# Entomopathogenic nematodes and soil-dwelling predatory mites: Suitable antagonists for enhanced biological control of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)?

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## Summary

*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (**W**estern **F**lower **T**hrips, WFT) is one of the most important pests damaging a wide range of economic important crops in protected cultures worldwide. Its cryptic life cycle, combined with a very short generation time and the ability to rapidly develop resistances against insecticides, are characteristics that make pest control extremely difficult. Moreover, commonly used natural enemies often do not lead to sufficient control levels. However it has been repeatedly reported that the life cycle of *F. occidentalis* includes a soil passage, which is still neglected in biocontrol strategies. Subjects of the present study were the quantification of the soil passage of *F. occidentalis* and the evaluation of the effectiveness of predatory soil mites and entomopathogenic nematodes (EPN) as natural enemies against WFT. Thus, microcosm experiments were carried out on individual potted plants, which had been covered with acryl-glass tubes. The extent of the soil passage and the control success for synchronised and mixed WFT populations was quantified by the use of photo eclectors. Additionally, the suitability and susceptibility of the soil-dwelling thrips instars towards predation or infestation by predatory mites or EPN was investigated in arena experiments. The soil passage was quantified with *Phaseolus vulgaris* and three ornamentals (*Tagetes patula nana*, *Saintpaulia ionantha* and *Dendranthema x grandiflorum*) as host plants. The results show that only a small part of a *F. occidentalis* population pupates on the plant and that the variation between host plant species is small. On *P. vulgaris,* only 1-3 % of the thrips population remained on the plant for pupation. With flowering ornamental host plants the proportion increased slightly on *D. grandiflorum* (4.6%) and *S. ionantha* (6.8%) and was highest on *T. patula nana* (7.15%). Flowers were not preferred as the pupation site, a maximum of one third of the thrips that pupated on the plant were located in the flowers.

The introduction of five predatory mites to the soil caused a thrips mortality of at least 44.9%. Doubling the predator density to 10 *Hypoaspis miles* increased thrips mortality to 61%. A maximum thrips mortality of 80.5% was achieved by 20 *H. aculeifer*. Over all, *H. aculeifer* showed a higher efficiency as a natural enemy against soil-dwelling thrips instars than *H. miles*. Nevertheless, at least a small part of the thrips population escaped from predation in spite of the fact that in arena experiments high predation rates of *Hypoaspis* spp. were observed. All thrips instars were suitable as prey and for the maintenance of reproduction for both *Hypoaspis* species. The number of killed thrips larvae, prepupae or pupae was similar for both *H. miles* and *H. aculeifer*. On average, females of the latter preyed on 3.5 thrips instars and laid 2.5 eggs per day, whereas females of

*H. miles* preyed on 1.61 thrips and laid 0.8 eggs per day. Males of both species killed 0.6 thrips per day. Cannibalism, as a possible reason for a reduced efficacy of the predatory mites against WFT, could be excluded since the cannibalism rate of both *Hypoaspis* spp. was very low and rarely exceeded one conspecific individual within three days. Only *H. aculeifer* nymphs showed a cannibalism rate of on average one conspecific egg per day. In the presence of alternative prey, cannibalism never occurred. Conceivable reasons for the limited thrips control in microcosm experiments are discussed.

Possible alternative natural enemies of thrips instars in the soil are EPN. To investigate the efficiency of EPN against thrips instars in the soil, six different strains were selected which included two *Heterorhabditis bacteriophora* strains (*H. bacteriophora* HK3 (H.b H) and *H. bacteriophora* HB Brecan (H.b B)), three *Steinernema feltiae* strains (*S. feltiae* Sylt (S.f S), *S. feltiae* OBSIII (S.f O) and *S. feltiae* strain CR (S.f C)) and one *S. carpocapsae* strain (DD136 (S.c D)). All tested thrips instars were susceptible to all EPN strains. The most virulent strains were S. *feltiae* (S), S. *carpocapsae* (D) and H. *bacteriophora* (H), but a high concentration of 400 infective juveniles (IJ) per $cm^2$ was necessary to obtain high thrips mortality rates of at least 65%. Nevertheless, dose rates of 100–200 IJs/$cm^2$ already caused 30–50% mortality in WFT. The efficacy of S. *feltiae* (S) against different thrips instars in the soil revealed that improved thrips control was realized if the proportion of second instar larvae in the thrips population was low. WFT prepupa and pupa were similarly susceptible to *S. feltiae* and their proportion in the population did not affect the mortality caused by EPN. The highest mortality rate (80%) was recorded for populations consisting only of prepupae and/or pupae. In microcosm experiments, the impact of S. *feltiae* (S), S. *carpocapsae* (D) and H. *bacteriophora* (H) at concentrations of 400 and 1000 IJ/cm² was investigated against the thrips stages in the soil. All tested EPN strains at both dose rates significantly reduced WFT populations. Up to 70% reduction in the WFT population was obtained with the higher EPN concentrations.

Over all, both *Hypoaspis* species and the selected EPN strains can substantially reduce a thrips population and might be important antagonists to optimise *F. occidentalis* control in protected crops. However, both soil dwelling antagonists seem not to be able to keep *F. occidentalis* below an economic threshold level. It is recommended to combine the tested soil foraging thrips antagonists with predators acting on the plant parts above soil.

Keywords: *Frankliniella occidentalis*, Biological Control, *Hypoaspis* spp., Entomopathogenic Nematodes

## Zusammenfassung

*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) befällt weltweit als einer der bedeutendsten Schädlinge ein weites Spektrum an ökonomisch bedeutenden Kulturpflanzen. Seine versteckte Lebensweise in Kombination mit einer kurzen Generationsfolge und der Fähigkeit, sehr rasch Resistenzen gegenüber Insektiziden zu entwickeln, machen eine Bekämpfung extrem schwierig. Auch die allgemein gebräuchlichen natürlichen Gegenspieler führen häufig zu keiner ausreichenden Kontrolle. Darüber hinaus wird wiederholt von einer Bodenpassage im Lebenszyklus von *F. occidentalis* berichtet, welche jedoch bei der biologischen Bekämpfung vernachlässigt wird. Gegenstand der vorgestellten Arbeit war u.a. die Quantifizierung der Bodenpassage und die Untersuchung der Effektivität von räuberischen Bodenmilben und entomopathogenen Nematoden (EPN) als natürliche Gegenspieler von *F. occidentalis*. Dazu wurden Mikrokosmos-Experimente mit in Acryl-Glas-Röhren eingeschlossenen Pflanzen durchgeführt. Der Umfang der Bodenpassage und der Bekämpfungserfolg wurde bei synchronisierten und gemischten Populationen mit Hilfe von Photoeklektoren quantifiziert. Zusätzlich wurde die Eignung der Thrips-Bodenstadien als Beute bzw. Wirt für Raubmilben und EPN in Arena-Experimenten untersucht. Die Bodenpassage wurde an *Phaseolus vulgaris* und drei Zierpflanzen (*Tagetes patula nana*, *Saintpaulia ionantha* und *Dendranthema x grandiflorum*) als Wirtspflanze quantifiziert: Mehr als 90% einer Population von *F. occidentalis* verpuppte sich im Boden. Bei *P. vulgaris* blieben lediglich 1-3% auf der Pflanze. Der Anteil der Thripse, die auf den Zierpflanzen blieb, war etwas höher: Bei *D. grandiflorum* 4.6%, bei *S. ionantha* 6.8% und bei *T. patula nana* mit 7.15%. Dabei wurden die Blüten als Verpuppungsort nicht bevorzugt, maximal ein Drittel der Thripse, die sich auf der Pflanze verpuppten, war in den Blüten zu finden.

Der Einsatz von fünf Raubmilben führte zu einer Thripsmortalität von mindestens 44,9%. Durch eine Verdopplung der Prädatordichte von *Hypoaspis miles* konnte die Thripsmortalität auf 61% gesteigert werden. 20 *H. aculeifer* führten zu einer Mortalität von bis zu 80,5%. *H. aculeifer* zeigte gegenüber Bodenstadien eine größere Wirksamkeit als *H. miles*. Dennoch entkam zumindest ein kleiner Teil der Thripspopulation den Prädatoren, obwohl in Arena-Experimenten eine hohe Prädationsraten von *Hypoaspis* spp. beobachtet wurde. Alle Thripsstadien waren als Beute und für eine Aufrechterhaltung der Reproduktion beider *Hypoaspis*-Arten geeignet. Die Anzahl der getöteten Thripse war bei *H. miles* und *H. aculeifer* ähnlich: *H. aculeifer* Weibchen erbeuteten 3,5 Thripsstadien und legten 2,5 Eier pro Tag, während weibliche *H. miles* 1,61 Thripse töteten und 0,8 Eier legten. Männchen beider Arten töteten 0,6 Thripse pro Tag. Kannibalismus schied als Ursa-

che für eine begrenzte Wirksamkeit der Raubmilben gegenüber *F. occidentalis* aus, da die Kannibalismusrate bei beiden *Hypoaspis*-Arten sehr gering war und nur selten einen Artgenossen innerhalb von drei Tagen überstieg. Lediglich *H. aculeifer*-Nymphen verzehrten durchschnittlich ein Ei der eigenen Art pro Tag, wohingegen es in Anwesenheit von Alternativbeute bei beiden nie zu Kannibalismus kam. Mögliche Ursachen für eine verminderte Thripsbekämpfung im Mikrokosmos-Experiment werden diskutiert.

