium* (Artyukhovsky) (Anomali strain [SAA]) showed moderate mortality (40-45%) while *Steinernema* spp. Travassos (Morocco strain [SM]) and *H. bacteriophora* (commercial formulation Nematop® [HBN]) had no effect on the prepupae and pupae of WFT. Late L2 proved to be more susceptible to SFN, *H. bacteriophora* (Hk3 strain [HBHK3]) and HBHDO1 than pupal stages. Results from a dose response study conducted with concentrations of 100, 400 and 800 infective juveniles (IJs) per cm^2 soil of the same three EPN strains indicated that the most effective dose against pupal stages of WFT was 400 IJs/cm^2 soil. Post application short-term persistence of HBHK3 and SFN were studied three and six days after initial inoculation

---

[1] To be published as Premachandra, W.T.S.D.,Borgemeister, C., Berndt, O. and Poehling, H.-M. Efficacy and short-.term persistence of entomopathogenic nematode strains against soil-inhabiting stages of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae): A laboratory evaluation. To be submitted to Biological Control

against late L2. Results showed that under laboratory conditions both strains can persist at least up to six days in the soil without loosing virulence (expressed in terms of mortality).

Key words: *Biological control; dose response; entomopathogenic nematodes;* Frankliniella occidentalis; Heterorhabditis bacteriophora; Steinernema arenarium; S. carpocapsae; S. feltiae; *short-term persistence; soil dwelling stages; western flower thrips*

2.2 Introduction

Western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), one of the predominant thrips species in the world, is a major pest associated with a wide range of crops of economic importance in the field and greenhouses (Yudin *et al.,* 1986; Brødsgaard, 1989; Tommasini and Maini, 1995). Crop damage is principally caused by this pest through direct feeding on leaves, flowers and fruits. Moreover, WFT is an efficient vector of lethal plant viruses, such as tomato spotted wilt virus (TSWV) (Broadbent *et al.*, 1987).

Control strategies have been developed in many directions against this pest. However, chemical control is hampered by rapid development of resistance against major groups of insecticides (Broadbent and Pree, 1997). Short generation time, high fecundity of WFT (Robb and Parrella, 1987) coupled with excessive use of insecticides (Zhao *et al.*, 1995) facilitates the development of resistance against insecticides. Moreover, WFT eggs are inserted into plant tissues, larvae feed in protected areas of the plants, pupal stages are inhabiting in the soil, leaf litter or protected areas in the foliage, and adult thrips also feed in protected areas (Palmer, 1989). All these factors render chemical control of WFT very difficult.

In the scope of biological control, several efficient natural enemies like predatory mites and bugs have been identified and are used in management programs against foliar-feeding life stages, i.e. larval instars and adults of WFT (Bennison and Jacobson, 1991; Chambers *et al.*, 1993; Williams, 2001). However, at present, no single biological control technique can efficiently control WFT, particularly not on high value crops like ornamentals.

In recent years entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabtiditae are acquiring considerable attention as potential biological control agents against soil-inhabiting insect pests (Klein, 1990; Kaya and Gaugler, 1993). The only free living stage, the so-called infective juveniles (IJs) of EPNs locate suitable hosts in the soil and initiate parasitism by which host death occurs within 24-48 hours (Dunphy and Thurston, 1990). Previous investigations have proved that EPNs have the potential for controlling soil-dwelling stages of WFT (Chyzik *et al.*, 1996; Helyer *et al*; 1995; Tomalak, 1994; Ebssa *et al.*, 2001b). However, until now only a limited number of EPN strains have been screened for efficacy against WFT.

The objective of this present study was to evaluate the efficacy of selected EPN strains (including two commercial products) against different life stages of WFT in a Petri dish bioassay. We further tested under laboratory conditions post application short-term persistence of EPNs, against late L2 of WFT.

2.3 Materials and methods

2.3.1 Nematodes

The species name, strain, origin of the nematodes and the abbreviation used are listed in **Table 2.1**. All EPN strains were obtained from Dr. Ralf-Udo Ehlers, Christian-Albrechts–

University Kiel, Germany. The commercial formulations SFN (Nemapuls®) and HBN (Nematop®) (E-Nema, Raisdorf, Germany) were obtained as powders and stored at 4°C until used. In order to assure the consistency of results, efforts were always made to conduct experiments with freshly obtained products. The products were dissolved in sterile distilled water (SDW) and the nematodes were separated using a modified Bearmann funnel.

The other EPN strains were obtained as IJs in March 2000 and were continuously reared in the laboratories of the Institute of Plant Diseases and Plant Protection (IPP) in Hanover, Germany, at 23 ± 2°C in final instars of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), following the method of Kaya and Stock (1997). Nematodes were stored at 6°C in SDW in tissue culture flasks until used, and nematodes of less than three weeks age were used in the tests. The nematode suspensions were allowed to acclimate at ambient room temperature for 24 hours prior to exposure to WFT. Quantification of nematodes in the suspension was done using standard procedures (Kaya and Stock, 1997).

Table 2.1 Investigated EPN species, strains, origin and their abbreviations

Nematode species	Strain	Origin	Abbreviation
Steinernema arenarium	Anomali	Russia	SAA
Steinernema carpocapsae	Dd136	USA	SCD
Steinernema carpocapsae	Agriotos	Russia	SCA
Steinernema feltiae	Sylt	Germany	SFS
Steinernema spp.	Marocco	Morocco	SM
Heterorhabditis bacteriophora	Hdo1	Germany	HBHDO1
Heterorhabditis bacteriophora	Hk3	Germany	HBHK3
Steinernema feltiae	Hybrid[1]	Europe	SFN
commercial product Nemaplus®			
Heterorhabditis bacteriophora	Hybrid[2]	Europe + USA	HBN
commercial product Nematop®			

1 - Hybrid of several strains of *S. feltiae* isolated in several European countries
2 - Hybrid of several strains of *H. bacteriophora* isolated in several European countries and in the USA

2.3.2 Western flower thrips

WFT were reared on pods of green beans (*Phaseolus vulgaris* L.) in glass jars in a climatic chamber (23 ± 2°C, 50-60% relative humidity (RH) and 18:6h L:D photo period) in the laboratories of IPP according to the protocol of Ullman *et al.* (1997). Only thrips with uniform–age and development stage structure were used in the bioassays.

2.3.3 Bioassay protocol

In all experiments under laboratory conditions, the following protocol was used. The bioassay system consisted of a plastic Petri dish (5.8 × 1.5 cm) filled with 8g of non-sterilised Fruhstorfer Erde Type P, a commercial growing substrate frequently used in German horticulture (Archut GmbH, Lauterbach - Wallenrod, Germany). The soil constituted of a mixture of humus, clay and peat in the proportion of 15:35:50 and $N:P_2O_5:K_2O$ of 150:150:250 mg/l, respectively, and a pH of 5.9. For the present study the

bioassay protocol developed by Ebssa (2000), used in a previous study at IPP, was modified in several aspects. Our overall aim was to create a set-up that closely mimics field conditions. Late L2 of WFT (i.e. nine days after hatching) were introduced into the arena and allowed to penetrate into the soil to continue their development. In order to avoid age heterogeneity, only eggs that had been laid within of a period of 24 hours were used in the experiments. The centre of the lid of the Petri dish was perforated (diameter ~ 7 mm) and covered with a nylon tissue (pore size ~ 64 µm) to allow ventilation and at the same time prevent escaping of WFT. The inner side of the lid, except for the nylon tissue, was painted with insect glue (Temmen GmbH, Hattersheim, Germany) to trap emerging adult thrips from the soil (so called 'sticky traps'). In order to avoid emerging thrips to escape through the lid, the inner edge of it was lined with modelling clay, which assured that the Petri dish was tightly closed.

Nematode suspensions or distilled water (as control) were distributed evenly to the top of the soil in which thrips were undergoing their development according to the treatments. After inoculation of EPNs to the soil, the Petri dishes were sealed and incubated at 23 ± 2°C, 18:6h L:D photo period and 60-90% RH in the climatic chamber. After seven days, the adult WFT that got stuck on the sticky traps and/or were wandering or found dead on the nylon tissue or on the soil surface were recorded. Data collection continued until no more adults were found. Five replicates per treatment and control were used, respectively. The experiments were arranged using a completely randomised block design.

Pre-experiment - Timing of EPN application

For an optimisation of the time of EPN application, first the thrips development within the soil of the bioassay units needed to be studied. Therefore, initially 20 late L2 were released into the soil and subsequently the number of larvae, prepupae, pupae and the

emerged adults (dead/alive) inside the soil as well as individuals that were trapped in the sticky traps were continuously counted from the first to the eighth day after introduction of the thrips using destructive sampling techniques. The initial moisture content (MC) of the soil was adjusted to 60%(w/w). The mean percent proportion of thrips in the different development stages was computed in relation to the time.

2.3.4 Main Experiment I- Efficacy of EPN strains against different life stages of WFT

Efficacy of six EPN strains, i.e. HBN, SFN, HBHDO1, SAA, SCA and SM were assessed in this trial. Twenty late L2 were released into the soil, and three days later (for the details refer the result section) EPN suspensions were evenly distributed on the soil surface, using a concentration of 7,200 IJs/Petri dish (i.e. 300 IJs/cm^2) in 2.5 ml of distilled water (final MC of the soil was about 65%). Only 2.5 ml of distilled water was applied to the control treatment. Data collection was identical to the one described in section 2.3.3.

2.3.4.1 Screening of six EPN strains against pupal stages of WFT

In this experiment, efficacy of the EPN strains which caused highest mortality in the experiment 2.3.4.1 (SFN and HBHDO1 - for the details see the results section) were compared with HBHK3, SCD and SFS. The latter three strains had been successfully used in a previous study against WFT (Ebssa, 2000 and 2001b). All strains were tested against pupal stages of WFT using a concentration of 300 IJs/cm^2 soil.

2.3.4.2 Comparison of effectiveness of the most efficient EPN strains against pupal stages of WFT

In this experiment, efficacy of the EPN strains which caused highest mortality in the experiment 2.3.4.1 (SFN and HBHDO1 - for the details see the results section) were

compared with HBHK3, SCD and SFS. The latter three strains had been successfully used in a previous study against WFT (Ebssa, 2001b). All strains were tested against pupal stages of WFT using a concentration of 300 IJs/cm^2 soil.

2.3.4.3 Efficacy against late L2 of WFT

Out of the EPN strains assessed in experiment 2.3.4.2, the strains causing highest mortality of pupal stages of WFT were selected (HBHK3, SFN and HBHDO1) (for the details refer to the results section) to evaluate the efficacy against late L2. EPN suspensions (300 IJs/cm^2 soil) were introduced one day after releasing of late L2 into the assay arena.

2.3.4.4 Dose rate study of EPN strains against pupal stages of WFT

In order to identify the minimum dose rate of EPNs that causes highest mortality in pupal stages of WFT, the relationship between EPN concentrations and WFT mortality was tested. The same EPN strains as in experiment 2.3.4.3 were used (i.e. HBHK3, SFN and HBHDO1), and the effects of three dose rates, i.e. 0 (control), 100, 400 and 800 IJs/cm^2 were tested against pupal stages of WFT.

2.3.5 Main Experiment II - Investigations on post application short-term persistence of EPN strains

Quantification of persistence can be ascertained by recovering of IJs after a given time after the application using extraction techniques (e.g. Baermann funnel). However, in this trial, the post application short-term persistence was evaluated in an indirect way by comparing the ability of two EPN strains (HBHK3 and SFN) to successfully parasitise and subsequently, kill late L2 WFT at different time intervals after an initial application onto the soil. The experiment consisted of three treatments: In the first treatment, the

EPNs and 20 late L2 were applied simultaneously to the soil. In the second treatment, only ten L2 were applied at the time of the EPN application; three days later the remaining ten L2 were applied to the soil. In the third treatment, again ten L2 were applied together with the EPNs, and the remaining L2 were introduced six days later **(Table 2.2)**. In all treatments mortality of WFT was recorded seven days after introduction of thrips larvae into the assay arena. This implies that for treatment two and three the duration of the trial was longer than in treatment one. Based on the results of the previous dose rate study, the two EPN strains were applied at a concentration of 400 IJs/cm^2 (for the details refer to the results section). The experimental design was similar to the one described in section 2.3.3.

Table 2.2 Description of treatments and controls for the investigation of post application short-term persistence of EPNs against late L2 of WFT

Trail	Days	Assay arena		
		EPN suspension	Distilled water	Late L2
T1	0	-	-	20
C1	0	-	-	20
T2	0	-	-	10
	3	-	-	10
C2	0	-	-	10
	3	-	-	10
T3	0	-	-	10
	6	-	-	10
C3	0	-	-	10
	6	-	-	10

2.3.6 Statistical analysis

In all experiments mortality was expressed as percent mortality. Initially these mortality data were subjected to Shapiro-Wilk's Test test for normality and Brown and Forsythe's test for homogeneity of variance (PROC GLM; SAS Institute, 1999). Analysis of variance (ANOVA) was performed to test for differences in mortalities among the treatments. Means were separated using LSD (Least significant difference) multiple range test (Analyst; SAS Institute, 1999). Mortality data between the control and treatments was compared using Dunnett's test (Analyst; SAS Institute, 1999). Mortality data was transformed (arcsine of the square root) before performing ANOVA, if they violated normality and variance homogeneity. When more than one factor was found in treatments (EPN strains and doses / EPN strains and treatments/EPN strains and thrip developmental stages), factorial ANOVA was performed to test the interaction between the factors and when there was significant interaction was observed, means of the level of one factor was compared at each level of the other factor. Marginal means (i.e. means of the one factor were compared by ignoring the levels of other factor) were compared when interaction was not observed (Analyst; SAS Institute, 1999). All comparisons were made at the 0.05 level.

2.4 Results

Preliminary experiment – Timing of EPN application

Twenty-four hours after application of L2, the majority of them (74%) remained as L2. However, at day two and three a great proportion had already developed into prepupae. At day four almost equivalent proportions of prepupae and pupae and only very small numbers of L2 were observed. From day five onwards until day seven the proportion of pupae remained stable around 80%. The first adults were recorded on day seven **(Figure 2.1)**.

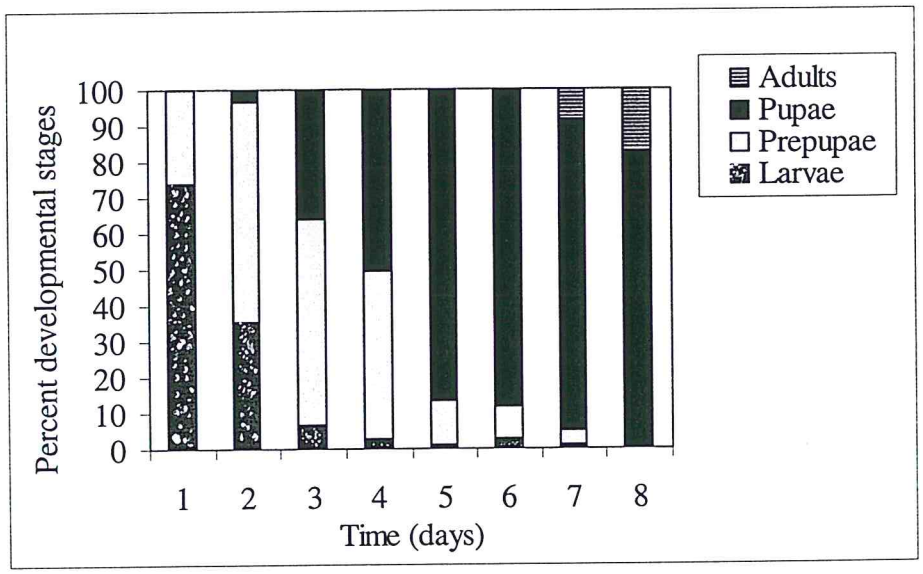

Figure 2.1 Different thrips (percent) stages recovered in soil with respect to 1-8 days after initial application of L2

2.4.1 Effectiveness of six EPN strains against pupal stages of WFT

Among the six EPN strains assessed, except for HBN ($P = 0.082$) and SM ($P = 0.075$) all other EPN strains tested caused significantly higher mortality (HBHDO1, SFN and SCA $P < 0.0001$ and SAA $P = 0.0005$) compared to the natural mortality in the control (3%,

SE = 2). Significant differences in percent mortalities were observed among the EPN strains. With 65% and 59% high mortalities were induced by SFN and HBHDO1, respectively. SAA and SCA caused moderate levels of mortality (41 and 46%) **(Figure 2.2).**

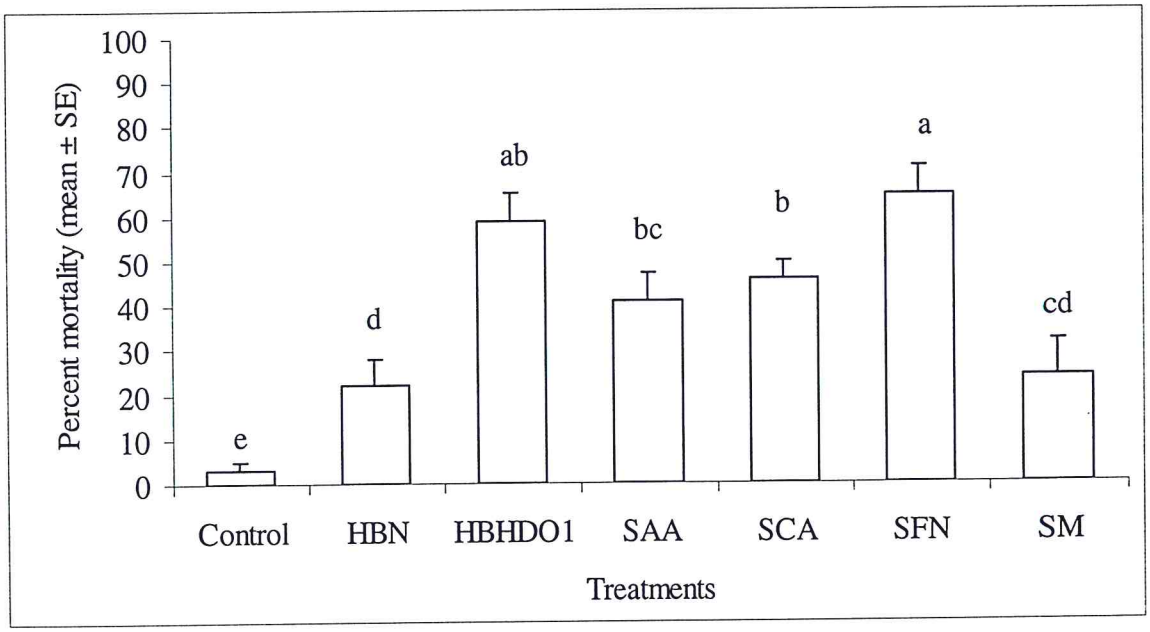

Figure 2.2 Effect of six EPN strains (concentration 300 IJs/cm2 soil) on the mortality of pupal stages of WFT in the soil. Columns represent mean percent mortality (± SE); columns marked with the same letter are not statistically different at $P < 0.05$ (LSD multiple range test). Details on the EPN strains are provided in Table 2.1.

2.4.2 Comparison of efficacy of most efficient EPN strains against pupal stages of WFT

The efficacy of the EPN strains that induced highest mortality in WFT pupa in experiment 2.3.4.1 (i.e. SFN and HBHDO1) was compared with HBHK3, SCD and SFS. In this trail no mortality was recorded in the control treatment. All five EPN strains tested produced significantly higher mortality ($P < 0.0001$) than in the control. Significant differences in mortalities were recorded among the EPN strains tested. With 74% the

highest mortality was observed in HBHK3, followed by SFN (62%) and HBHDO1 (56%). Moderate mortality was detected in SFS (49%) and the lowest mortality was recorded in SCD (24%) **(Figure 2.3)**.

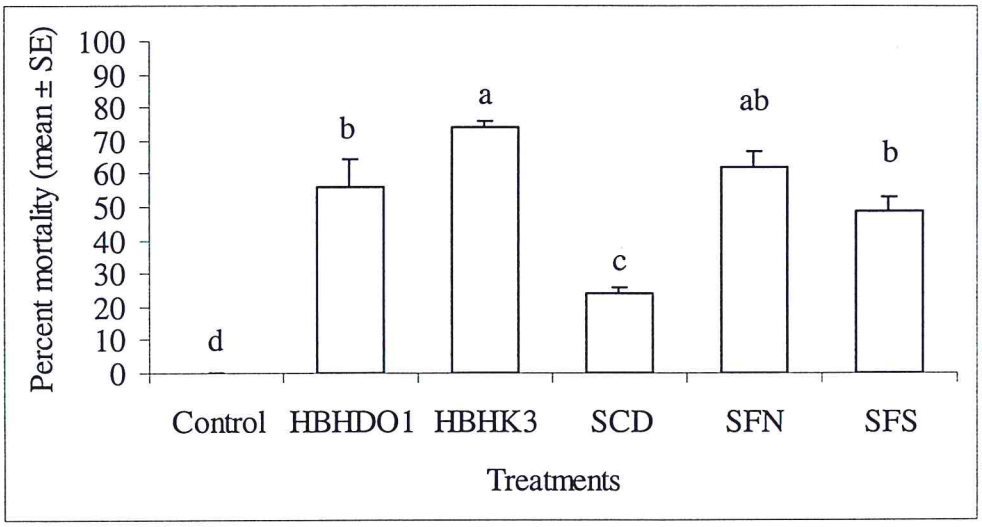

Figure 2.3 Effect of five EPN strains (concentration 300 IJs/cm^2 soil) on the mortality of pupal stages of WFT in the soil. Columns represent mean percent mortality (± SE). Columns marked with the same letter are not statistically different at $P < 0.05$ according to the LSD multiple range test. Details on the EPN strains are provided in Table 2.1.

2.4.3 Efficacy of EPNs against late L2 of WFT

Based on the results of the previous experiment, the three EPN strains that caused mortality higher than 50%, i.e. HBHK3, HBHDO1 and SFN, were tested at a concentration of 300 IJs/cm^2 against late L2. All three strains caused significantly higher mortality ($P < 0.0001$) than in the untreated control where 5% mortality (SE = 2.24) was recorded. In all three treatments high mortality was observed, with 93%, 86% and 79% for HBHK3, HBHDO1 and SFN, respectively **(Figure 2.4).**

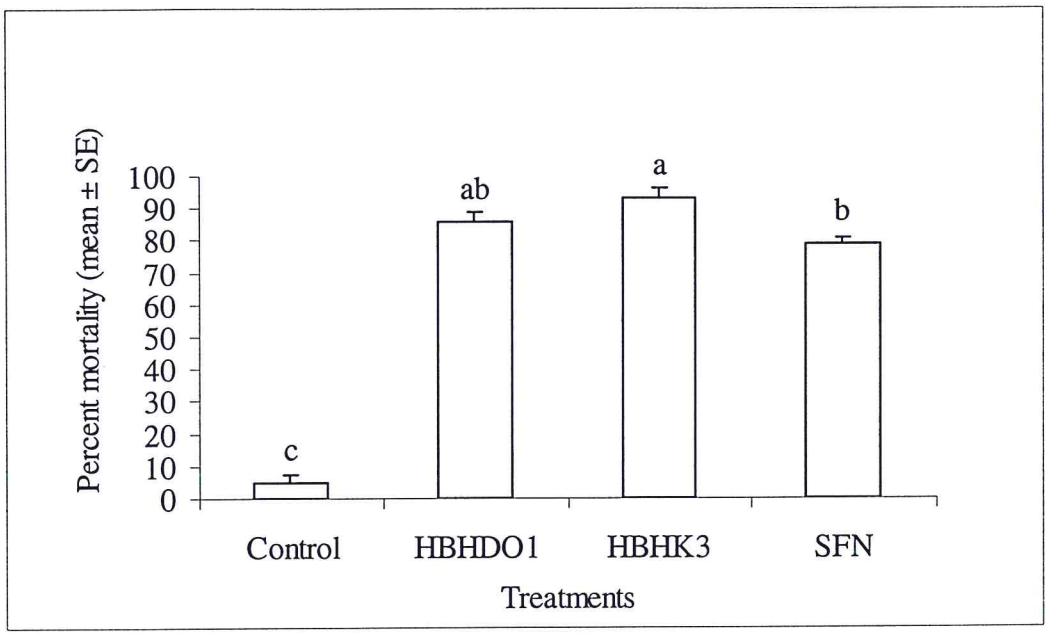

Figure 2.4 Effect of three EPN strains (concentration 300 IJs/cm^2 soil) on the mortality of late L2 of WFT in the soil. Columns represent mean percent mortality (± SE). Columns marked with the same letter are not statistically different at $P < 0.05$ (LSD multiple range test). Details on the EPN strains are provided in Table 2.1.

2.4.4 Comparison of efficacy of EPNs against larvae and pupal stages of WFT

The efficacy of HBHK3, HBHDO1 and SFN against larval and pupal stages of WFT was assessed by comparing mortality data from experiments 2.3.4.2 and 2.3.4.3 (prior analysis revealed no temporal effect between the two experiments and thus the data were pooled). No significant interaction was found between the thrips developmental stage and the EPN strains tested (df = 2.24, F = 1.16; P = 2.24). Therefore, mortalities caused by EPN strains were compared irrespective of the developmental stage as well as the mortalities of developmental stage were compared irrespective of the EPN strains (i.e. marginal means). Significant differences in mortality were found among the three EPN strains tested **(Table 2.3)**. Mortality caused by EPNs was significantly higher against late L2 than against pupal stages of WFT **(Table 2.4).**

Table 2.3 Mean percent mortality (± SE) caused by three EPN strains irrespective of the thrips developmental stages. Means followed by the same letter are not statistically different at $P < 0.05$ (LSD multiple range test). Details on the EPN strains are provided in Table 2.1

EPN strain	Mean percent mortality ± SE (irrespective of the thrip developmental stages)
HBHK3	83.5 (± 3.58)[a]
HBHDO1	71.0 (± 6.62)[b]
SFN	70.5 (± 3.83)[b]
LSD(∝= 5%)	9.50

Table 2.4 Mean percent mortality (± SE) of late L2 and pupal stages of WFT caused by three EPN strains irrespective of the EPN strains. Means followed by the same letter are not statistically different at $P < 0.05$ according to LSD multiple range test.