EPN könnten alternativ als natürliche Gegenspieler der Thripse im Boden herangezogen werden. Sechs ausgewählten EPN Stämmen wurden in ihrer Wirksamkeit gegenüber den Bodenstadien der Thripse untersucht: Zwei *Heterorhabditis bacteriophora* Stämme (*H. bacteriophora* HK3 (H.b H) und *H. bacteriophora* HB Brecan (H.b B)), drei *Steinernema feltiae* Stämme (*S. feltiae* Sylt (S.f S), *S. feltiae* OBSIII (S.f O) und *S. feltiae* CR (S.f C)) und der Stamm *S. carpocapsae* DD136 (S.c D). Alle untersuchten Thripsstadien waren anfällig gegenüber den EPN. Die Stämme mit der größten Virulenz waren S.f S, S.c D und H.b H.. Allerdings waren Konzentrationen von 400 infektiösen juvenilen Nematoden (IJ) pro cm² erforderlich, um eine Thripsmortalität von mindestens 65% zu erzielen. Aber bereits 100 bis 200 IJ/cm² führten zu einer Thripsmortalität von 30-50%. Die Wirksamkeit von S.f S gegenüber den unterschiedlichen Thripsstadien im Boden war umso höher je geringer der Anteil an L2-Stadien an der Thripspopulation war. Präpuppen und Puppen waren hingegen gleich anfällig, und ihr Anteil an der Population wirkte sich nicht auf die EPN-bedingte Thripsmortalität aus. Die höchste Thripsmortalität (80%) war zu beobachten, wenn sich die Population lediglich aus Präpuppen und/oder Puppen zusammensetzte. Ferner wurde in Mikrokosmos-Experimenten der Einfluss von S.f S, S.c D und H.b H in den Konzentrationen 400 und 1000 IJ/cm² gegenüber den Bodenstadien der Thripse untersucht. Alle getesteten EPN-Stämme konnten bei beiden Dosierungen die Thripspopulation signifikant reduzieren. Die höhere Nematodenkonzentration führte zu einer Reduktion der Thripspopulation von bis zu 70%.

Insgesamt betrachtet, können sowohl die *Hypoaspis*-Arten als auch ausgewählte EPN Stämme Populationen von *F. occidentalis* deutlich dezimieren und als Gegenspieler im Unterglasanbau fungieren. Beide sind alleine jedoch nicht in der Lage, Thripspopulationen auf einem niedrigen, nicht schädigenden Niveau zu halten. Es wird empfohlen, diese bodenaktiven Thripsantagonisten in Kombination mit Prädatoren, die auf den oberirdischen Pflanzenteilen agieren, einzusetzen.

Schlagworte: *Frankliniella occidentalis*, Biologische Schädlingsbekämpfung, *Hypoaspis* spp., Entomopathogene Nematoden

## Contents

[1] based on: Berndt, O., Meyhöfer, R. & Poehling, H.-M., Importance of soil passage in the ontogenesis of *Frankliniella occidentalis* and a comparison of *Hypoaspis miles* and *H. aculeifer* as predators of soil dwelling thrips stages. Submitted to *Biological control*

[2] based on: Denecke, A., 2001. Entwicklung des Kalifornischen Blütenthripses *Frankliniella occidentalis* (Thysanoptera: Thripidae) an ausgewählten Zierpflanzen unter besonderer Berücksichtigung der Bodenpassage. Diploma Thesis, Institute of Plant Diseases and Plant Protection, University of Hanover, Germany

[3] based on: Berndt, O., Meyhöfer, R. & Poehling, H.-M., Cannibalism among soil dwelling predatory mites: a reason for low predation efficiency of *Hypoaspis* spp. against thrips stages in the soil? Submitted to *Experimental and Applied Acarology*

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[5] based on: Ebssa, L., Borgemeister, C., Berndt, O. & Poehling, H.-M., 2001. Impact of entomopathogenic nematodes on different soil-dwelling stages of Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), in the laboratory and under semi-field conditions. *Biocontrol Science and Technology*, 11, 515-525

[6] based on: Ebssa, L., Borgemeister, C., Berndt, O. & Poehling, H.-M., 2001. Efficacy of entomopathogenic nematodes against soil-dwelling life stages of Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Invertebrate Pathology*, 78, 119-127

## Abbreviations

| | |
|---|---|
| EPN | Entomopathogenic Nematodes |
| GRSV | Groundnut Ringspot Tospovirus |
| H.b B | *Heterorhabditis bacteriophora* Brecon |
| H.b H | *Heterorhabditis bacteriophora* Poinar HK3 |
| IJ | Infective juveniles |
| INSV | Impatiens Necrotic Spot Tospovirus |
| IPM | Integrated Pest Management |
| L:D | Relation of light to darkness |
| L1 | First instar larva |
| L2 | Second instar larva |
| RH | Relative humidity |
| S.c D | *Steinernema carpocapsae* (Weiser) DD136 |
| S.f C | *Steinernema feltiae* (Filipjev) CR |
| S.f O | *Steinernema feltiae* OBSIII |
| S.f S | *Steinernema feltiae* Sylt |
| TCSV | Tomato Chlorotic Spot Tospovirus |
| TSWV | Tomato Spotted Wilt Virus |
| WFT | Western Flower Thrips |
| n.d. | No data |

# 1 Introduction

The Western Flower Thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (described by Pergande, 1895) was found in California in orchards and on various weeds. It was first seen in Europe 1983 in *Saintpaulia ionantha* Wendl nurseries in the Netherlands (Vrie, 1987, Mantel & Vrie, 1988, Vierbergen & Ulenberg, 1988). Since it was introduced nearly 20 years ago, it has spread rapidly throughout Europe and was soon valued to be one of the most important pests in protected plant production. Until now it has not lost its pest status (Parker *et al.*, 1995, Tommasini & Maini, 1995, Lewis, 1997a, 1998, Lenteren & Loomans, 1998). Nowadays, WFT is a serious pest on a wide range of crops throughout the world causing substantial economic losses (Shipp *et al.*, 1991, Robb & Parrella, 1995, Tommasini & Maini, 1995, Lewis, 1997a, b, 1998).

Being extremely polyphagous, *F. occidentalis* feeds on a wide range of wild plants and cultivated crops, including vegetables and ornamentals. The degree of damage depends on the plant tissue that is affected, the developmental stage of the plant, susceptibility of the cultivars or species attacked and salivary toxicity (Bournier, 1983 Childers, 1997). According to Kirk (1997a), the feeding behaviour of Thysanoptera is best described as piercing-sucking. Thus, *F. occidentalis* causes direct damage to the host plant by mechanically destroying the cells and damging the tissue of leaves, fruits or petals (Kirk, 1997a). The surface of the leaf exhibits emptied and discoloured cells, initially adopting a mother-of-pearl appearance and subsequently turning brown. If plants are massively infested, the leaves may wither and fall (Tommasini & Maini, 1995). The result of the feeding can vary from a deformation to the total destruction of the developing leaves or flower organs and subsequently scarring and deformation of fruits. Streaking, browning and distortion of leaves and/or petals or even buds of various flowering ornamentals (e.g. roses, gerberas, chrysanthemums, carnations, geraniums, pansies and marigolds) are frequent symptoms of thrips damage (Oetting *et al.*, 1993, Childers, 1997). Thrips feeding e.g. on immature cucumber fruits can result in silvery scarring, or even malformation of the fruit (Rosenheim *et al.*, 1990, Shipp *et al.*, 2000) and feeding on immature nectarine fruits causes minute scarring, which can develop into serious surface russetting on the mature fruit. Such fruit is generally downgraded at sale, but if damage is serious enough, the fruit is culled (Pearsall, 2000). The feeding on the foliage may also have an adverse impact on leaf size and photosynthesis, and eventually results in significant yield loss (Welter *et al.*, 1990, Shipp *et al.*, 1998, Shipp *et al.*, 2000).

The damage to leaves, flowers and fruits, which is caused by sap ingestion and puncturing the plants surface, is aggravated in dry climates and seasons, especially under glasshouse conditions, when heavily attacked plants lose moisture rapidly. Under these conditions, infestation can seriously reduce yields and sometimes render crops uneconomic (Lewis, 1997a).

*F. occidentalis* also feeds on pollen in anthers and on pollen scattered over floral surfaces (Kirk, 1997a) and often spreads the pollen over petals. This dislocated pollen, together with the presence of brownish faecal drops, is a clear indication of thrips infestation (Tommasini & Maini, 1995). Furthermore, WFT is able to induce indirect damage to the infested plant. Thysanoptera feed by first injecting saliva into plant cells. Lysins in the saliva kill the cell and their contents are then sucked in by the thrips. This feeding pattern means that the insects can acquire and consequently transmit virus-induced diseases. In fact, larvae puncture the virus-infected plant, thus absorbing viruses which go into the haemocoelic cavity through the digestive tube and finally into the salivary glands, from which they are then reinjected into healthy plants. Fifteen minutes are necessary for the larvae to acquire the virus. The time necessary for the virus to reach the saliva glands usually coincides with the time taken for the insect to develop into an adult, at which stage the virus has become highly virulent. The maximum period of transmission of the virus is 3-4 weeks after acquiring it (Tommasini & Maini, 1995). The period between acquisition and transmission, i.e. in which the thrips is not infectious, ranges from 4 to 14 days. Where development of first instar larvae into second instar larvae is slowed down by temperature factors, the second instar larvae are themselves already infesting agents (Bournier, 1983). If the infection by the insect has not occurred at the larval stage, adults do not contract the virus. Once infested, the thrips can remain virulent throughout its lifespan without transmitting the viral cells to its offspring (Ie, 1970, Tommasini & Maini, 1995).

Viruses transmitted in a persistent way like this are Tomato Spotted Wilt Virus (TSWV), Impatiens Necrotic Spot Tospovirus (INSV), Groundnut Ringspot Tospovirus (GRSV) and Tomato Chlorotic Spot Tospovirus (TCSV) all belonging to the genus *Tospovirus* (family Bunyaviridae) (Allen & Broadbent, 1986, Ullman *et al.*, 1997 and articles mentioned there).

Moreover, thrips may predispose plant tissues to subsequent invasion by bacterial or fungal pathogens as a result of the inflicted feeding injuries (Lewis, 1973, Ananthakrishnan, 1980, Bournier, 1983). Bacteria can probably penetrate into the plant through the

punctures made by thrips and fungal spores can easily be trapped in the bristles of thrips species and consequently deposited on healthy plants (Ghabn, 1932, Yarwood, 1943, Ondrej, 1973, Bournier, 1983, Tommasini & Maini, 1995, Childers, 1997).

At present, none of the available control options provides satisfactory results (e.g., Parrella *et al.*, 1999). A number of different characteristics make WFT an extremely problematic pest (Lewis, 1997a, 1998, Pearsall, 2000, Jensen, 2000a, b). Its minute size accommodates its repeatedly mentioned thigmotaxis, and the combination of both these factors makes even the recognition of an infestation extremely difficult, let alone its control. The eggs are laid in the plant tissue, where they are protected against any environmental rigours. The subsequent post-embryonic development involves two larval instars, a prepupa and a pupa stage before the adult stage (Tommasini & Maini, 1995, Moritz, 1997). Both the adults and the juvenile instars are thigmotacic and, therefore, particularly attracted to enclosed microhabitats, where they live and feed. Upon maturity, the larvae display a positive geotaxis coupled with a negative phototaxis, thus they move away from the flower or the plant towards the soil or hide in crevices on the plant (Tommasini & Maini, 1995). In the soil or leaf litter the larvae develop into prepupa and finally pupa. In these niches the thrips instars are well sheltered towards most chemical or biological attacks (Lewis, 1997c, Jensen, 2000a, b).

Sheltered thrips instars hardly come in contact with insecticides. Thus, chemical treatments have to be repeated frequently to reach all thrips, causing residue problems and disrupt Integrated Pest Management programmes that have been adopted (Riudavets, 1995). Thus, attempts to control WFT and thereby distribution of transmitted virus diseases by means of chemical treatments against the vectors are often not successful (Riudavets, 1995, Lewis, 1998). However, the management with chemicals is made difficult not only by the thrips' way of life but also by wide spread pesticide resistances (Immaraju *et al.*, 1992, Lenteren & Loomans, 1998, Jensen, 1998, 2000a, Kiers *et al.*, 2000, Jacobson *et al.*, 2001a). Insecticide resistance has evolved in many populations and to varyious active compounds (Immaraju *et al.*, 1992, Brødsgaard, 1994a, Robb *et al.*, 1995, Zhao *et al.*, 1995, Broadbent & Pree, 1997, Jensen, 1998, Kontesdalov *et al.*, 1998, Jensen, 2000b). The situation has become more serious since cross-resistance has been observed, e.g. resistance to methiocarb exists in populations of WFT never exposed to methiocarb before testing (Brødsgaard 1994a, Jensen, 2000b). Development of resis-

tance in *F. occidentalis* is fast because of the short generation time and high fecundity (Brødsgaard, 1989, Jensen, 2000b). In addition, the haplodiploid breeding system of *F. occidentalis*, in which resistance genes in the haploid males are directly exposed to selection following insecticide treatment, can accelerate the development of resistance (Denholm *et al.*, 1998, Jensen, 2000b). The latter is assisted by excessive pesticide use (Robb *et al.*, 1988, Riudavets, 1995). As the knowledge of these problems increases, the demand for other forms of pest control, in particular biological control, increases too.

Polyphagous predators offer the greatest potential for biological control of WFT on protected crops (Courcy Williams, 2001). The most widely employed species are phytoseiid mites of the genera *Neoseiulus* and *Amblyseius* (Manjunatha *et al.*, 1998, Courcy Williams, 2001, Jacobson *et al.*, 2001b) and anthocorid flower bugs of the genus *Orius* (Riudavets, 1995, Sabelis & Rijn, 1997, Wittmann & Leather, 1997, Riudavets & Castane, 1998, Meiracker & Sabelis, 1999, Courcy Williams, 2001). Both of these groups of natural enemies have been used extensively in biological control programmes on protected edible crops, such as sweet pepper and cucumber (Jacobson, 1997). Recently there has been considerable interest in transferring these methods of biological control to ornamental crops (Wardlow *et al.*, 1992, 1993, Brødsgaard, 1995, Murphy & Broadbent, 1996, Courcy Williams, 2001). *Amblyseius* (or *Neoseiulus*) *cucumeris* Oudemans and *Amblyseius barkeri* Hughes were found to reduce the populations of thrips, but they are only able to prey on first stage larvae, and most phytoseiid strains enter diapause at low temperature and short photoperiod conditions (Bakker & Sabelis, 1989, Courcy Williams, 2001). These Phytoseiidae are released in very high numbers as a biological pesticide and they are easy and economical to rear. Since the control effect of Phytoseiidae is sometimes limited, Anthocoridae (*Orius* spp.) became more important (Riudavets, 1995). However, anthocorid bugs often tend to leave the crop or even the glasshouse. Nevertheless, they are commonly released as biocontrol agents. There are occasions, however, when control with these biocontrol agents fails. This is usually due to the application of fungicides that are harmful to Phytoseiidae, or because of the abiotic conditions in the glasshouse environment (high temperatures, short photoperiod) or the sudden immigration of large numbers of *F. occidentalis* from other crops or from outside (Malais & Ravensberg, 1992, Shipp & Houten, 1996, Tommasini & Nicoli, 1996, Shipp *et al.*, 2000, Kiers *et al.*, 2000, Jacobson *et al.*, 2001a). In order to reduce the risk of failing or insufficient control measures, alternative and additional control strategies are needed.

A characteristic feature of the commonly utilised thrips antagonists described above is that they prey only on the plant inhabiting developmental thrips stages. Although it is known that a considerable part of a *F. occidentalis* population withdraws to the soil for pupation (Palmer, 1989, Childers *et al.*, 1994, Helyer *et al.*, 1995, Kirk, 1996). Thus, at least two developmental stages of *F. occidentalis* are excluded from common beneficials attack. This soil passage in the life cycle of *F. occidentalis* is neglected in control strategies, even though it offers the opportunity to search for antagonists foraging in the same environmental area. Most encouraging are predatory soil mites of the genus *Hypoaspis* and entomopathogenic Nematodes (EPN).

In one section of the presented research *Hypoaspis* mites were examined. The genus *Hypoaspis* is cosmopolitan and includes exclusively predatory mites found in glasshouses as well as outside. *Hypoaspis aculeifer* (Canestrini) and *H. miles* (Berlese) (Acarina: Laelapidae) are of special interest: They prey on a wide spectrum of organisms in the soil, including small insects, mites, nematodes and Enchytreae (Keith *et al.*, 1964, Barker, 1969, Karg, 1982, Ragusa *et al.*, 1986, Sardar & Murphy, 1987, Ragusa & Zedan, 1988, Gillespie & Quiring, 1990, Glockemann, 1992, Chambers *et al.*, 1993, Lesna *et al.*, 1995, 1996). *Hypoaspis* mites are already used as biocontrol agents to control mushroom flies and root mites especially in plant breeding and mushroom production (Gillespie & Quiring, 1990, Chambers *et al.*, 1993, Lesna *et al.*, 1995, Jess & Kilpatrick, 2000). In the literature an impact on thrips is also mentioned (Glockemann, 1992, Brødsgaard *et al.*, 1996). Despite frequent success in controlling Sciaridae (Chambers *et al.*, 1993, Wright & Chambers, 1994, Enkegaard *et al.*, 1997, Ali *et al.*, 1999, Jess & Kilpatrick, 2000) or bulb mites (Lesna *et al.*, 1995, 1996), the efficiency of *Hypoaspis* spp. often is limited (Gillespie & Quiring, 1990, Glockemann, 1992, Wright & Chambers, 1994, Conijn *et al.*, 1997, Ydergaard *et al.*, 1997, Lesna *et al.*, 2000). Therefore, the aim of the project was to elucidate the ability of these predatory soil mites to control *F. occidentalis*.

*Phaseolus vulgaris* plants were utilised as a model system to examine whether the part of a thrips population, leaving the host plant and pupating in the soil, is large enough to justify any attempts to control this pest by ground foraging predators like *H. miles* and *H. aculeifer*. Subsequently it was examined whether these mites had any impact on synchronised populations of WFT in the model system. In a further series of experiments, the population dynamics of WFT were examined to find out whether the soil passage also takes place on ornamental plants with varying habits and, if so, to what extent it occurs. Moreover, it was examined how many thrips are actually consumed by *Hypoaspis* mites

and whether a reproduction exclusively preying on thrips instars is possible. Finally experiments were conducted to investigate whether cannibalism among the different developmental stages of *H. miles* and *H. aculeifer* might be the reason for limited predation efficiency on a target pest.

Entomopathogenic nematodes and their utilisation for thrips control was the subject of the second section of the presented investigation. Among the about 30 families of Nematodes associated with insects, most attention has been focused on two families: Steinernematidae and Heterorhabditidae. These entomopathogenic nematodes (EPN) are associated with pathogenic symbiotic bacteria that enable them to rapidly kill infested hosts. Several species of the two genera *Steinernema* and *Heterorhabditis* are used as commercial pest control agents (Driesche & Bellows, 1996). According to Poinar (1986), the following attributes make them ideal candidates for the biocontrol of various soil inhabiting pests: Most species show a wide host range, an infected host is usually killed very fast, they are easily propagated in artificial media enabling large scale production, the applicable infective stages can be stored for a long period of time, and finally their application is apparently safe for the environment.

The infective stages invade the host through natural openings or even the cuticle and penetrate into the haemocoel. Here they release the symbiotic bacteria they mutualistically carry with them in a specially built section of their gut. These bacteria are pathogenic for the host and quickly kill it within two days. The bacteria now multiply and prepare the ground for the nematodes development inside the dead host where the nematodes live saprophytically on the decomposing host tissues and the bacteria. Inside the cadaver the EPN develop to adults, mate and reproduce, their progeny develops to infective stages, which leave the cadaver to invade new hosts (Driesche & Bellows, 1996). Best results in pest control are achieved by EPN application against root weevils of the genus *Otiorhynchus* (Coleoptera: Curculionidae) (Smith, 1999, Long *et al.*, 2000, Fitters *et al.*, 2001, Gill *et al.*, 2001) and against mushroom flies (Diptera: Sciaridae) (Hay & Richardson, 1995, Harris *et al.*, 1995, Peters, 1996, Scheepmaker *et al.*, 1996, 1997, 1998).

Even though varying species and biotypes of EPN infest a wide range of insects, only a small number of attempts have been made to control soil-dwelling thrips stages (Tomalak, 1994, Helyer *et al.*, 1995, Chyzik *et al.*, 1996). Nearly nothing is known about their ability to infest the different developmental thrips instars in the soil and only little research has been done on suitable EPN strains. A small number of strains has been tested with regard to their ability and virulence to control WFT. For this reason in the pre-

sented experiments a few selected nematode strains were examined at varying densities with regard to their impact on different soil-dwelling thrips stages. Moreover, arena and microcosm experiments were conducted to investigate the nematode strains with respect to their efficacy towards mixed population structures of soil-dwelling development stages of WFT.