Thrip developmental stage	Mean percent mortality ± SE (irrespective of the EPN strains)
Late L2	86.0 (± 2.25)[a]
Pupal stages	64.0(± 4.60)[b]
LSD (∝=5%)	7.75

2.4.5 Dose rate study of EPN strains against pupal stages of WFT

Average mortality in the untreated control was 8% (SE = 3.39). All three EPN strains tested caused significantly higher mortality ($P < 0.0001$) than in the untreated control. No significant interaction was recorded between the EPN strains (SFN, HBHDO1 and HBHK3) and the doses tested (100, 400 and 800 IJs/cm^2) ($df = 4, 36$; $F = 2.32$; $P = 0.0757$). Therefore, EPN strains were compared regardless of the doses and vice versa (for details refer to section 2.3.6). Significant differences in pupal mortality of WFT were

found among the EPN strains, with maximum mean percent mortality in HBHK3 (85%) and minimum in SFN (51%) **(Figure 2.5)**. EPN-induced mortalities increased only up to a dose rate of 400 IJs/cm^2 soil and further increase of the concentrations did not produce significantly higher mortality of pupal stages of WFT **(Figure 2.6)**.

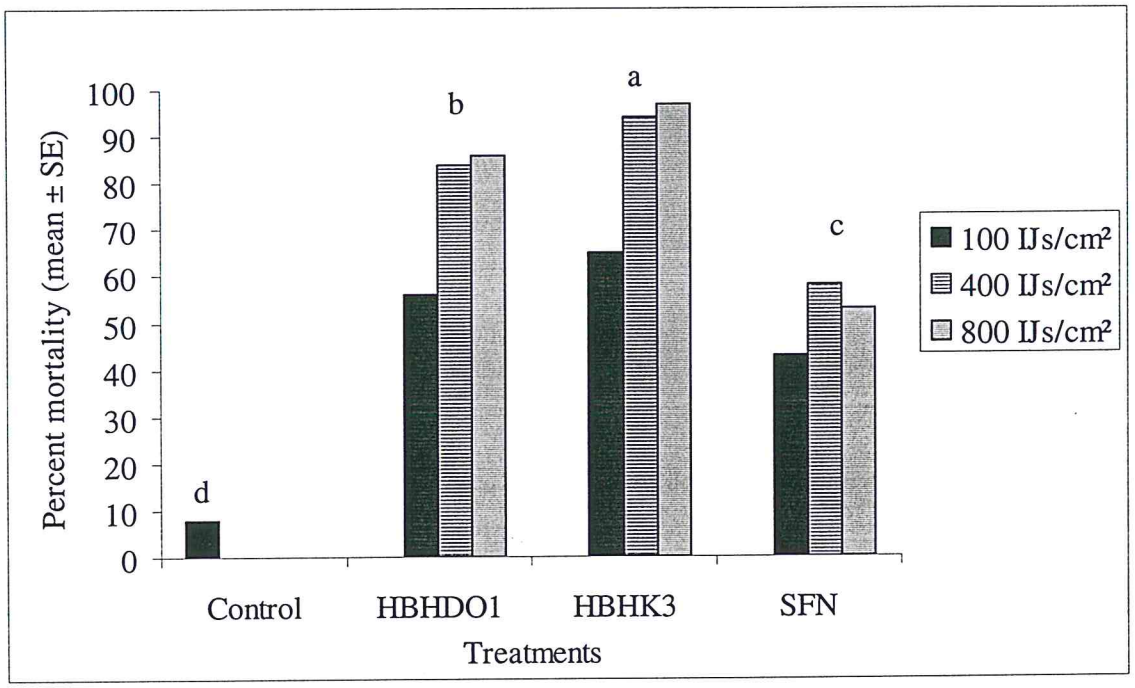

Figure 2.5 Mean percent mortality of pupal stages of WFT caused by three EPN strains at concentrations of 100, 400 and 800 IJs/cm^2 soil and in the untreated control. No significant interaction was found between the dose rates and the EPN strains tested. Therefore, comparisons of EPN strains were performed irrespective of the dose rates. Same letters above groups of three individual columns each and the control indicate no significant difference ($P < 0.05$) (LSD multiple range test). Details on the EPN strains are provided in Table 2.1.

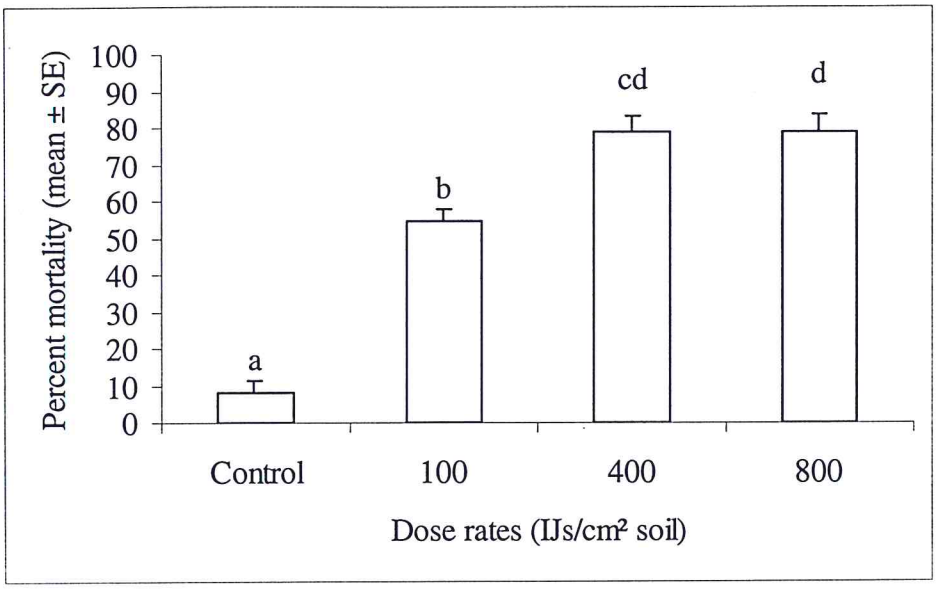

Figure 2.6 Mean percent mortality (± SE) of pupal stages of WFT at concentrations of 100, 400 and 800 IJs/cm^2 soil caused by three EPN strains (i.e. SFN, HBHDO1 and HBHK3). No significant interaction was found between the dose rates and EPN strains tested. Therefore, comparisons of doses were performed irrespective of the EPN strains. Same letters above the columns indicate no significant difference ($P < 0.05$) (LSD multiple range test). Details on the EPN strains are provided in Table 2.1.

2.4.6 Post application short-term persistence of EPN strains against late L2

Prior to the analysis percent mortality data were transformed (arcsine of the square root). Both HBHK3 and SFN caused significantly higher mortality ($P < 0.0001$) than in the untreated controls of the respective time treatments. The mortalities in three controls were 10% (SE = 2.74) in T1 and T2 and 15% (SE = 1.58) in T3. No significant interaction was found between the time treatments and strains (df = 2.24; F = 0.29; P = 0.7524). Therefore, EPN strains were compared regardless of the time treatments and vice versa (for details refer to section 2.3..6). Significant differences in mortality values were recorded between HBHK3 (94%) and SFN (58%) **(Figure 2.7)**. However, no significant differences were detected among the three time treatments **(Figure 2.8).**

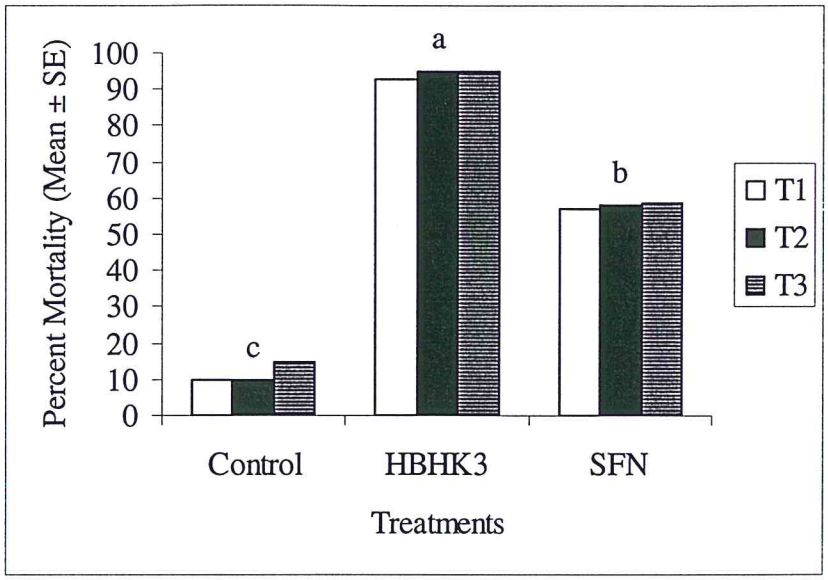

Figure 2.7 Mean percent mortality of late L2 of WFT caused by two EPN strains (concentration of 400 IJs/cm^2 soil) in relation to three time treatments (T1- 20 L2 and EPNs were introduced simultaneously into the soil; T2- ten L2 and EPNs were initially introduced together and three days later the remaining ten L2 were applied; T3- ten L2 and EPNs were initially introduced together and six days later the remaining ten L2 were applied). No significant interaction was found between the time treatments and EPN strains tested. Therefore, EPN strains were compared irrespective of the time treatments. Same letters above groups of three individual columns each indicate no significant difference ($P < 0.05$) (LSD multiple range test). Details on the EPN strains are provided in Table 2.1.

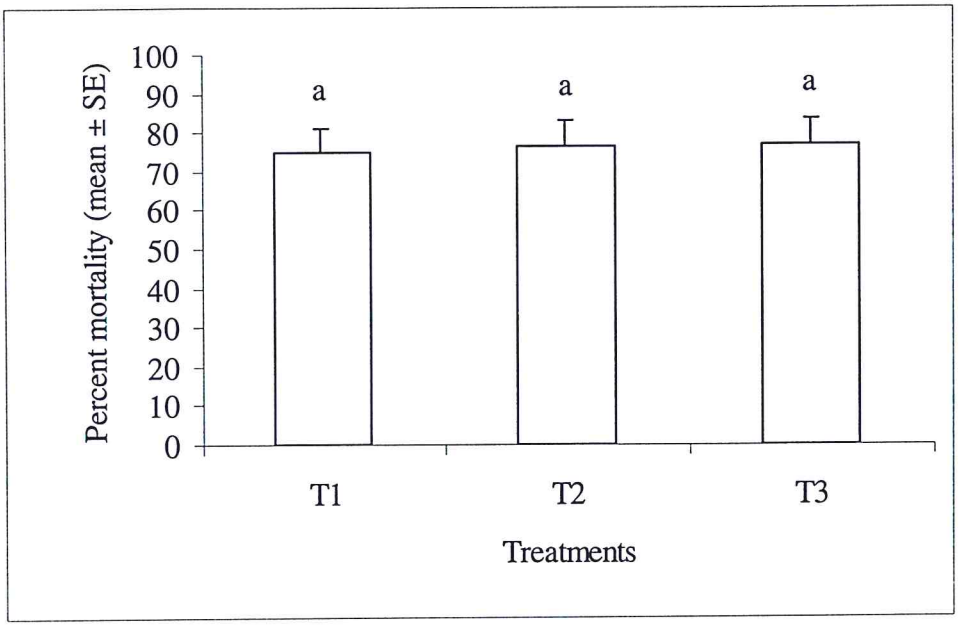

Figure 2.8 Mean percent mortality (± SE) of late L2 of WFT caused by two EPN strains (concentration of 400 IJs/cm^2 soil) in relation to three time treatments (T1- 20 L2 and EPNs were introduced simultaneously into the soil; T2- ten L2 and EPNs were initially introduced together and three days later the remaining ten L2 were applied; T3- ten L2 and EPNs were initially introduced together and six days later the remaining ten L2 were applied). No significant interaction was found between the time treatments and EPN strains tested. Therefore, comparisons of time treatments were performed irrespective of the EPN strains. Columns marked with the same letter are not statistically different ($P < 0.05$) (LSD multiple range test). Details on the EPN strains are provided in Table 2.1

2.5 Discussion

In this study the EPN strains tested varied in their ability to infect pupal stages of WFT in the soil. Among the six strains evaluated two strains, i.e. HBHDO1 and the commercial product SFN, were the most effective agents against pupal stages of WFT. We further observed that virulence (expressed in terms of mortality) of different strains (HBN, HBHDO1 and HBHK3) of the same EPN species (*H. bacteriophora)* also differed significantly. Our findings corroborate results of a previous study, in which different

strains of *S. feltiae* caused considerably different levels of mortality in WFT (Ebssa *et al.*, 2001b). In addition, Bracken (1990) reported that virulence of different EPN strains of the same species can differ substantially.

Host location strategies determine to a large extent the efficacy of EPNs. According to Grewel *et al.* (1995) *S. carpocapsae* is an ambush forager i.e. sit and wait strategy, while *H. bacteriophora* is a cruiser forager i.e. actively pursuing its hosts. Moreover, *H. bacteriophora* and *S. feltiae* respond to host cues, whereas *S. carpocapsae* does not. *Steinernema feltiae* possesses some characteristics of ambush foragers and some of cruise foragers (Grewel *et al.*, 1995). Also, *S. feltiae* has been reported to nictate only for shorter periods of time (Kondo and Ishibashi, 1986). Cruisers are usually more active in searching for subterranean hosts, while ambush foragers are effective searching mobile hosts on the surface. In the methodological set-up used in the present study, the searching ability of EPNs play an important role because late L2 penetrate into the soil and settle at different positions within the soil column of about 1.5 cm. The high pupal mortality by SFN and HBHDO1 is most likely due to their host location strategies. Although being a cruiser, HBN did not cause high mortality in WFT pupae. Other factors like production and formulation technologies, and/or EPN strain differences (e.g. the pathogenicity of the associated symbiotic bacteria) might be responsible for the comparatively low virulence of HBN.

Ebssa *et al.* (2001a and b) showed that HBHK3, SCD and SFS have a great potential for controlling WFT under laboratory as well as semi-field conditions. Their bioassay protocol was slightly different from the one used in the present study. In our protocol, late L2 were introduced onto the soil in order to first promote penetration into the soil and then the development into prepupal and pupal stages. This methodological set-up enabled

us to closer mimic field conditions. SCD, which caused high mortality against pupal stages in a previous study (Ebssa *et al.*, 2001b) did not show an equal response when using our slightly modified experimental protocol. This may be associated with the ambush foraging behaviour of SCD. In our study mortalities caused by SCA and SFS did not differ to those of HBHDO1 and SFN. Moreover, the higher mortality of HBHK3 compared to HBHDO1 and SFN indicates a more pronounced cruising behaviour of HBHK3 than in the two other strains. The present results as well as the ones by Ebssa *et al.* (2001 a and b) stress the importance of the host location strategy for the efficacy of EPNs as biological control agents.

Age-related variation in susceptibility of insect hosts to steinernematids and heterorhaditids has been well studied and documented. Lebeck *et al.* (1993) reported that in the leaf miner *Liriomyza bryoniae* (Kaltenbach) older third instar larvae and pupae were less susceptible than second and early third instar larvae. Older larvae of the West Indian weevil *Diaprepes abbreviatus* L. were less susceptible to EPNs than younger ones (Shapiro *et al.*, 1999). These results corroborate our observations that late L2 were more susceptible to EPNs than pupal stages of WFT. However, Glazer and Navon (1990) showed that early larval stages of *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) were more susceptible to steinernematids than older ones. As such, the age-related susceptibility is species-specific to some extent. Differences in size, structure and behaviour of the insect host might be attributed to the variation of susceptibility related to insect age. Generally, insect larvae are more sensitive to penetration by IJs due to their thinner cuticle, the inter-segmental membranes and the more frequent body openings compared to the pupal stages. Moreover, the size of the insects and the insects' immune response are also important factors determining the susceptibility to EPNs (Kaya, 1990). Small-sized insect species or small insect development stages can physically inhibit

nematode infection or development (Jackson and Brooks, 1995). Yet, as insect larvae grow and age, their immune systems generally become stronger and hence less likely to become infected by pathogens (Watanable, 1987).

In the dose-response experiment, mortality of pupal stages increased with increasing dosages up to a concentration of 400 IJs/cm^2 soil. In some pathogen species, once a mortality-producing threshold dose is reached, a further increase in concentration will not yield a further increase in mortality (Tanda and Fuxa, 1987). Our results confirm earlier findings by Chyzik *et al.* (1996) who also recorded higher mortality of WFT pupae and prepupae at 400 than at 20, 40 and 200 IJs/cm^2 soil, respectively. However, at present a concentration of 400 IJs/cm^2 soil seems to be rather high and hardly economical. Yet, the control level of the tested EPN strains in our study at concentrations below 400 IJs/cm^2 was insufficient. At present we are investigating possibilities of combined releases of EPNs with other natural enemies like the predatory mites, *Hypoaspis* spp. Here, possibly lower EPN concentrations may suffice to successfully control soil-dwelling life stages of WFT.

Results of the persistence study show that IJs of both HBHK3 and SFN can persist for at least six days in the soil, and are still able to successfully parasitise and kill late L2 of WFT. In their study with mature larvae of the Mediterranean fruit fly *Ceratitis capitata* Wiedemann, kept in plastic containers with clay loam soil, Gazit *et al.* (2000) recorded that the persistence of the *S. riobrave* (Texas strain) extends over five days and thereafter gradually declined; no activity was recorded after 14 days. In contrast, our results show that the two EPN strains did not lose their activity after six days. However, these results were obtained under laboratory conditions and need to be confirmed at least under semi-field conditions.

3 Compatibility of entomopathogenic nematodes and the predatory mite, *Hypoaspis aculeifer* Canestrini (Acarina: Laelapidae) on soil-dwelling stages of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae): semi-field conditions[2]

3.1 Abstract

The effect of the predatory mite *Hypoaspis aculeifer* Canestrini (Acarina: Laelapidae) on a mixture of soil-dwelling stages of western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and its compatibility with two entomopathogenic nematodes (EPNs) *Heterorhabditis bacteriophora* Poinar (Hk3 strain [HBHK3])) and *Steinernema feltiae* (Filipjev) hybrid (commercial formulation, Nemaplus® [SFN]) were investigated in pot trials using seedlings of green beans (*Phaseolus vulgaris* L.) as model plant species. This is the first report on combined releases of a soil-dwelling predatory mite and EPNs for the control of WFT. Ten *H. aculeifer* adults and infective juveniles (IJs) of the entomopathogenic nematode strains at a concentration of 400 IJs/cm² per pot were used. In comparison to the untreated control *H. aculeifer* alone reduced the adult emergence of *F. occidentalis* by 46% while the EPN strains reduced adult WFT emergence by 46% and 61% for SFN and HBHK3, respectively. Significant differences in adult WFT emergence were found between combined treatments of EPNs and *H. aculeifer* and individual treatments of EPNs and/or

---

[2] To be published as Premachandra W.T.S.D., Borgemeister, C., Berndt, O., and Poehling, H.-M. Compatibility of entomopathogenic nematodes and the predatory mite *Hypoaspis aculeifer* Canestrini (Acarina: Laelapidae) on soil-dwelling stages of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae): semi-field conditions. To be submitted to Bio Control.

H. aculeifer. No evidence for antagonistic reactions between the two biocontrol agents were observed. These results highlight the potential for combined releases of EPNs and *H. aculeifer* for control of WFT.

Key words: *Compatibility, entomopathogenic nematodes;* Hypoaspis aculeifer Frankliniella occidentalis; Heterorhabditis bacteriophora; Steinernema feltiae; *soil dwelling stages; western flower thrips*

3.2 Introduction

Sustainable systems for biological pest control often require the integration of several natural enemies like predators, parasitoids and/or entomopathogens for the successful management of a particular pest species. For biological control of western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), a devastating pest of vegetables and ornamental crops in the field and in greenhouses, polyphagous predatory mites and anthocorid predatory bugs have shown great potential for pest control, particularly in protected cultivation. Most commonly several *Amblyseius* spp. (Acarina: Phytoseiidae) and *Orius* spp. (Heteroptera: Anthocoridae) are used; both groups of predators prey on the foliar dwelling life stages of WFT, i.e. larval instars and adults (Ramakers, 1995; Riudavets, 1995; Sabelis and Van Rijn, 1997). However, augmentative releases of predatory mites and bugs do not provide sufficient control of WFT, particularly in high value crops like ornamentals (Gillespie and Ramey, 1988; Bakker and Sabelis, 1989). Thus, additional biological control agents are urgently needed for successful management of WFT populations.

In, WFT the majority of the late second larval stage (late L2) leave the plant and pupate in the soil; thus thrips spend about one-third of their life cycle (mainly as prepupae and pupae) off the plants in the soil (Tommasini and Maini, 1995). This previously often

ignored 'soil passage' in WFT opens up new venues for biological control by targeting at these soil-dwelling life stages of the pest.

Oligophagous predatory mite species of the genus *Hypoaspis* (Acarina: Laelapidae) are soil inhabitants and feed on different developmental stages of thrips, mites, collembola and other small arthropods (Insera and Davis, 1983). Recent studies showed that *Hypoaspis aculeifer* Canestrini and *H. miles* (Berlese) (Acarina: Laelapidae) are promising predators against soil-dwelling stages of WFT (O. Berndt (Institute of Plant Diseases and Plant Protection (IPP), personal communication; Glockemann, 1992; Gillespie and Quiring 1990).

Entomopathogenic nematodes (EPNs) (Rhabditida: Heterorhabditidae and Steinernematidae) are obligate pathogens of insects and occur naturally in the soil (Kaya, 1990). Many EPN species form a specific symbiosis with bacteria (*Photorhabdus* spp. or *Xenorhabdus* spp.) and are able to kill their hosts within 24-48 hours (Kaya and Gaugler, 1993). Several previous studies have revealed virulence of several EPN species against soil-dwelling stages of WFT (Ebssa *et al.*, 2001b; Tomalak 1994; Heyler *et al.*, 1995; Chyzik *et al.*, 1996).

In this study the effect of combined releases of *H. aculeifer* and some selected EPN strains for control of soil-inhabiting life stages of WFT were investigated under semi-field conditions.

3.3 Materials and methods

3.3.1 Nematodes

Heterorhabditis bacteriophora Poinar (Hk3 strain) (HBHK3) and *Steinernema feltiae* (Filipjev) (commercial formulation Nemaplus®) (SFN) (E~Nema, Raisdorf, Germany)

were used in the experiments (for the details refer to Table 2.1 in section 2.3.1). Both EPN strains (SFN as a powder and HBHK3 as IJs) were obtained from Dr. Ralf-Udo Ehlers, Christian-Albrechts–University Kiel, Germany. Descriptions of the rearing procedure for HBHK3, storage conditions and quantification methods can be found in section 2.3.1.

3.3.2 Western flower thrips

The rearing procedure of WFT was identical to that described in section 2.3.2.

3.3.3 *Hypoaspis aculeifer*

Specimens of *H. aculeifer* were obtained from Mr. O. Berndt (IPP, University of Hanover, Germany). The mites were reared using the nematode *Turbatrix silusiae* (de Man) (Nematoda: Rhabditida: Cephalabidae) as the prey, following the protocol developed by O. Berndt (IPP), University of Hanover, Germany.

3.3.4 Bioassay system and protocol

Plastic pots (11 × 7.5 × 8.5 cm), filled with soil (for the details refer to section 2.3.3) and having a 74 cm^2 upper soil surface were used as experimental units. One green beans (*Phaseolus vulgaris* L.) seedling was transplanted at two-leaf stage and subsequently the pot was covered with a plexy glass cylinder (Ø 10 cm, 30 cm in height). The space between the cylinder and the pot was sealed using modelling clay to prevent escape of thrips. Similarly, the upper opening of the cylinder was covered by nylon tissue (64 µm pore size), which facilitated ventilation in the cylinder and at the same time prevented any escape of thrips. Four side-holes (Ø 3 cm) were formed both at the upper and the lower section of the cylinder and only two side-holes at each section were covered by nylon tissue for additional air supply. The remaining side-holes were covered by a piece of glue

tape on to which a piece of paper was fixed to prevent thrips got stuck onto it. These holes were used to release thrips (from the upper side-holes) and/or application of EPN suspensions/distilled water and to release *H. aculeifer* (both from the lower side-holes). The pots were placed on a plastic tray in which water was supplied for the bean plants.

Twenty female and three male WFT of synchronised age (23 days after hatching) were released into each cylinder. In preliminary experiments the majority of the F1 commenced pupation eight to ten days after releasing the adult thrips. Therefore, twelve days after introducing the adult thrips EPNs (SFN and HBHK3 at a concentration of 400 IJs/cm^2), dissolved in 13 ml of distilled water, or 13 ml of distilled water only as control were applied onto the soil. Five minutes after the EPN application, the soil surface was additionally irrigated with 20 ml of distilled water in order to rinse the EPNs to the layer where WFT preferentially pupate, i.e. to a depth of 1.5-2.0 cm. To avoid possible water logging as well as to prevent an overflow of the irrigation water, plants were not watered two days prior to the application of EPNs. This additionally facilitated a rapid percolation of the EPN suspension into the deeper soil layers and also potential losses of EPNs due to overflowing. Thirteen days after introducing the adult thrips, ten unsexed adult *H. aculeifer* of mixed age but no older than one month, were released onto the soil. On the fifteenth day after introducing the adult WFT, the plexy glas cylinder was removed and the shoot part of the bean plants were cut. Subsequently, the pot was covered with a photo eclector. Photo eclectors consisted of an inverted pot the same type as used in the experiments where the base had been removed. An inverted Petri dish lid (86 × 15 mm) was placed on the hole. Four holes (Ø 2 cm), covered with nylon tissue (64 µm pore size), were drilled into the sides of the eclector to allow ventilation. The inner side of the Petri dish lid was painted with insect glue to trap emerging adult thrips from the soil (thereafter referred to as 'sticky traps'). Trapped adult WFT were counted daily until no adults were

observed i.e. at least for a period of 10 days after setting-up the eclector. In addition, in all treatments dead adults on the soil surface were also counted at the end of the experiment in controls as well as in each treatment.