The main aim of the present thesis was the analysis of the vulnerability of populations of *F. occidentalis* towards natural antagonists acting in the soil subsystem, which may open a new opportunity for biological control of this pest. Ecological aspects of *Hypoaspis* mites with respect to their suitability as biocontrol agent against *F. occidentalis* and the overall impact of selected strains of entomopathogenic nematodes (EPN) on soil-dwelling thrips instars were also examined.
Prerequisite for these innovative control attempts was that the majority of a thrips population actually enters the soil for pupation; therefore the soil passage was explored intensively with *P. vulgaris* as model plants and varying ornamentals.

# 2 Importance of soil passage in the ontogenesis of *Frankliniella occidentalis* and a comparison of *Hypoaspis miles* and *Hypoaspis aculeifer* as predators of soil-dwelling thrips stages

## 2.1 Introduction

In the early 80s *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) was introduced to Europe (Strassen, 1986, Mantel & Vrie, 1988, Vierbergen & Ulenberg, 1988, Brødsgaard, 1989) and today the Western Flower Thrips (WFT) is one of the most important insect pests in greenhouses world-wide (Shipp *et al.*, 1991, Robb & Parrella, 1995, Tommasini & Maini, 1995, Lewis, 1997a, 1998). Pest control with chemicals appears to be difficult for the above-mentioned reasons (Chapter 1).

Biological control of WFT with predatory mites (*Amblyseius* spp.) or anthocorids (*Orius* spp.) was successful in a few glasshouse crops like sweet pepper and eggplant (Ramakers *et al.*, 1989, Rijn & Sabellis, 1990, Morewood & Gilkeson, 1991, Jarosik & Pliva, 1995, Jacobson, 1995). Both antagonist groups are efficient natural enemies of leaf-dwelling instars of WFT, i.e. larval stages and adults. But at least a substantial part of the thrips population regularly escapes from predation because late second instar larvae of WFT leave the plant for pupation in the soil. Here WFT spends more than one third of its developmental cycle. Prepupae and pupae remain mobile in the soil but usually just move when disturbed (Childers *et al.*, 1994, Helyer *et al.*, 1995, Kirk, 1996). Although the soil passage is mentioned repeatedly (Childers *et al.*, 1994, Helyer *et al.*, 1995, Kirk, 1996) the proportion of a thrips population entering the soil is unknown. The soil passage in the life cycle of thrips offers the opportunity to screen for new effective antagonists. Promising antagonists are predatory soil mites. These mites are abundant throughout the world and play an important role as regulators in soil ecosystems (Karg, 1998). For biocontrol in greenhouses members of the genus *Hypoaspis*, especially *Hypoaspis aculeifer* (Canestrini) and *H. miles* (Berlese) (Acari: Laelapidae) seem to be of special importance. Both mites are polyphagous predators foraging on the soil surface and in upper soil layers. Both are successfully promoted and commercialised for biological control of mushroom flies (Diptera, Sciaridae) (Wright & Chambers, 1994, Enkegaard *et al.*, 1996, Ydergaard *et al.*, 1997, Folker-Hansen & Krogh, 1998, Ali *et al.*, 1999, Jess & Kilpatrick, 2000). In first choice experiments with eight different prey species Brødsgaard *et al.* (1996) could demonstrate that also *F. occidentalis* pupae are accepted as prey by *H. miles*. Specific information on their forging behaviour, prey preference and efficacy as

predator of thrips is still rare. Karg (1995) showed that *Hypoaspis* spp. preys on most soil organisms like Nematoda, Enchytraeidae, Acari, Collembola and small insect larvae. Glockemann (1994) could verify that WFT, *F. occidentalis*, at least belonged to the prey spectrum of *Hypoaspis* mites in greenhouses. For the following studies it is hypothesised that a soil passage is preferred by most thrips species particularly WFT and that soil-dwelling stages of WFT are accepted as prey from *Hypoaspis* spp. which may open a new opportunity for biological control of this pest in the soil subsystem. Therefore the first aim of our study was to quantify the proportion of WFT larvae entering the soil for pupation. The second aim was to evaluate the overall impact of soil-dwelling predatory mites on WFT and to compare the efficiency of both species, *H. miles* and *H. aculeifer*.

## 2.2 Materials and Methods

Rearing of *F. occidentalis*

The rearing procedure was based on a protocol of Bailey and Smith (1956) and slightly modified. *F. occidentalis* was reared in 0.75 l glass jars (Leifheit, Germany) with aluminium lid as rearing units and kept under controlled conditions (photoperiod 16:8 L:D, 24±1°C, 60±10% RH). For ventilation a hole was cut in the lid (diameter 5 cm) and covered with Nylon tissue (mesh width 63 µm, Sefar, Swizerland). On the bottom of the jars two layers of folded filters were spread as hiding place.

Bean pods (*Phaseolus vulgaris*, var. 'Marona') served as food and oviposition site. Before they were transferred in the jars, pods were washed in 1% sodium hypochlorite as surface sterilisation. Pollen (collected from birch or pine trees) was offered and the bean pods were dipped in honey water solution as additional food source.

About 100 adult *F. occidentalis* were enclosed in each jar. For two days the adult thrips are allowed to feed on the beans pods and to lay eggs in the tissue. After two days the bean pods were taken out and subsequent to removing the adults transferred in new glass jars. Adult thrips were provided with fresh bean pods. Two days later larvae started to emerge, another one to two days later the larvae developed to the second stage larvae. At an age of about 9 days the larvae started to crawl around in the jars, this is the stage the larvae search for a hiding place for pupation. Thrips moult to adults from the $13^{th}$ day onwards after leaving the eggs. Between the $9^{th}$ and the $13^{th}$ day they pass the prepupa and the pupa stage.

Rearing of *H. miles and H. aculeifer*

*Hypoaspis* mites were taken from a laboratory rearing that was established at the Institute of Plant Diseases and Plant Protection (University of Hanover, Germany). Both species were reared in plastic containers (12 cm diameter and 8 cm high) with an airtight lid and exclusively fed on the saprophytic nematode, *Turbatrix silusiae* (de Man) (Nematoda: Cephalobidae). Nematode prey was reared with an artificial diet consisting of milk, rolled oats and yeast (54:36:1). The bottom of the *Hypoaspis* rearing units was covered with a 1 to 2 cm thick layer of plaster of Paris mixed with charcoal (7:1). For ventilation a hole (1.5 cm diameter) was cut in the middle of the lid and covered with nylon tissue (64 µm mesh width). The plaster was irrigated two to three times a week to maintain high humidity inside the containers. In the rearing units, a surplus of prey was always available. The rearing units were stored in a climate chamber at 24 ± 0.5 °C with a photoperiod of 14:10 hours (L:D) and a relative humidity of 70 ± 10 %. The humidity inside the rearing units was not recorded.

Experimental design

To investigate (I) the extent of WFT soil passage and (II) the efficiency of *Hypoaspis* spp. against soil-dwelling developmental stages of WFT two separate microcosm experiments on potted French bean plants (*P. vulgaris*) were conducted. In the first experiment *H. miles* and in the second *H. aculeifer* was examined.

Bean plants were cultivated in unsterilised standard pricking ground. Ten days old single plants (two-leaf stage) were separately enclosed in an Acryl glass tube (diameter 10 cm, length 30 cm) serving as microcosm. For ventilation eight holes (35 mm diameter) were cut in the side of each tube, four in the upper and four in the lower third. The holes and the top of the tubes were closed by Nylon tissue (mesh width 63 µm) glued to the tubes by special Acryl glass glue (Acrifix® Röhm GmbH, Darmstadt, Germany). Only one hole was left open and later closed with a piece of paper fixed by sticky tape and served as a window to transfer thrips and mites onto the enclosed plants or the soil, respectively. The tubes, exactly fitting to the margin of the 11 cm pots the plants were grown in, were put over the plants and sealed with modelling clay.

Synchronised WFT larvae two days past hatching were deposited using a fine Kolinsky hairbrush directly on bean leaves inside the microcosm. Adult predatory mites directly taken from the stock culture were released three days later (i.e. five days past hatching) directly on the soil surface. To prevent escape of thrips and mites from the microcosm the

acryl-glass tube was attached to the pot with a tightly fitting layer of modelling clay and the bottom wholes of pots were sealed with nylon tissue.

To evaluate possible density depend variation in predator efficacy different numbers of prey and predators were encountered. Three predator densities (5, 10, 20) on two densities of WFT (10 and 50) were combined and 15 replications with *H. miles* and 13 with *H. aculeifer* respectively were conducted. Both experiments lasted for 21 days. Control treatments without predatory mites allowed the quantification of the proportion of WFT entering the soil and estimates of the intrinsic mortality rates. In all experiments the thrips population density in the soil and on the plant parts was recorded separately: Plants were cut at ground level. Soil and plant parts (stem and leaves) were placed separately in photo-eclectors to catch the emerging adults in insect glue (Fig. 2.1). The photoeclector makes use of the fact that the adult thrips after emerging (in contrast to the larvae) behave positive phototactic and fly or crawl towards the light. Standard planting pots (10cm diameter) were modified and used as eclectors: The bottom of the pots was cut off and they were put upside down on the soil with the thrips instars inside and sealed with modelling clay. Petri dishes painted with transparent insect glue (Temmen GmbH, Hattersheim, Germany) on the inside were attached on top to close the eclectors as transparent lids. Three holes (diameter 10 mm) were drilled in the side of the eclectors and covered with Nylon tissue (mesh width 63 µm) as ventilation openings. Thrips adults - after emerging from the soil - head towards the light and stick irreversible to the glue on the inside of the lid where they are counted and removed every day. The number of predatory mites inside the soil was not estimated.

All experiments were carried out in a climate chamber at 24 ± 1 °C and relative humidity of 60 ± 5% with a photoperiod of 16h light.