Reproduction of WFT and the population structure of the foliage-feeding life stages of the thrips were recorded in the control 15 days after the initial release of the adults. The cut plant parts (stems and leaves) were first dipped into a water-soap solution. Thereafter, the solution was filtered onto a filter paper on which a drop of alcohol (70% ethanol) was added to immobilise the thrips and the number of all development stages on the foliage (larvae, prepupae, pupae and adults) were counted under a stereo microscope. In addition, in the control treatment the number of emerged adults from the soil were counted on the sticky traps and on the soil surface. The efficacy of the mite and EPN treatments, as well as the combined releases of mites and EPNs were then calculated by relating the number of emerged WFT in those treatments with that recorded in the untreated control. Details of all treatments are provided in **Table 3.1**. The experiments were completely randomised with five replications for each treatment.

Table 3.1 Description of different treatments in the study of compatibility of EPNs and *H. aculeifer* against soil-dwelling stages of WFT in pot experimentation

Treatment acronyms	Description of treatments	
	Treatment details	Time of application (days after WFT application)
1 SFN	SFN only (400 IJs/cm^2 soil)	12 days
2 HBHK3	HBHK3 only (400 IJs/cm^2 soil)	12 days
3 H	*H. aculeifer* only (10 mites/pot)	14 days
4 H-SFN	SFN (400 IJs/cm^2) and *H. aculeifer* (10 mites/pot)	12 days 14 days
5 H-HBHK3	HBHK3 (400 IJs/cm^2) and *H. aculeifer* (10 mites/pot)	12 days 14 days
Untreated control	Distilled water (13 ml)	12 days

3.3.5 Statistical analysis

Data on the number of emerging adults were first subjected to a square root transformation. Analysis of variance (ANOVA) was performed to test the differences among the different treatments. Means of different treatments were compared by using the LSD (least significant difference) multiple range test while data on adult emergence between the control and the EPN/mite treatments were compared using Dunnett's test (PROC GLM; SAS Institute, 1999).

3.4 Results

Based on the comparison of the number of thrips counted on the plants with the adult WFT that subsequently got stuck on the sticky traps and the additionally recorded dead adults on the soil surface, in the control treatment 15 days after the introduction of the

adult WFT i.e. at the time when the bean seedlings were cut, 65% of the total thrips population was found on the plants. The remaining 35% had left the plants for pupation in the soil. On the foliage the majority of the thrips proportion were L2 (63%) (**Figure 3.1**). However, it should be stressed that with our methodology we could not assess the naturally occurring mortality of thrips in the soil.

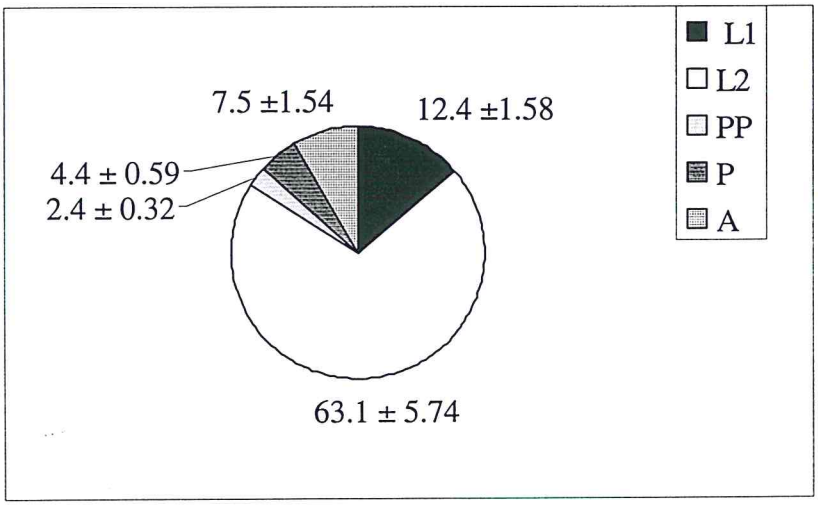

Figure 3.1 WFT developmental stages (percent mean ± SE) recorded on the foliage 15 days after the introduction of adult WFT; L1 = 1st larval instar; L2 = 2nd larval instar; PP = prepupae; P = pupae; A = adults).

When considering only the proportion of the F1 that had already developed into prepupae and pupae, 15 days after the initial introduction of adult WFT about 90% (SE = 1.32) had left the plants, while the remaining 10% (SE = 1.32) were recorded on the foliage. At this time approximately 12% of all WFT counted on the plants were adults (**Figure 3.1**). Continuous counting of adult WFT on the sticky traps revealed, that from the 16th day after the initial introduction of adult WFT the F1 adults started to emerge. Peak emergence was recorded around 19 days after the initial introduction of **(Figure 3.2).**

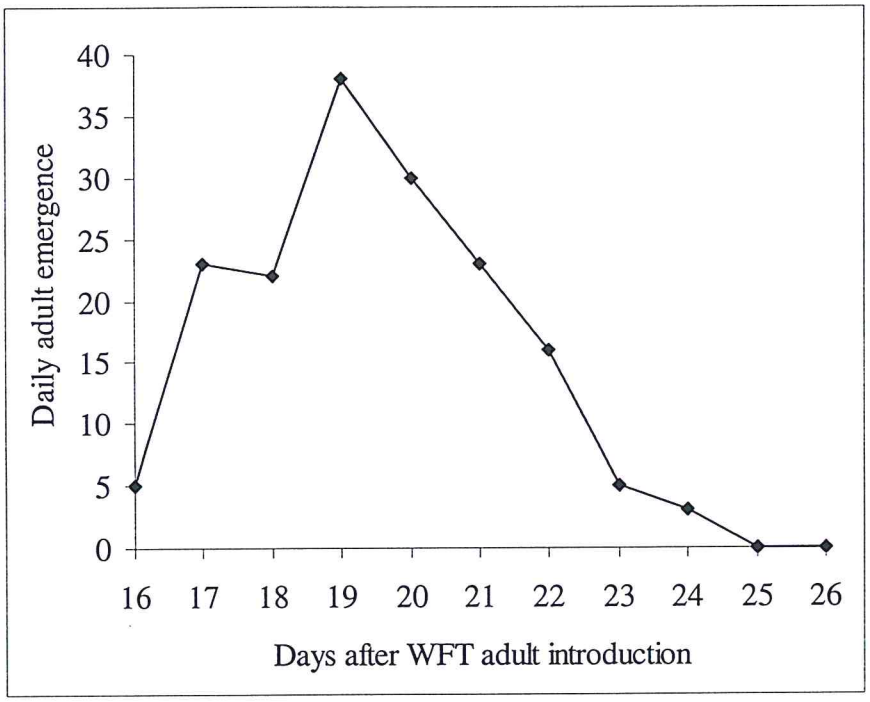

Figure 3.2 Daily emergence of adult WFT (sticky trap counts).

Compared to the untreated control adult emergence was significantly lower in all treatments (H-HBHK3 and H-SFN $P = 0.0001$; H $P = 0.0345$; HBHK3 $P = 0.0017$; SFN $P = 0.0234$). In addition, significant differences in adult emergence were also recorded between the different EPN and *H. aculeifer* treatments. No significant differences were recorded between sole releases of EPNs (SFN and HBHK3) and *H. aculeifer*. However, a combined application of SFN and *H. aculeifer* significantly reduced the number of emerged adult WFT compared to SFN and *H. aculeifer* only. The combined releases of *H. aculeifer* and HBHK3 significantly reduced the number of emerged adult WFT compared to all sole treatments but not the combination of SFN and *H. aculeifer*. Compared to the untreated control the two EPN strains alone reduced WFT adult emergence by 46% and 61% for SFN and HBHK3, respectively. Releases *H. aculeifer* reduced adult emergence by 46% compared to the untreated control. Finally the combined

application of EPNs and *H. aculeifer* impaired adult emergence in WFT by 71% and 82% for H-SFN and H-HBHK3, respectively (**Figure 3.3**).

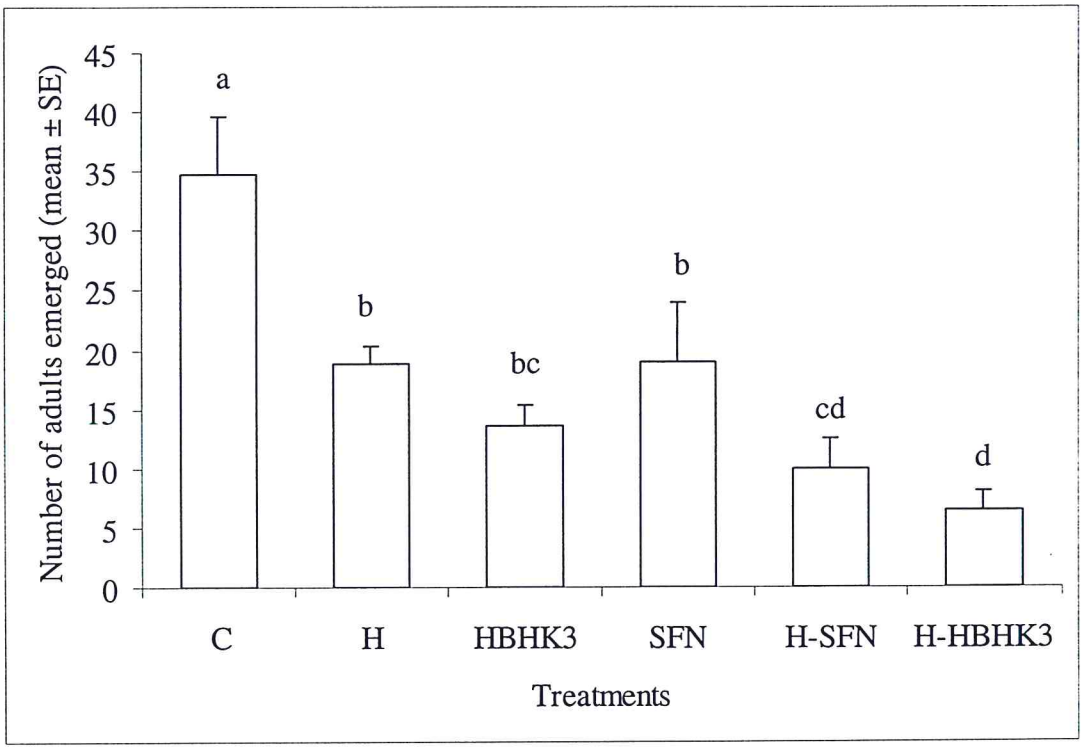

Figure 3.3 Effect of EPN strains and *H. aculeifer* on adult emergence of WFT (sticky traps counts) (non-transformed data was used). Columns represent mean number of adults emerged (± SE) Columns marked with the same letter are not significantly different at $P < 0.05$ (LSD multiple range test; after square root transformation) (HBHK3 = HBHK3 only; SFN = SFN only; H = *H. aculeifer* only; H-HBHK3 = combined application of HBHK3 and *H. aculeifer*; H-SFN = combined application of SFN and *H. aculeifer*).

No significant differences were found in the proportion of dead WFT adults on the soil surface between the control and the two EPN treatments. However, in all treatments where *H. aculeifer* was released no dead adult WFT were encountered on the soil surface (**Figure 3.4**).