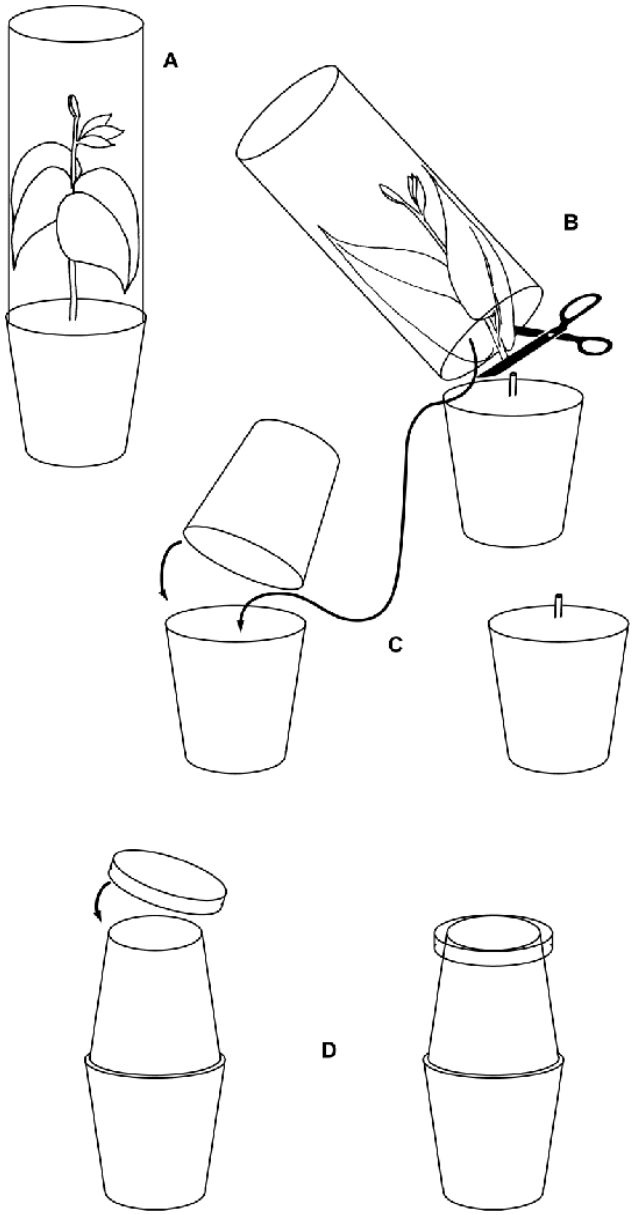



**Fig. 2.1 Experimental set up of microcosm experiments. Single bean plants were enclosed in acryl-glass tubes (A) and predatory mites, as well as thrips were transferred to the tubes. For quantification of thrips pupation sites and thrips mortality, respectively, plants were cut at ground level (B). Foliage was transferred in one, and soil was enclosed in a second photo-eclector (C). Emerging adult thrips were caught on insect glue applied on Petri dishes serving as lid (D).**

Statistical analysis

Thrips mortality was calculated as percentage of introduced thrips that could not be recaptured within the photo-eclectors. Mortality rates were Arcsine transformed. Normal distribution was tested using Kolmogorov-Smirnov-Test. In case of normal distribution differences among treatments were analysed with univariate analysis of variance, ANOVA and Post-Hoc-multiple comparison by Bonferroni-test. Pairwise comparison was done with T-test. If not normal distributed the comparison was done using Kruska-Wallis test and Mann-Whitney-U-test for couple comparison (Sokal & Rohlf, 1995). Subsequent to the

statistical analysis thrips mortality rates were corrected according to Schneider Orelli´s formula (Schneider & Orelli, 1947) to estimate the efficiency of the antagonists.

## 2.3 Results

Soil passage and intrinsic mortality rate of WFT

Second instar larvae (L2) of WFT started to leave the plants for pupation in the soil six days after hatching. Two days later most thrips larvae have left the plants. First adult thrips emerged from the substrate 12 days after hatching from the eggs. Emergence continued until day 20. Nearly 80% of the thrips emerged between the 14$^{th}$ and 17$^{th}$ day after leaving the eggs (Fig. 2.2).

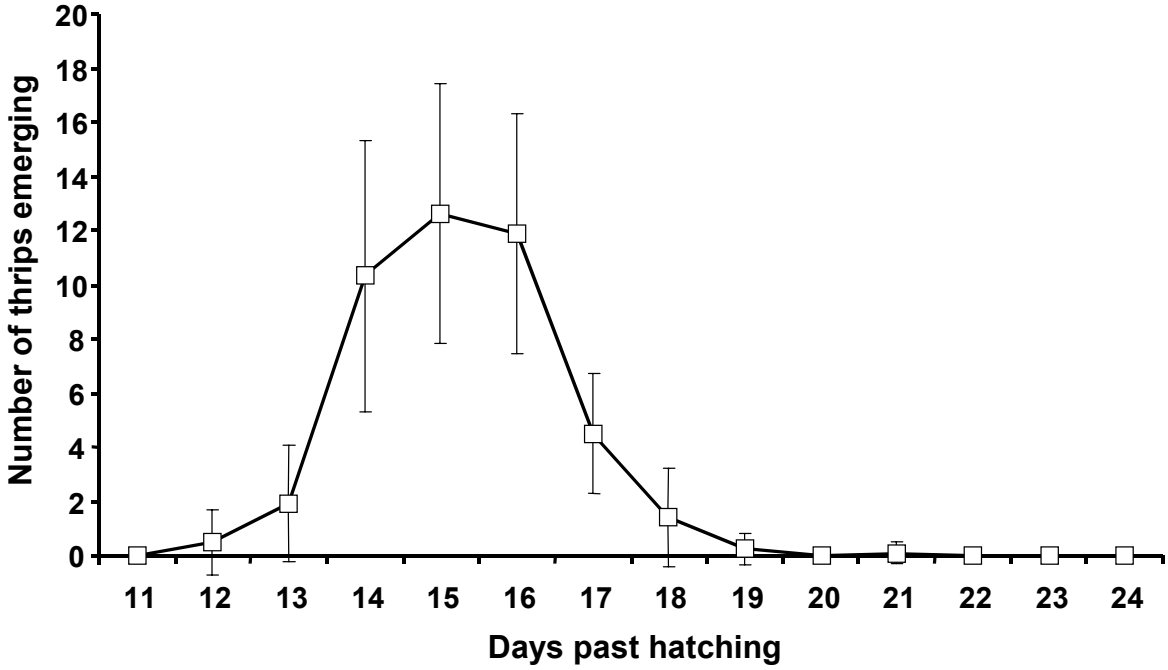



**Fig. 2.2 Temporal course of adult *Frankliniella occidentalis* emerging from the soil in *Hypoaspis* experiments. For each day, the mean number of recaptured thrips individuals per replicate (± SD N=56) is shown. The x-axis indicates days after thrips hatched from the eggs.**

In the control treatment without predatory mites the total number of recaptured thrips consisted of the number of thrips emerging from the soil (caught in sticky traps above the substrate) plus the number of thrips, which completed their life cycle on the plant (caught in sticky traps above the plant material (Fig. 2.1)). The intrinsic mortality represents the sum of natural and experimental mortality and was calculated as difference between the number of introduced and recaptured thrips. Overall 87.5% of the introduced thrips could

be recaptured in the control treatments without antagonists. The intrinsic rate of mortality was similar in the control treatments of the two series of experiments and not affected by the different initial densities of 10 or 50 thrips larvae (H-Test according to Kruskal-Wallis-Test, Chi²=2.916, *df*=3, P=0.405). In the different treatments intrinsic mortality rates varied between 9.69% and 15.39%.

The soil passage seems to be obligatory for WFT on *P. vulgaris* plants. About 98% of the introduced thrips emerged from the soil substrate, while only 2% emerged from the plant material (Fig. 2.3). This ratio was not affected by the different initial thrips densities and was the same in the control treatments of both series of experiments (H-Test according to Kruskal-Wallis-Test, Chi²=3.649, *df*=3, P=0.302).

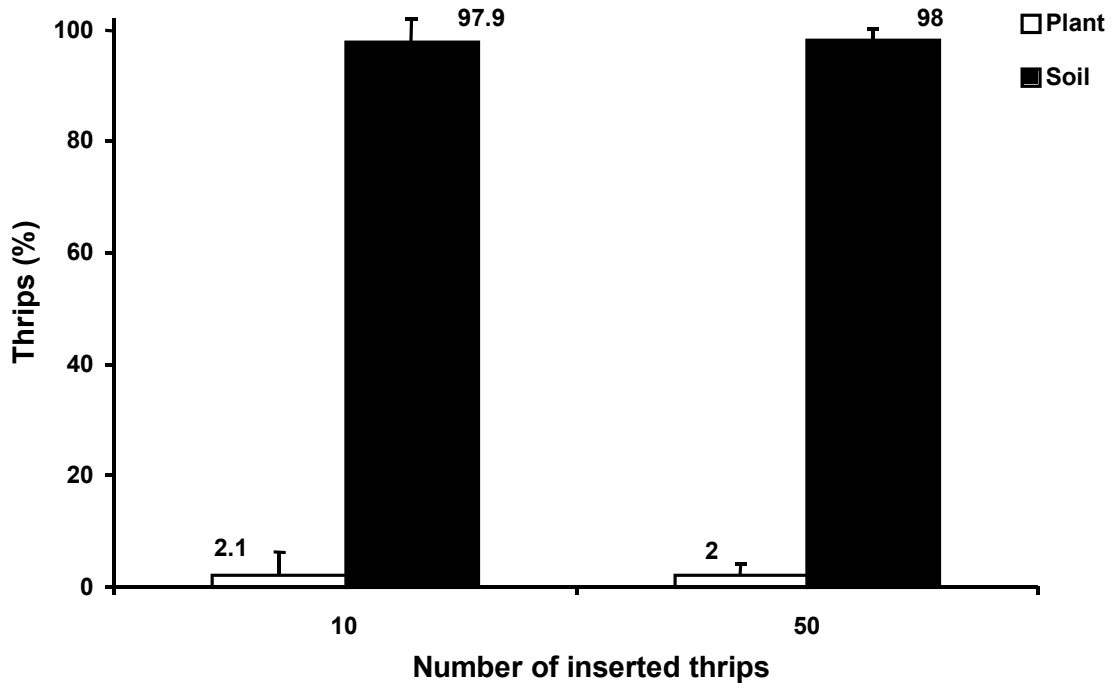



**Fig. 2.3 Preferred pupation site of *Frankliniella occidentalis* at two different initial population densities in *Hypoaspis* experiments. Mean percentages (± SD) of thrips emerging from the substrate (soil) and from the foliage (plant) are given in the graph (N=56).**

Efficacy of predatory mites

The efficacy of *Hypoaspis* spp. as predator of soil-dwelling developmental stages of WFT was tested with different predator-prey ratios in microcosm experiments. Thrips introduced in the microcosm two days old thrips started to enter the substrate at an age of six days. Since most adult thrips emerged from day 14 to 17 past hatching (Fig. 2.2) ground foraging predatory mites introduced three days after thrips release could encounter different thrips instars above and in the ground for approximately seven to eight days.