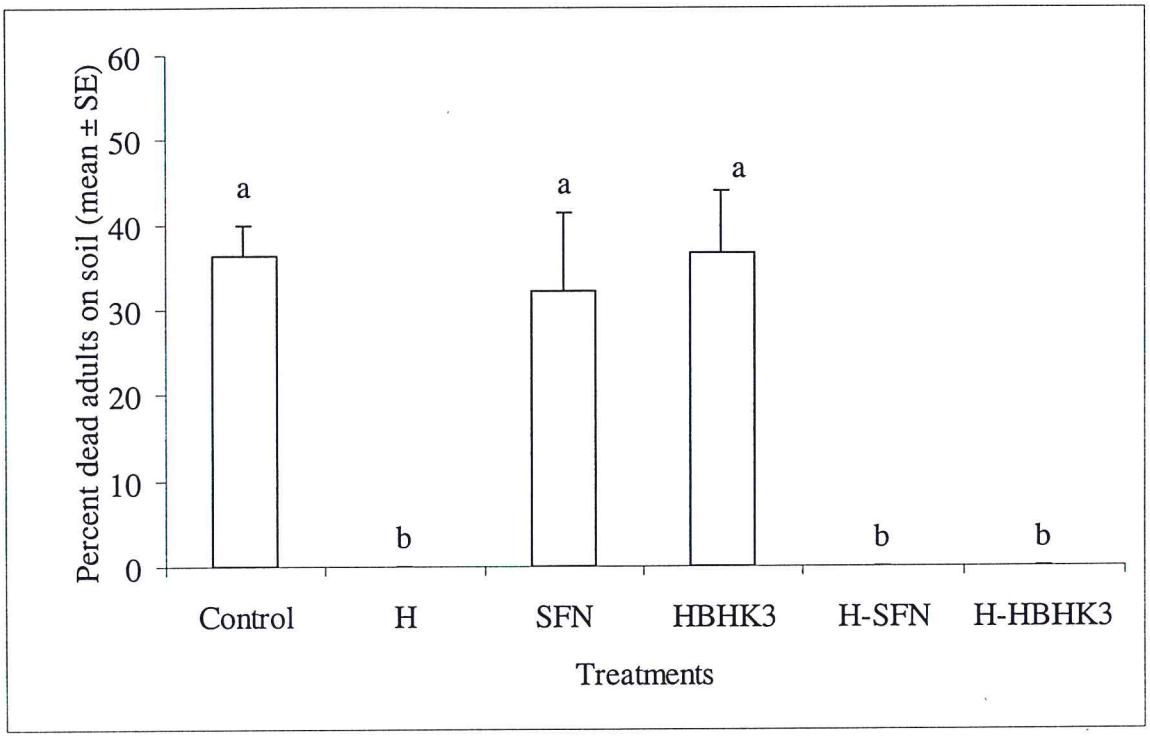

Figure 3.4 Mean percent dead WFT adults (± SE) recorded on the soil surface. (non transformed data was used). Columns marked with the same letter are not significantly different at $P < 0.05$ (LSD multiple range test) (HBHK3 = HBHK3 only; SFN = SFN only; H = *H. aculeifer* only; H-HBHK3 = combined application of HBHK3 and *H. aculeifer*; H-SFN = combined application of SFN and *H. aculeifer*).

3.5 Discussion

This is the first report comparing individual and combined releases of the soil-dwelling predatory mite *H. aculeifer* and EPNs for the control of WFT.

In a similar semi-field trial Ebssa *et al.* (2001a) recorded about 38% corrected mortality of WFT after an application of HBHK3. Compared to the untreated control in our study the two EPN strains alone, reduced adult emergence by 46% and 61% for SFN and HBHK3, respectively, applied at a concentration of 400 IJs/cm² soil. Individual releases of ten *H. aculeifer* per bean plant reduced adult emergence in WFT by 46%. In laboratory

investigations, releases of *H. aculeifer* using predator: prey ratios of 1:1 and 1:5 resulted in 66% and 80% mortality, respectively, of soil-dwelling development stages of WFT (O. Berndt (IPP), personal communication). Innundative releases of *H. aculeifer* at a rate of 1,600 mites per cucumber plant reduced WFT emergence to about 30% compared to the untreated controls over a 40 days trial period (Gillespie and Quiring, 1990). Glockemann (1992) reported that weekly application of ten females of *H. aculeifer* per *Sainpaulia ionantha* (Wendl) (Gesneriaceae) plant over a period of ten weeks slowed down the increase of WFT populations for some weeks and population peaks were diminished. Although we used a different experimental set-up than Glockemann (1992), similar release rates of *H. aculeifer* considerably reduced adult emergence of WFT with beans as host plants.

Using more than one natural enemy species to control an insect pest is potentially advantageous, provided that the two or more species are compatible (Miller, 1983; Kakehashi *et al.*, 1984). Rosenheim *et al.* (1995) defined the term "intraguild predation" (IGP), as being a situation whereby two species share a common host/prey and therefore may compete or engage in a trophic interaction with each other. In the present study, we integrated two natural enemies, i.e. two EPN strains as parasites and a predator (*H. aculeifer*) to control soil-dwelling stages of WFT. Both natural enemy groups share WFT as a common host/prey but in the soil encounter a mixture of different developmental stages of WFT. The compatibility of natural enemies employed in biological control depends on the minimization of niche overlap. This is achieved by mechanisms such as minimization of the overlap of the preferred host/prey stage, microhabitat or temporal influences (Ehler, 1979; Kistler, 1985).

Rosenheim *et al.* (1995) described that in pathogen/parasite–natural interactions, IGP occurs because the pathogens/parasites usually have broad host ranges. EPNs in the families of Steinernematidae and Heterorhabditidae posses a broad host range (Kaya and Gaugler, 1993). Variable responses have been reported relating to the susceptibility of beneficial insects and other arthropods to EPNs. Ishibashi *et al.* (1987) found no deleterious effects of EPNs on collembolans and mites. Bathon (1996) reviewed the literature on non-target effects of EPNs and concluded that non-target impacts were negligible. However, Kaya and Hotchkin (1981) and Kaya (1978a and 1978b) reported that EPNs could affect the interactions between braconid and tachinid parasitoids and their hosts. Moreover, Shannag *et al.* (2000) observed that *S. carpocapsae* is directly or indirectly detrimental to the development of *Cardiochiles diaphaniae* Marsh (Hymenoptera: Braconidae), a parasitoid of the melon worm *Diaphania hyalinata* (L.) and the pickleworm *D. nitidalis* Stoll (Lepidoptera: Pyralidae). No published records indicate a susceptibility of mites to EPNs. However, mites have been related to population reductions of EPNs. Ishibashi *et al.* (1987) conducted a study on predators of EPNs and observed that collembolans, mites, tardigrades and nematodes (mononchid and dorylaimid) preyed upon *S. carpocapsae*. Moreover, Epsky *et al.* (1988) demonstrated that the mesostigmatid mite, *Gamasellodes vermivorax* Walter (Acarina: Ascidae) reduced *S. carpocapsae* efficacy against *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae. In addition, they observed that the endeostigmatid mite *Alycus roseus* Koch (Acarina: Bimichaeliidae) completed its development from late instar nymphs to adults and produced viable eggs when feeding on IJs of *S. carpocapsae*. Finally, Insera and Davis (1983) reported that *Hypoaspis* spp. feed on plant parasitic nematodes.

In our study we observed a significantly reduced emergence of adult WFT after combined releases of EPNs and *H. aculeifer* compared to individual releases of both types of natural

enemies, indicating no antagonistic reactions between EPNs and *H. aculeifer* and thus no sign of IGP. Under field conditions WFT pupate in the soil at a depth of 1.5-2.0 cm (Tommasini and Maini, 1995). *Hypoaspis* spp. inhabits the top-soil layer (at about 1.3 cm depth) where they search for their prey. Microhabitats of EPNs differ according to their host locating behaviour. HBHK3 is a cruiser forager and searches actively for subterranean hosts. SFN possess both cruiser and ambush foraging behaviour (often termed 'sit and wait behaviour', and lives mostly on the surface layers of the soil) (Grewel *et al.*, 1995). Glockemann (1992) and Gillespie and Quiring (1990) showed that *Hypoaspis* spp. can be used against WFT, but only in the pupal stage, which occurs in the soil. Moreover, Altena and Mulder (2000) reported that *H. aculeifer* and *H. miles* consume thrips pupae. However, detailed information on the prey preference for different developmental stages of WFT in *Hypoaspis* spp. were lacking. In contrast, the effect of EPNs on different soil-dwelling stages of WFT is well documented (Ebssa *et al.*, 2001 a and b; Tomalak, 1994; Heyler *et al.*, 1995; Chyzik *et al.*, 1996). In our efficacy study we found that EPNs have the capability of infecting late L2, prepupae and pupae of WFT, and the susceptibility of late L2 to HBHK3 and SFN was significantly higher than pupal stages (refer the section 2.4.4 for details). In contrast Ebssa *et al.* (2001b) reported that both prepupae and pupae of WFT were equally susceptible to *S. feltiae* (Sylt strain). The possible reasons of lacking IGP between EPNs and *H. aculeifer* in the present study are: (a) minimum overlap of microhabitats, i.e. *H. aculeifer* attacks WFT on the surface of the soil or just beneath, whereas the two EPN strains used in our study search for their hosts in deeper layers; moreover, additional irrigation caused passive movement of EPNs to deeper layers of soil where WFT preferably pupates; (b) minimum temporal influence, i.e. application of EPNs prior to releases of *H. aculeifer* (two days early); this allowed the EPNs to settle in deeper layers of the soil before possibly encountering *H. aculeifer;* (c)

availability of additional food sources for *H. aculeifer*, because the peat used in our study provided plenty of organic matter as an additional food source for the mites.

Under natural conditions, populations of WFT comprise of different life stages. At the time when the plants were cut we observed a mixture of all thrips developmental stages, i.e. larvae, prepupae, pupae and adults, on the foliage. Hence a similar mixture of late L2, prepupae and pupae existed in the soil when the EPNs and *H. aculeifer* were released, thus mimicking natural conditions. However, in our methodological set-up we could not assess natural mortality of soil-dwelling life stages of WFT, leading to an overestimation of the treatment effect.

The efficacy of EPNs, *H. aculeifer* and combined releases of both natural enemy groups was calculated by comparing the adult emergence data between the untreated control and the different treatments. In all treatments where no mites were employed (i.e. the untreated control and the two EPN treatments), dead WFT adults were found on the soil surface. However, in all *H. aculeifer* treatments i.e. individual and combined releases, no adult WFT were recorded on the soil surface, indicating that the mites had fed on emerging WFT adults. Thus due to this methodological constraint we underestimated the impact of *H. aculeifer* on WFT in our experimental set-up. Ignatowicz (1974) also observed that *H. aculeifer* feeds on injured or freshly dead arthropods.

In conclusion our results indicate a new venue for biological control of WFT through combined releases of EPNs and *H. aculeifer*. In ongoing experiments we are investigating possibilities of using joint releases of EPNs and *H. aculeifer* against soil-dwelling life stages of WFT and additional releases of predatory bugs and mites against the foliage-feeding life-stages of *F. occidentalis*.

4 General discussion

In the Petri dish bioassay we introduced 20 late L2 of WFT into the soil and after one week recorded in the untreated controls an adult emergence of 85% and 100% as minimum and maximum, respectively. In a similar study Ebssa *et al.* (2001b) recorded after one week in control treatments a proportion of adult emergence in WFT of 70% and 80-90% after initially applying larvae and pupae and prepupae, respectively. Soria and Mollema (1995) detected about 43% mortality of WFT on susceptible cucumber genotype and also found that L2 was the most critical stage. Heyler *et al.* (1995) reported 68% adult emergence from WFT pupae in compost. In our Petri dish study we initially introduced L2 into the soil, thereby allowing them to enter the later developmental stages and thus as far as possible mimicking natural conditions in our methodological set-up. The data on WFT adult emergence in the untreated controls suggest that our experimental design was well suited for this kind of investigations.

The high proportion of peat in our test substrate resulted in a high field capacity of the soil. Therefore, in all experiments of the Petri dish study soil moisture content was maintained at about 65% (w/w). Moisture content of the soil is one of the most important factors for the survival and movement of EPNs (Gaugler, 1988). Maximum movement of IJs is expected when soils are at the field capacity and this in turn causes high rates of host infection by EPNs. However, species and strains of EPNs differ in their specific responses to abiotic factors (Curran, 1993). Movement of IJs in the soil is more important for cruisers than ambush foragers. This is possibly the reason why the two *Heterorhabtidis* strains, i.e. HBHK3 and HBHDO1 (cruisers), and SFN (possessing both ambush and cruiser foraging behaviours) benefited most from the high moisture content in the test substrates.

EPN-induced mortality was higher in the Petri dish study than in the pot experiments. Georgis and Gaugler (1991) showed that mortality caused by EPNs in the field was inferior to that recorded in laboratory assays, corroborating our findings in the Petri dish study and the pot experiments. In the Petri dish bioassay, the area of the soil surface and depth of the soil was comparatively smaller and less profound than in the pot experiments. In our study the soil depth in the Petri dish was about 1.5 cm. Georgis and Poinar (1983) showed that EPNs introduced 14 cm below the soil surface (using silica sand as substrate) successfully infected *G. mellonella* pupae located 10 cm above and below the point of application. Because of the smaller soil depth in our Petri dish experiments, EPNs were able to reach their target hosts more easily and at the same time by spending comparatively less searching energy than in the pot environments which might have been the reason for the higher mortality of WFT in the Petri dish bioassay than in the pot experiments.

One critical objective of combined releases of more than one natural enemy specie for biological pest control is to lower the dose rate or number of each enemy released compared to single applications. This is of paramount importance from an economical point of view as well as for avoidance of possible inter-specific interference. In our study we applied the two EPN species at a rate of 400 IJs/cm^2 soil and ten adult *H. aculeifer* per pot and found that the combined effect of two natural enemies was significantly higher than the use of either species alone. Selection of EPN dose rate was based on the results of our efficacy studies (refer to section 2.4.5) as well as on the results of previous studies (Ebssa, 2001b; Chyzik *et al.*, 1996). Abou-awad *et al.* (1989) reported that females of *Hypoaspis* spp. are more voracious than males. Hence, in future studies the effect of female *H. aculeifer* for control of WFT need to be investigated. Moreover, more attempts

should be made to further reduce the dose rate of IJs in order to render such an approach more economic.

5 Literature cited

Abou-awad, B.A., Nasr, A.K., Gomaa, E.A., and Abou-elela, M.M. 1989. Feeding, development and reproduction of the predatory mite, *Hypoaspis vacua* on various kinds of food substrates (Acari: Laelapidae). *Insect Science Application* **10**, 503-506.

Allen, W.R., and Broadbent, A.B. 1986. Transmission of tomato spotted wilt virus in Ontario greenhouses by the western flower thrips, *Frankliniella occidentalis* Pergande. *Canadian Journal of Plant Pathology* **8**, 33-38.

Altena, K., and Mulder, S. 2000. *Hypoaspis aculeifer* – a new dimension for thrips control. Koppert on line. http://www.koppert.nl/hypoaspis.html. Accessed on 07.01.2000.

Anonymous, 2000. Thrips and their Biocontrol. http://florawww.eeb.uconn.edu/ipm Accessed on 06.01.00.

Bakker, F.M., and Sabelis, M.W. 1989. How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. *Entomologica Experimentalis et Applicata* **50**, 47-51.

Bathon, H. 1996. Impact of entomopathogenic nematodes on non-target hosts. *Biocontrol, Science and Technology* **6**, 421-434.

Bennison, J.A., and Jacobson, R.J. 1991. Integrated control of *Frankliniella occidentalis* Pergande in UK cucumber crops-evaluation of a controlled release system of introducing *Amblyseius cucumeris*. *Medelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit, Gent* **56**, 251-258.

Boemare, N., Laumond, C., and Mauleon, H. 1996. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. *Biocontrol, Science and Technology* **6**, 333-345.

Bracken, G.K. 1990. Susceptibility of first-instar cabbage maggot, *Delia radicum* (L.) (Anthomyiidae: Diptera), to strains of entomogenous nematodes *Steinernema feltiae* (Filipjev), *S. bibionis* (Bovien), *Heterorhabditis bacteriophora* Poinar, and *H. heliothidis*. *Canadian Entomologist* **122**, 633-639.

Broadbent, A.B., and Pree D. 1997. Resistance to insecticides in populations of *Frankliniella occidentalis* Pergande (Thysanoptea: Thripidae) from greenhouses in the Nigara region of Ontario. *Canadian Entomologist* **129**, 907-913.

Broadbent, A.B., Allen, W.R., and Foottit, R.G. 1987. The association of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) with greenhouse crops and the tomato spotted wilt virus in Ontario. *The Canadian Entomologist* **119**, 501-503.

Brødsgaard, H.F. 1989. *Frankliniella occidentalis* (Thysanoptera: Thripidae)- a new pest in Danish glasshouses.: a review. *Tidsskrift foer Planteavl* **93**, 83-91.

Brødsgaard, H.F. 1993. Cold hardness and tolerance to submergence in water in *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Environmental Entomology* **22**, 647-653.

Burman, M. 1982. *Neoaplectana carpocapsae*: Toxin production by axenic insect parasitic nematodes. *Nematologica* **28**, 62-70.

Campbell, L.R., and Gaugler, R. 1991. Role of the sheath in the desiccation tolerance of two entomopathogenic nematodes. *Nematologica* **37**, 324-332.

Campbell, L.R., and Gaugler, R. 1993. Nictation behaviour and its ecological implications in the host search strategies of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Behaviour* **126**, 155-169.

Chambers, R.J., Long, S., and Helyer, N.L. 1993. Effectiveness of *Orius laevigatus* for the control of *Frankliniella occidentalis* on cucumber and pepper in the UK. *Biocontrol, Science and Technology* **3**, 295-307.

Cho, J.J., Mau R.F., German, T.L., Hartmann, R.W., Yudin, L.S., Gonsalves, D., and Provvidenti, R. 1989. A multidisciplinary approach to management of tomato spotted wilt virus in Hawaii. *Plant Disease* **73**, 375-383.

Chyzik, R., Glazer, I., and Klein, M. 1996. Virulence and efficacy of different entomopathogenic nematode species against Western Flower Thrips (*Frankliniella occidentalis*). *Phytoparasitica* **24**, 103-110.

Curran, J. 1993. Post-application biology of entomopathogenic nematodes in soil. In "*Nematodes and the biological control of insect pests*" (R. Bedding, R. Akhurt, and H.K. Kaya, Eds.), pp. 67-78, CSIRO publications, East-Melbourne, Australia.

Downes, M.J., and Griffin, C.T. 1996. Dispersal behaviour and transmission strategies of the entomopathogenic nematodes *Heterorhabditis* and *Steinernema. Biocontrol, Science and Technology* **6**, 347-356.

Drees, M.B., and Cole, L.C. 1988. Western Flower Thrips on Ornamental Plants. In "Proceedings of the Fourth Conference on Insect and Disease Management on Ornamentals" (T.G. Ali, M.S. Hall, and M.P. Parella, Eds.), Society of American Florists. http://entowww.tamu.edu/extension/bulletin/uc/uc-017.htr. Accessed on 24.11.99.

Dunphy, G.B., and Webster, J.M. 1984. Interaction of *Xenorhabdus nematophilus* subsp. *nematophilus* with the haemolymph of *Galleria mellonella. Journal of Insect Physiology* **30**, 883-889.

Dunphy, G.B., and Thurston, G.S. 1990. Insect immunity. In "*Entomopathogenic nematodes in biological control*"(R. Gaugler, and H.K. Kaya, Eds.), pp. 301-318, CRC Press, Boca Raton, FL.

Ebssa, L. 2000. The potential of entomopathogenic nematodes to control soil-borne stages of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). M.Sc Thesis, University of Hanover, Germany.

Ebssa, L., Borgemeister, C., Berndt, O., and Poehling, H.-M. 2001a. Impact of different soil-dwelling stages of wesstern flower thrips, *Frankliniella occidentalis* () (Thysanoptera: Thripidae) in the laboratory and under semi-field conditions. *Biocontrol, Science and Technology* **11**, 515-525.

Ebssa, L., Borgemeister, C., Berndt, O., and Poehling, H.-M. 2001b. Efficacy of entomopathogenic nematodes against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Submitted for the publication in the *Journal of Invertebrate Pathology*.

Ehler, L.E. 1979. Utility of facultative secondary parasite in biological control. *Environmental Entomology* **8**, 829-832.

Ehlers, R.-U. 1996. Current and future use of nematodes in biocontrol: Practice and commercial aspects with regard to regulatory policy issues. *Biocontrol, Science and Technology* **6**, 303-316.

Endo, B.Y., and Nickle, W.R. 1994. Ultra-structure of the buccal cavity region and oesophagus of the insect parasitic nematode, *Heterorhabditis bacteriophora*. *Nematologica* **40**, 379-398.

English-Loeb, G., Villani, M., Martison, T., Forsline, A., and Consolie, N. 1999. Use of entomopathogenic nematodes for control of grape phylloxera (Homoptera: Phylloxeridae): A laboratory evaluation. *Environmental Entomology* **28**, 890-894.

Epsky, N.D., Walter, D.E., and Capinera, J.L. 1988. Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes (Rhabditida: Steinernematidae and Heterorhabditidae). *Journal of Economic Entomology* **81**, 821-825.

Gaugler, R. 1981. Biological control potential of neoaplectanid nematodes. *Journal of Nematology* **13**, 241-249.

Gaugler, R. 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. *Agriculture, Ecosystem and Environment* **24**, 351-436.

Gaugler, R., Bednarek, A., and Campbell, J.F. 1992 . Ultraviolet inactivation of heterorhabditid and steinernematid nematodes. *Journal of Invertebrate Pathology* **59**, 155-160.

Gaugler, R., Lewis, E., and Stuart, R.J. 1997. Ecology in the service of biological control: the case of entomopathogenic nematodes. *Oecologia* **109**, 483-489.

Gaugler, R., Grewel, P., Kaya, H.K., and Smith-Fiola, D. 2000. Quality assessment of commercially produced entomopathogenic nematode. *Biological Control* **17**, 100-109.

Gaugler, R., Leback, L., Nakagaki, B., and Boush, G.M. 1980. Orientation of the entomogenous nematode *Neoaplectana carpocapsae* to carbon dioxide. *Environmental Entomology* **9**, 649-652.

Gazit, Y., Rössler, Y., and Glazer, I. 2000. Evaluation of entomopathogenic nematodes for the control of Mediterranean fruit fly (Diptera: Tephritidae), *Biocontrol, Science and Technology* **10**, 157-164.

Georgis, R., and Poinar, G.O. 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). *Journal of Entomology* **15**, 308-311.

Georgis, R., and Gaugler, R. 1991. Predictability in biological control using entomopathogenic nematodes. *Journal of Economic Entomology* **84**, 713-720.

Gillespie, D.R., and Ramey, C.A. 1988. Life history and cold storage of *Amblyseius cucumeris* (Acarina: Phytoseiidae). *Journal of the Entomological Society of British Colombia* **85**, 71-76.

Gillespie, D.R., and Quiring, D.M.J. 1990. Biological control of fungus gnats, *Bradysia* spp. and western flower thrips, *Frankliniella occidentalis*, in glasshouses using a soil dwelling predatory mite, *Geolaelaps* sp. Nr *aculeifer*. *Canadian Entomologist* **122**, 975-983.

Glazer, I. 1996. Survival mechanisms of entomopathogenic nematodes, *Biocontrol, Science and Technology* **6**, 373-378.

Glazer, I., and Navon, A. 1990. Activity and persistence of entomopathogenic nematodes tested against *Heliothis armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **83**, 1795-1800.

Glockemann, B. 1992. Biological control of *Frankliniella occidentalis* on ornamental plants using predatory mites. *EPPO Bulletin* **22**, 397-404.

Grewal, P.S, Gaugler, R., and Georgis, R. 1995. Predictors of foraging strategy in entomopathogenic nematodes. In "*Ecology and transmission strategies of entomopathogenic nematodes*" (C.T. Griffin, R.L. Gwynn, and J.P. Masson, Eds.), pp. 95-104, Brussels, Luxembourg.

Grewal, P.S., Lewis, E.E., and Gaugler, R., and Campbell, J.F. 1994. Host finding behaviour as a predator of foraging strategy in entomopathogenic nematodes. *Parasitology* **108**, 207-215.

Heyler, N.L., Brobyn, P.N., and Edmondson, R.N. 1995. Control of western flower thrips (*Frankliniella occidentalis* Pergande pupae in compost. *Annals of Applied Biology* **127**, 405-412.

Hominick, W.M., and Reid, A.P. 1990. Perspectives on entomopathogenic nematology. *In* "Entomopathogenic Nematodes in Biological Control" (R. Gaugler, and H.K. Kaya, Eds.), pp. 327-345, CRC Press, Boca Raton, FL.

Hominick, W.M., Reid, A.P., Bohan, D.A., and Briscoe, B.R. 1996. Entomopathogenic Nematodes: Geographical distribution and the convention on biological diversity. *Biocontrol, Science and Technology* **6**, 317-331.

Hominick, W.M., Hunt, D.J., Reid, A.P., Briscoe, B.R., and Bohan, D.A. 1999. Biosystematics, phylogeny and population genetics of entomopathogenic nematodes. In *"Taxonomy, phylogeny and gnotobiological studies of entomopathogenic nematode bacterium complexes cost 819 Entomopathogenic nematodes"* (N. Boemare, P. Richardson, and F. Coudert, Eds.), pp. 45-53, European Commission, Rue de la Loi/Wetstraat 200, B-1049, Brussels.

Ignatowicz, S. 1974. Observations on the biology and development of *Hypoaspis aculeifer* Canestrini, 1885 (Acarina: Gamasides). *Zoologica poloniae* **24**, 41-59.

Immaraju, J.A., Paine, T.D., Bethke, J.A., Robb, K.L., and Newman, J.P. 1992. Western flower thrips (Thysanoptera: Thripidae) resistance to insecticides in coastal California greenhouses. *Journal of Economic Entomology* **85**, 9-14.

Insera, R.N., and Davis, D.W. 1983. *Hypoaspis* nr *aculeifer*: a predation on root-knot and cyst nematodes. *Journal of Nematology* **15**, 324-325.

Ishibashi, N., Young, F., Nakashima, M., Abiru, C., and Haraguchi, N. 1987. Effects of application of DD-136 on silkworm, *Bombyx mori*, predatory insect, *Agriosphodorus dohrni*, parasotiod, *Trichomalus apanteloctenus*, soil mites, and other non-target soil arthropods, with brief notes on feeding behaviour and predatory pressure of soil mites, tardigrades, and predatory nematodes on DD-136 nematodes. *In* "Recent advances in biological control of insect pests by entomogenous nematodes in Japan" (N. Ishibashi, Eds.), pp. 158-164, Ministry of Education, Culture, and Science, Saga, Japan.

Jackson, J.J., and Brooks, M.A. 1995. Parasitism of western corn rootworm larvae and pupae by *Steinernema carpocapsae*. *Journal of Nematology* **27**, 15-20.

Jacobson, R.J. 1997. Integrated pest management (IPM) in glasshouses. In *"Thrips as Crop Pests"* (T. Lewis, Eds.), pp. 639-666, CAB International, Wallingford, UK.

Jensen, S.E. 2000. Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integrated Pest Management Reviews* **5**, 131-146.