Because intrinsic mortalities and duration of soil development of thrips was not different for the control treatments data from both succeeding experiments were used for analysis (ANOVA). The results showed significant differences among the treatments (ANOVA, F=40.98, *df*=15, P<0.001). Thrips mortality was significantly influenced by predator density and species but not by prey density (Tab. 2.1). Moreover factors did not interact with each other (Tab. 2.1). Subsequently data could be analysed separately for each predator species. Consequently mortality rates at the two different prey densities were pooled. Finally the efficiency of the two predator species at the different predator densities were compared by two samples T-tests.

**Table 2.1 Statistical analysis for predation efficiency of *Hypoaspis miles* and *H. aculeifer*. Results of univariate analysis of variance with tests of effects between the examined subjects (number of thrips, number of mites, mite species)**

| Source of variation | *df* | Mean square | F-value | P-value |
|---|---|---|---|---|
| Number of thrips | 1 | 48.79 | 0.41 | 0.520 |
| Number of mites | 3 | 23396.89 | 198.78 | 0.000 |
| Mite species | 1 | 924.93 | 7.86 | 0.006 |
| Number of thrips * Number of mites | 3 | 84.81 | 0.72 | 0.541 |
| Number of thrips * Mite species | 1 | 75.62 | 0.64 | 0.424 |
| Number of mites * Mite species | 3 | 72.70 | 0.62 | 0.604 |
| Number of thrips * Number of mites * Mite species | 3 | 213.39 | 1.81 | 0.146 |

Thrips mortality was significantly influenced by the density of the predatory mite *H. miles* (ANOVA, F=111.07, *df*=3, P<0.001). Compared to the control treatment thrips mortality significantly increased with *H. miles* densities (Fig. 2.4). The presence of five predatory mites in the soil caused a corrected thrips mortality of approx. 44.9%. With a fourfold predator density the mortality increased by 30.8% to in total 75.7%. At all predator densities the efficiency of *H. miles* was significantly different (Tab. 2.2).

**Tab. 2.2 Analysis of differences between treatments with regard to the predation efficiency of different predator densities. All treatments are compared to each other by Post-Hoc-multiple comparison according to Bonferroni subsequent to an analysis of variance (ANOVA) (standard error calculated for *H. miles* = 2.66, and for *H. aculeifer* = 3.18).**

| Mite species | Compared treatments | | P-value |
|---|---|---|---|
| | Number of mites | Number of mites | |
| ***H. miles*[1]** | 0 | 5 | 0.000 |
| | 0 | 10 | 0.000 |
| | 0 | 20 | 0.000 |
| | 5 | 20 | 0.000 |
| | 10 | 5 | 0.018 |
| | 10 | 20 | 0.004 |
| ***H. aculeifer*[2]** | 0 | 5 | 0.000 |
| | 0 | 10 | 0.000 |
| | 0 | 20 | 0.000 |
| | 5 | 20 | 0.000 |
| | 10 | 5 | 0.169 |
| | 10 | 20 | 0.165 |

[1] For *H. miles* N=30. [2] For *H. aculeifer* N=26.

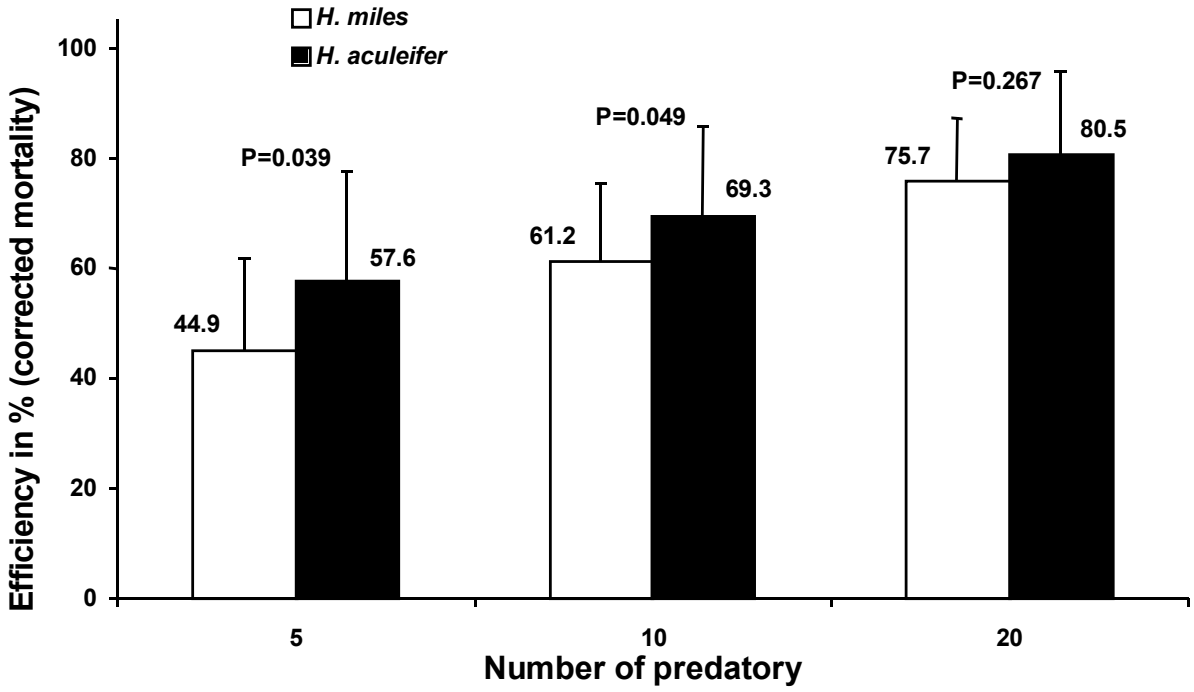



**Fig. 2.4 Predation efficiency of *Hypoaspis miles* and *H. aculeifer* on thrips stages in soil with three predator densities. The efficiency was calculated as corrected thrips mortality (according to Schneider & Orelli, 1947) (± SD). P-values above bars indicating significant differences between the *H. miles* and *H. aculeifer* treatment (N=15 in case of *H. miles* and N=13 with *H. aculeifer*).**

In correspondence to *H. miles* density of *H. aculeifer* also affected thrips mortality significantly (ANOVA, F=88.69, *df*=3, P<0.001). Compared to the control treatment *H. aculeifer* increased thrips mortality at any tested density (Fig. 2.4) but there were no significant differences in mortality if 5 or 10 predatory mites were present. Only a fourfold predator density resulted in a significant increase in thrips mortality (Tab. 2.2). In total efficiency of *H. aculeifer* increased from 58% with 5 mites to 80.5% with 20 mites (Fig. 2.4).

Both *Hypoaspis* species considerably differ in their efficiency as thrips antagonist at low prey densities, being significantly higher with *H. aculeifer* compared to *H. miles*. At the highest density of 20 predatory mites the species did not differ in their efficiency (Fig. 2.4). This effect becomes also obvious if the difference in mortalities caused by both species is plotted against *Hypoaspis* densities (Fig. 2.5).

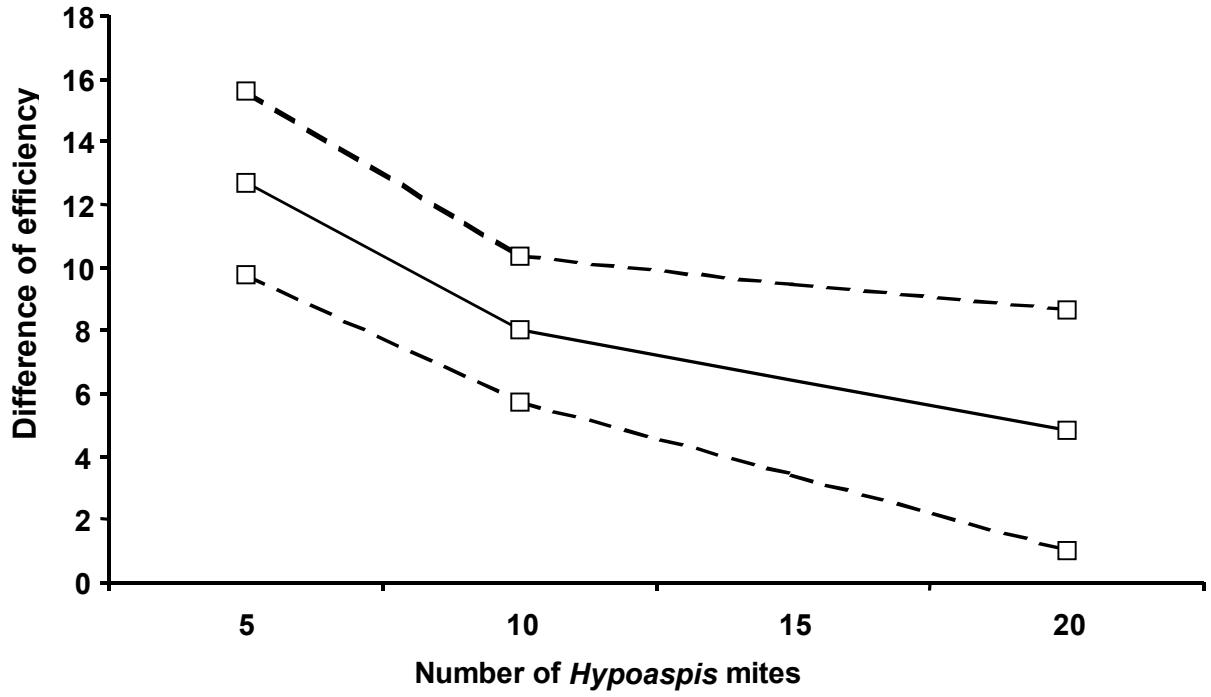



**Fig. 2.5 Comparison of *Hypoaspis miles* and *H. aculeifer*. Lines show the difference between efficiencies (thrips mortality caused by *H. miles* minus *H. aculeifer*) of both predatory mites as a function of the predator density. Solid line indicates differences of mean efficiency, broken lines show differences of upper and lower limits, respectively, calculated from the standard deviation of mean efficiency.**

## 2.4 Discussion

In many greenhouse cultures, ornamentals as well as vegetables, the western flower thrips, *Frankliniella occidentalis*, is an important pest species. The current pest status refers to problems in chemical control (i.e. resistance management) as well as problems in biological control efforts (Chapter 1).

Biological control of thrips has to be done almost exclusively with predatory antagonists (i.e. *Amblyseius* spp., *Orius* spp. *Chrysoperla carnea*) foraging in the crop canopy, and recently a fungal antagonist is available (i.e. *Verticillium lecanii*) (Brownbridge *et al.*, 1999). But the use of the mentioned predators against thrips is often not satisfying for the growers, since thrips densities cannot always be reduced below critical thresholds particularly on ornamental plants. Thrips escape from predation because they use the soil to complete their life cycle. And predators often show restrictions in preferred prey species or instar, respectively (Bakker & Sabelis, 1989, Hoeven & Rijn, 1990, Higgins, 1992, Chyzik *et al.*, 1995, Saminathan *et al.*, 1999, Venzon *et al.*, 1999). Both reasons will be discussed in detail below to stress how biological control of thrips can be optimised with natural enemies against the soil-dwelling developmental stages of WFT, e.g. with *Hypoaspis* mites.

The life stages of WFT include the egg, two larval stages, a prepupal stage, the pupa and the adult. At the end of its development the second larval stage moves to convenient places for pupation (see below). They spent this resting period in concealed places in or near their feeding habitats (Lewis, 1973, Varatharajan & Daniel, 1984, Helyer *et al.*, 1995, Tommasini & Maini, 1995). Our results showed a strong preference of WFT for the soil as pupation habitat. 98% of a *F. occidentalis* population left the host plant and completed their life cycle in the soil. Within the family Thripidae the soil passage is rather common (Lewis, 1973, Varatharajan & Daniel, 1984, Helyer *et al.*, 1995, Tommasini & Maini, 1995) but it remains unclear whether entering the soil for pupation is obligatory for most species belonging to the family Thripidae. For example the soil passage is obligatory for *Taeniothrips inconsequens* (Uzel), a serious pest of sugar maple. This monovoltine species enters the soil in autumn for hibernation (Parker *et al.*, 1992, Skinner & Parker, 1992, Brose *et al.*, 1993). Even for WFT it is too early to conclude from our studies that soil passage is obligatory since small and simple structured host plants were used, which may force WFT to select the soil for pupation. A lot of alternative pupation sites may be available directly on the plant if plant architecture or phenology is more complex (Lewis, 1973, Grout *et al.*, 1986, Kirk, 1996). Suitable microhabitats are flowers, buds, leaf axils, and any structure

on the stem. Pupae of WFT were found frequently on the bark of apple trees (Terry, 1988). The citrus thrips, *Scirtothrips citri* (Moulton), often pupates within the tree rather than in the ground (Grout *et al.*, 1986). French beans used in our experiments were not flowering and seem to offer only few possibilities for pupation. Since WFT has a broad host range it can be hypothesized that the proportion of a population, which enters the soil is reduced on a plant species with a more complex architecture (e.g. flowers of Asteraceae).

In Kirks (1996) opinion the soil passage is - as far as flower dwelling thrips are concerned - an adaptation to the short period of presence of the preferred flower habitat. Additionally the adaptive value of the soil passage within the life cycle of thrips might be related to protection against unfavourable abiotic conditions on the plant and against natural enemies. A balanced humid environment in the soil hinders desiccation of thrips. Moreover protection against natural enemies is especially important for prepupal and pupal stages because they are less mobile and almost defenceless (Lewis, 1973, Kirk, 1996). Therefore it is not expected that WFT would prefer to pupate on the plant rather than in the soil even though complex plant structures were available.

In consequence, while pupating in the soil a more or less large proportion of a thrips population regularly escapes predation by natural enemies which are today commercially used for biocontrol and which are foraging on upper plant parts (e.g. *Chrysoperla carnea*, *Amblyseius* sp., *Orius* sp.). Moreover thrips larvae often do not belong to the preferred prey of lacewing larvae (Saminathan *et al.*, 1999) or predatory bugs (Chyzik *et al.*, 1995, Venzon *et al.*, 1999) and *Amblyseius* spp. only attack first instar thrips (Bakker & Sabelis, 1989, Hoeven & Rijn, 1990, Higgins, 1992). These drawbacks often result in low efficacy of beneficials and failure of biocontrol.

Efficacy of WFT biocontrol could be improved if the soil reservoir was eliminated. In the soil nematodes, pathogens or arthropods could attack thrips. Pathogens, i.e. *Verticillium lecani* (Zimmermann) (Brownbridge *et al.*, 1999), and *Beauveria bassiana* (Balsamo) (Jacobson *et al.* 2001a) were recently propagated as effective antagonists. Moreover screening for entomopathogenic nematodes revealed some promising isolates causing high mortality with thrips larva and pupa (Chyzik *et al.*, 1996, Bennison *et al.*, 1999, Ebbsa *et al.*, 2001a, b). In this study it was focused on predatory mites of the genus *Hypoaspis* that forage for prey in the soil and on the soil surface. These mites are commercially available and primarily used to control fungus gnats (Sciaridae) (Ydergaard *et al.*, 1997, Ali *et al.*, 1999) or bulb mites (*Rhizoglyphus robini*) (Lesna *et al.*, 1996, 2000, En-

kegaard *et al.*, 1997). Side effects on other pest species like thrips are mentioned repeatedly in the literature (Gillespie & Quiring, 1990, Glockemann, 1994), but the efficiency of the polyphagous predatory mite *Hypoaspis* sp. was not quantified until now. *Hypoaspis* spp. are polyphagous and prey on almost every small animal present in the soil, e.g. Nematodes, Enchytraeids, Collembola, Diptera, and mites (Karg, 1995). Although thrips pupae are accepted as prey by *H. miles* (Glockemann, 1994, Brødsgaard *et al.*, 1996, Enkegaard & Brødsgaard, 2000) they seem to be an alternative rather than an essential food source. *H. miles* developed best with Collembola, Sciaridae, or bulb mites as prey (Brødsgaard *et al.*, 1996, Enkegaard *et al.*, 1997, Enkegaard & Brødsgaard, 2000).

Our results show that both species, *H. miles* and *H. aculeifer*, are obviously able to reduce a WFT population by feeding on the soil-dwelling stages. At a density of 5 predatory mites per microcosms (which is equivalent to 700 mites per $m^2$) the population density of thrips could be reduced by approx. 44.9% and 57.6% by the introduction of *H. miles* and *H. aculeifer*, respectively. Control efficacy could be enhanced by an increase in predator density to approx. 80% thrips mortality. In addition *H. miles* and *H. aculeifer* show different predation rates. While *H. miles* had the potential to kill up to 2 thrips larvae per day *H. aculeifer* killed up to 3 thrips larvae per day in a Petri dish experiment (Chapter 5). These findings confirm the observations of Brødsgaard *et al.* (1996) who found *H. miles* feeding on up to two thrips pupae per day. Furthermore the statistical analysis of our data leads to the conclusion that *H. aculeifer* should be preferred because of its higher predation efficiency. At application rates of 700 mites per $m^2$ *H. aculeifer* showed a 1.28 times higher efficiency than *H. miles*. However at a 4 times higher application rate (2800 mites per $m^2$) the differences in the predation efficiency of the two predatory mite species were minimal (Fig. 2.4 and 2.5). This shows again that the potential of *Hypoaspis* spp. to control thrips in general is limited. At least 20 – 25% of a thrips population were able to complete their life cycle even in the presence of predatory mites and could recolonise the plants. The following reasons – not excluding each other – may account for this effect:

(1) Thrips seem to be not the preferred prey species for *Hypoaspis* spp. (see above). Alternative prey species present in the soil can reduce the efficiency of the predator. Since not sterilised soil was used, at least nematodes and Collembola are expected to be present in the soil.

(2) Encounter rates between predator and prey might be low. Because the ability of *Hypoaspis* spp. to locate thrips at their pupation site might be the limiting factor.

(3) In preliminary experiments a very low tendency of *Hypoaspis* spp. to show cannibalism was found (Berndt unpublished). Even though a high cannibalism rate can be excluded it might be possible that interference between individuals reduce their overall efficiency.

Nevertheless *Hypoaspis* mites have advantages compared to other thrips antagonists. They are long-living arthropods, able to starve for a long time, and to survive even under unfavourable abiotic conditions. The polyphagous species can easily prey on other arthropods present in the soil and therefore survive even after the pest population decreased. *Hypoaspis* mites are therefore ideal candidates for thrips control and can even be used for preventive releases because of their broad prey range.

In conclusion it is obvious that ground dwelling predators can substantially contribute to reduce a source population of thrips in the well protected refuge soil, but are not able to reduce the pest population to low not damaging levels. It could be suggested that combinations of different antagonists, attacking different thrips instars living in the soil as well as on the leaves above ground, may result in a more successful strategy to optimise biological control of thrips. From combinations of leaf and soil-dwelling predators, desired additive effects could be expected since compared to combinations of above ground antagonists the interspecific competition (i.e. intraguild predation) should be neglectable due to the different foraging patches.

# 3 Extent of the soil passage in the life cycle of the Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) on selected ornamentals

## 3.1 Introduction

*Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) originated most likely in North America. It was introduced to the Netherlands in the early 80s and became one of the most important glasshouse pests in Europe (Tommasini & Maini, 1995). Short developmental times, a wide range of host plant species, and especially its cryptic life style promoted its permanent pest status. Larvae and adults often hide in buds, flowers or axils (Böhmer, 1989; Tommasini & Maini, 1995) and the resting stages are reported to be found in the soil and in enclosed microhabitats like axils, leaf sheaths or crevices in the bark (Helyer *et al.*, 1995, Tommasini & Maini, 1995, Kirk 1997a, b). Because of this hidden life style, an infestation of a crop often remains unrecognised for a long period of time until the damage becomes severe, resulting in an enormously impeded chemical control (Chapter 1).