Kakehashi, N., Suzuki, Y., and Iwasa, Y. 1984. Niche overlap of parasitoids in host-parasitoid systems: its consequence to single versus multiple introduction controversy in biological control. *Journal of Applied Ecology* **21**, 115-131.

Kanagy, J.M.N., and Kaya, H.K. 1996. The possible role of marigold roots and ∝-tertheinyl in mediating host-finding by Steinernematid nematodes. *Nematologica* **42**, 220-231.

Kaya, H.K. 1978a. Interaction between *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) and *Apanteles militaris* (Hymenoptera:Brancinidae), a parasitoid of the armyworm, *Pseudaletia unipuncta*. *Journal of Invertebrate Pathology* **13**, 358-364.

Kaya, H.K. 1978b. Infectivity of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* to pupae of parasite *Apanteles militaris*. *Journal of Nematology* **10**, 241-244.

Kaya, H.K. 1990. Soil ecology. In "*Entomopathogenic nematodes in biological control*" (R. Gaugler, and H.K. Kaya, Eds.), pp. 93-115, CRC Press, Boca Raton, FL.

Kaya, H.K. 1993. Entomogenous and entomopathogenic nematodes in biological control. In *"Plant Parasitic Nematodes in Temperate Agriculture"* (K. Evans, D.L. Turdgill, and J.M. Webster, Eds.), pp. 101-106, CAB International, Wallingford, UK.

Kaya, H.K., and Hotchkin, P.G. 1981. The nematode *Neoaplectana carpocapsae* (Weiser) and its effect on selected ichneumonid and braconid parasites. *Environmental Entomology* **10**, 474-478.

Kaya, H.K., and Gaugler, R. 1993. Entomopathogenic nematodes. *Annual Review Entomology* **38**, 181-206.

Kaya, H.K., and Koppenhöfer, A.M. 1996. Effects of microbial and other antagonistic organism and competition on entomopathogenic nematodes. *Biocontrol, Science and Technology* **6**, 357-371.

Kaya, H.K., and Stock, S.P.1997 Techniques in insect nematology. In "*Manual of techniques in insect pathology*" (L. Lacey, Eds.), pp. 281-324, Academic Press, San Diego.

Kiman, Z.B., and Yeargan, K.V. 1985. Development and reproduction of the predator *Orius insidiosus* (Hemiptera: Anthocoridae) reared on diets of selected plant material and arthropod prey. *Annals of Entomological Society of America* **78**, 464-467.

Kirk, W.D. J. 1997a. Distribution, abundance and population dynamics. In "*Thrips as crop pests*" (T. Lewis, Eds.), pp. 217-258, CAB International, Wallingford, UK.

Kistler, R.A. 1985. Host-age structure and parasitism in a laboratory system of two hymenopterous parasitoids and larvae of *Zabrotes subfasciatus* (Coleoptera: Bruchidae). *Environmental Entomology* **14**, 507-511.

Klein, M.G. 1990. Efficacy against soil-inhabiting insect pests, In "*Entomopathogenic nematodes in biological control*" (R. Gaugler, and H. K. Kaya Eds.), pp. 195-214, CRC, Boca Raton, Florida.

Kondo, E., and Ishibashi, N. 1986. Nictataing behaviour and infectivity of entomogenous, *Steinernema* spp. to the larvae of common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), on the soil surface, *Applied Entomology and Zoology* **21**, 553-560.

Leback, L.M., Gaugler, R., Kaya, H.K., Hara, A.H., and Marshall, M.W. 1993. Host age suitability of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: steinernematidae). *Journal of Invertebrate Pathology* **62**, 58-63.

Lei, Z., Rutherford, T.A., and Webster, J.M. 1992. Heterorhabditid behaviour in the presence of the cabbage maggot, *Delia radicum* and its host plants. *Journal of Nematology* **42**, 9-15.

Lindquist, R.K., and Casey, M.L. 1990. Evaluation of oils, soaps and natural product derivatives for leaf miner, foxglove aphid, western flower thrips and greenhouse whitefly control. *Ohio Florist's Association Bulletin* **727**, 3-5.

Miller, J.C. 1983. Ecological relationships among parasites and the practice of biological control. *Environmental Entomology* **12**, 620-624.

Mollema, C., Steenhuis, M.M., Inggamer, H., and Soria, C. 1995. Evaluating the resistance to *Frankliniella occidentalis* in cucumber, methods, genotype variation and effects upon thrips biology. *IOBC/WPRS Bulletin* **16**, 77-82.

Moritz, A. 1997. Structure, growth and development. In *"Thrips as crop pests"* (T. Lewis, Eds.), pp. 15- 63, CAB International, Wallingford, UK.

Mracek, Z., Gerdin, S., and (Weiser), J. 1981. Head and cuticular structure of some species in the family Steinernematidae (Nematoda). *Nematologica* **27**, 443-448.

Murphy, G.D., and Broadbent, A.B. 1996. Adoption of IPM by the greenhouse floriculture industry in Ontario, Canada. *IOBC/WPRS Bulletin* **19,** 111-114.

Nealson, K.H., Schmidt, T.M., and Bleakly, B. 1990. Physiology and biochemistry of *Xenorhabdus.* In "*Entomopathogenic Nematodes in Biological Control*"(R. Gaugler, and Kaya, H.K, Eds.), pp. 271-284, CRC Press, Boca Raton, FL.

Palmer, J. 1989. *Frankliniella occidentalis*: the western flower thrips. *British Cactus and Succulent Journal* **7**, 33.

Parkman, J.P., Frank, J.H., Nguyen, K.B., and Smart, G.C. 1993. Dispersal of *Steinernema sacpterisci* (Rhabditida: Steinernematidae) after inoculative applications for mole cricket (Orthoptera: Gryllopalpidae) control in pastures. *Biological Control* **3**, 226-232.

Poinar, G.O. Jr. 1979. Nematodes for biological control of insects, CRC, Boca Raton, Florida.

Poinar, G.O. Jr. 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. In "*Entomopathogenic nematodes in biological control*" (R. Gaugler, and H. K. Kaya, Eds.), pp. 23-61, CRC Press, Boca Raton, Florida.

Poinar, G.O. Jr. 1993. Origins and phyletic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema*. *Fundamental and Applied Nematology* **16**, 333-338.

Ramakers, P.M.J. 1995. Biological control using oligophagous predators. In "*Thrips biology and management*" (B.L. Parker, M. Skinner, and T. Lewis, Eds.), pp. 225-229, Plenum Press, New York.

Riudavets, J. 1995. Predators of *Frankliniella occidentalis* Pergande and *Thrips tabaci* Lind.: a Review. *Wageningen Agricultural Papers* **95-1**, 43-87.

Robb, K.L., Parrella, M.P. 1987. Western Flower Thrips in California floriculture greenhouses. Typesscript, 10 pp. University of California, Riverside.

Rosenheim, J.A., Welter, S.C., Johnson, M.W., Man, R.F.L., and Gusukuma-Minuto, L.R. 1990. Direct feeding damage on cucumbers by mixed species infestations of *Thrips palmi* and *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Economic Entomology* **83**, 1514-1525.

Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J., and Jaffee, B.A. 1995. Intraguild predation among biological-control agents. Theory and evidence. *Biological Control* **5**, 303-335.

Sabelis, M.W., and Van Rijn, P.C.J. 1997. Predation by insects and mites. In "*Thrips as crop pests*" (T. Lewis, Eds.), pp. 259-354, CAB, Wallingford, Oxon, UK.

Salguero Navas, V.E., Finderburk, J.E., Beshear, R. J., Olson, S.M., and Mack, T.P. 1991. Seasonal patterns of *Frankliniella* spp. (Thysanoptera: Thripidae) in tomato flowers. *Journal of Economic Entomology* **84**, 1818-1822.

SAS Institute, 1999. "SAS/Stat Users Guide." SAS Institute Inc., Cary, NC, USA.

Schmidt, J., and All, J.N. 1979. Attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to common excretory products of insects. *Environmental Entomology* **8**, 55-61.

Selvan, S., Grewal, P.S., Gaugler, R., and Tomalak, M. 1994. Evaluation of steinernematid nematodes against *Popillia japonica* (Coleoptera: Scarabaeidae) larvae: species, strains and rinse after application. *Journal of Economic Entomology* **87**, 605-609.

Shannag, H.K., and Capinera, J.L. 2000. Interference of *Steinernema carpocapsae* (Nematoda: Steinernematidae) with *Cardiochiles diaphaniae* (Hymenoptera: Braconidae), a parasitoid of Melonworm and Pickleworm (Lepidoptera: Pyralidae). *Environmental Entomology* **29**, 612-617.

Shapiro, D.I., Cate, J.R., Pena, J., Hunsberger, A., and Mccoy, C.W. 1999. Effects of temperature and host age on suppression of *Diaprepes abbreviatus* (Coleoptera: Curculinidae) by entomopathogenic nematodes. *Journal of Economic Entomology* **5**, 1086-1092.

Smart, G.C. 1995. Entomopathogenic nematodes for the biological control of insects.Viewpoint. *Journal of Nematology* **27**, (Suppl. 4) 529-534.

Smits, P.H. 1996. Post-application persistence of entomopathogenic nematodes. *Biocontrol, Science and Technology* **6,** 379-387.

Soria, C., and Mollema, C. 1995. Life-history parameters of western flower thrips on susceptible and resistant cucumber genotypes. *Entomologica Experimentalis et Applicata* **74**, 177-184.

Spiridonov, S.E., Akhmedov, E.N., and Belostotsk, F.N. 1991. Proliferation of symbiotic bacteria in the intestinal vesicles of invasive larvae of *Neoaplectana* spp. (Nematoda: Steinernematidae). *Helminthologica* **28**, 141-142.

Strauch, O., Stoessel, S., and Ehlers, R.-U. 1994. Culture conditions define automictic or amphimictic reproduction in entomopathogenic rhabditid nematodes of the genus *Heterorhabditis*. *Fundamental and Applied Nematology* **17**, 575-582.

Stuart, R.J., and Gaugler, R. 1994. Patchiness in populations of entomopathogenic nematodes. *Journal of Invertebrate Pathology* **64**, 39-45.

Surrey, M.R., and Davis, R.J. 1996. Pilot scale liquid culture and harvesting of an entomopathogenic nematode *Heterorhabditis bacteriophora*. *Journal of Invertebrate Pathol*ogy **67**, 92-99.

Tanda, K., and Fuxa, J.R. 1987. The pathogen population. In "*Epizootiology of insect diseases*" (J.R. Fuxa, and K. Tanda, Eds.), pp. 113-157, Wiley, New York.

Timper, P., and Kaya, H.K. 1989. Role of the 2nd stage cuticle of entomogenous nematodes in preventing infection by nematophagous fungi. *Journal of Invertebrate Pathology* **54**, 314-321.

Tomalak, M. 1994. Genetic improvement *Steinernema feltiae* for integrated control of the western flower thrips, *Frankliniella occidentalis*. *IOBC/WPRS Bulletin* **17**, 17-20.

Tommasini, M.G., and Maini, S. 1995. *Frankliniella occidentalis* and other thrips harmful to vegetables and ornamental crops in Europe. *Wageningen Agricultural University Papers* **95-1**, 1-42.

Triggiani, O., and Poinar, G.O. Jr. 1976. Infection of adult lepidoptera by *Neoaplectana carpocapsae* (Nematoda). *Journal of Invertebrate Pathology* **27**, 413-414.

Ullman, D.E., Sherwood, J.L., and German, T.L. 1997. Thrips as vectors of plant pathogens. In "*Thrips as Crop Pests*" (T. Lewis, Eds.), pp. 539-565, CAB International, Wallingford, UK.

Vestergaard, S., Gillespie, A.T., Butt, T.M., Schreiter, G., and Eilenberg, J. 1995. Pathogenicity of the hypomycete fungi *Verticilium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *Frankliniella occidentalis*. *Biocontrol, Science and Technology* **5**, 185-192.

Wardlow, L.R., Davies, P.J., and Brough, W. 1992. Integrated pest management in protected ornamentals in England. *Bulletin OEPP/EPPO Bulletin* **22**, 493-498.

Watanable, H. 1987. The host population. In "*Epizooitiology of insect diseases*" (J.R. Fuxa, and Y. Tanda, Eds.), pp. 71-112, Wiley, New York.

Williams, C.M.E.De 2001. Biological control of thrips on ornamental crops: Interactions between the predatory mite *Neoseiulus cucumeris* (Acari: Phytoseiidae) and Western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on Cyclamen. *Biocontrol, Science and Technology* **11**, 41-55.

Woodring, J.L., and Kaya, H.K. 1988. Steinernematid and Heterorhabditid nematodes. A Handbook of biology techniques. *Southern Cooperative Series Bulletin* 331. Arkansas Agricultural Experiment Station, Fayetteville, AR.

Yudin, L.S., Cho J.J., and Mitchell, W.C. 1986. Host range of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) with special reference to *Leucaenea glauca*. *Environmental Entomology* **15**, 1292-1295.

Zhao, G., Liu, W., Brown, J.M., and Knowles, C.O. 1995. Insecticide resistence in field and laboratory strains of western flower thrips (Thysanoptea: Thripidae). *Journal of Economic Entomology* **88**, 1164-1170.