For this reasons, alternative control strategies have become more and more important during the last two decades. Primarily the focus was on natural enemies, such as predatory mites (Phytoseiidae) and bugs (Anthocoridae and Miridae), as biocontrol agent (Higgins, 1992, Riudavets, 1995, Castane *et al.*, 1996) but recently attention has been drawn to the soil passage of *F. occidentalis*. An increasing number of attempts has been made to use predators and pathogens against the soil-dwelling developmental stages. Most important in this context are predatory soil mites of the genus *Hypoaspis* (Glockemann, 1992), entomopathogenic nematodes (Helyer *et al.*, 1995, Ebssa *et al.*, 2001a, b) and entomopathogenic fungi (Helyer *et al.,* 1995, Jacobson *et al.,* 2001a).

Nevertheless, a control strategy focusing on developmental stages of thrips in the soil will be effective only if the majority of a population enters the soil for pupation. There are several studies that mention the soil passage as part of the life cycle of Thripidae (Childers *et al.*, 1994, Helyer *et al.*, 1995, Kirk, 1996) but it is also repeatedly stated that a complete development from egg to adult exclusively on the plant frequently occurs (Helyer *et al.,* 1995, Tommasini & Maini, 1995). However, quantitative data on thrips migration to the soil on different plant species is still missing. Previous experiments with *P. vulgaris* showed that more than 97% of the thrips population leave the plant to pupate in the soil (Chapter 2).

Since *F. occidentalis* is problematic not only in vegetables but also and especially in ornamentals (Klatt, 1998) it was important to verify the extent of the soil passage within the life cycle of *F. occidentalis* on ornamentals in order to analyse the possibility to control this thrips by beneficials acting in the soil. For this reason, the development of thrips populations was analysed on three ornamentals in comparison to *P. vulgaris* with special emphasize on the choice of the pupation site.

## 3.2 Materials and methods

Selection of plant species

The chosen host plants are popular ornamentals being very susceptible towards infestation with *F. occidentalis*. Moreover, plant species should differ in their plant architecture to offer varying kinds and different numbers of suitable pupation sites above soil (e.g. flowers, buds, axils, leaf sheaths).

The following plant species were chosen:

1.) *Saintpaulia ionantha* Wendl (Gesneriaceae), variety 'Mojo' characterised by small dark blue flowers and leaves covered with hairs in a rosettelike habitus, close to the soil.
2.) *Dendranthema x grandiflorum* (Asteraceae), variety 'Rega Davis' showing wide red composite flowers and an upright growth of slightly ramified shoots
3.) *Tagetes patula nana* (Asteraceae), variety 'Valencia' with close filled flowers and an upright bushy growth of richly ramified shoots and many leaf sheaths

In order to analyse the impact not only of the habitus but also of flowers as possible pupation site, the plants were used for the experiment as soon as blossoms started to open. Since different commercial growers supplied *S. ionantha* and *D. grandiflorum*, they did not reach the suitable stage for the experiments at the same time. Therefore the experiment was replicated for each plant species independently. The proportion of thrips leaving the plant for pupation is well known from earlier experiments using *Phaseolus vulgaris* var. 'Marona' as host plants (Chapter 2). To ensure the comparability of the results, a treatment with *P. vulgaris* plants as reference was included to all experiments.

*S. ionantha* and *D. grandiflorum* plants were purchased as rooted cuttings and were cultivated in the glasshouse (21°C ± 5°C) until use. All plants were potted in standard pricking soil (Fruhstorfer Erde Typ P, Archut GmbH, Lauterbach-Wallenrod, Germany). One week before the experiments started they were planted into 11 cm plastic pots. The drainage

holes in the bottom of the pots were covered with Nylon gauze to prevent thrips from escaping. *D. grandiflorum* plants were treated twice with Basacel® (Compo, Münster, Germany) to stunt the plant growth. For flower induction they were kept under short day conditions (photoperiod 8:16 L:D) for 3 weeks.

Rearing and synchronisation of thrips

Thrips were reared on *P. vulgaris* pods following the protocol described by Bailey and Smith (1956) (see also Chapter 2.2).

Experimental design

Set-up with synchronised thrips populations

The plants were enclosed in microcosms (Chapter 2.2) and treated as described above. In the first set of experiments, 25 and 50 five-day-old thrips larvae were transferred to the different plant species with a thin, moistened Kolinsky hairbrush. Control plants were left without artificial thrips infestation and ensured an assessment of baseline thrips infestation of plants and soil. Each of the five treatments (control, *P. vulgaris*, *S. ionantha*, *D. x grandiflorum*, *T. patula nana*) was replicated ten times. The microcosms were stored under controlled conditions in a climate chamber (photoperiod 16:8 L:D, 10.000 Lux, 24 ± 1°C and RH 60 ± 10%) and replicates of all treatments were arranged in a completely randomised design.

Starting at the 6$^{th}$ day after hatching, larvae searched for a pupation site and 10 days after hatching thrips at least entered the prepupal stage. At the 10$^{th}$ day the microcosms were carefully opened and plants were cut at ground level. For quantification of thrips pupating on the plant above ground material was cut in pieces and washed in warm water mixed with a few drops of usual washing-up liquid. The washing solution was poured through folded filter paper and thrips were counted under the microscope. The soil was enclosed in a photoeclector to collect the emerging adult thrips (Chapter 2.2). Numbers of emerging adults of WFT were recorded daily and removed from the insect glue. Utilization of a definite number of synchronised thrips enabled the calculation of the natural mortality rate within the experiment.

Set-up with mixed thrips populations

In the second set of experiments mixed populations consisting of 10 and 15 female plus 5 male *F. occidentalis* were put on the plants in microcosms resulting in higher population

densities compared to treatments with synchronised populations. Plants without artificial thrips introduction again served as the control treatment. Plant species and microcosms were treated and arranged as described above. Twenty days later, population growth was stopped and number of thrips on the plant material and soil enclosed in photoeclectors were quantified (Chapter 2.2). Additionally, the distribution of thrips pupating on the foliage and the flowers were assessed. Therefore, flowers were cut off the shoot and both samples were evaluated separately.

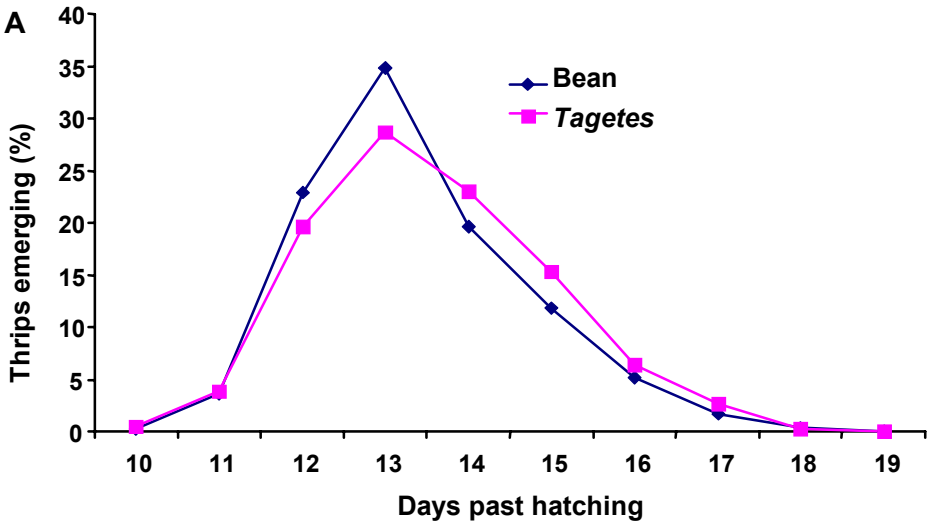
Statistics

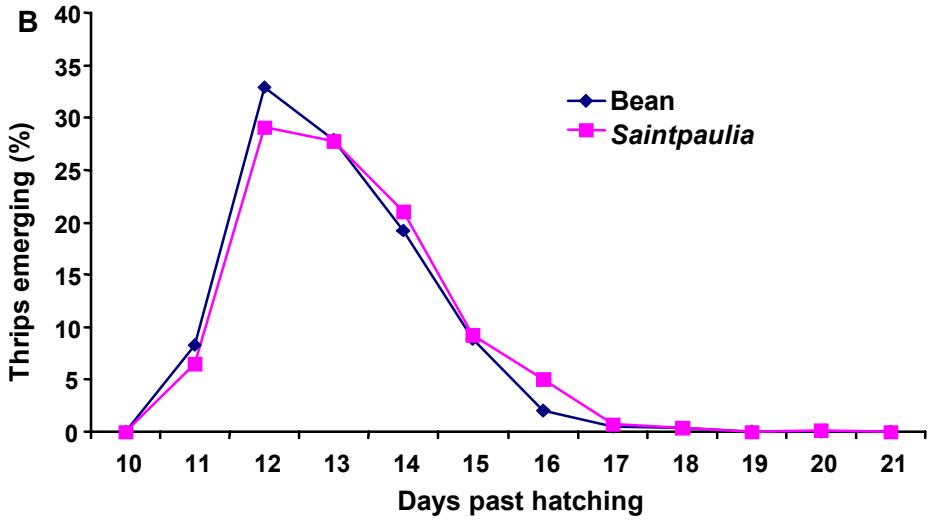
If plants had an initial thrips infestation at the beginning of the experiment, thrips counts were corrected by subtracting the mean thrips density from the values found in the treatments. Statistical analysis was done with SPSS 10.0 for Windows. Comparison among treatments of the first experiment was done with Mann-Whitney U-Test and among treatments of the second experiment with t-test according to Welch.

## 3.4 Results

Preferred pupation site of synchronised thrips larvae

The temporal pattern of adult thrips emerging from the soil was similar for all tested plant species. Emergence of adult thrips started 11 days after hatching from the eggs. The majority emerged between the $12^{th}$ and the $14^{th}$ day after hatching. With *D. grandiflorum* as host plant the peak of adult emergence was approximately one day later. Thrips emergence ended at the $19^{th}$ day after hatching (Fig. 3.1A-C). The developmental time from egg to adult on ornamental plants and *P. vulgaris* did not differ significantly (T-test: *T. patula nana* compared to *P. vulgaris* P=0.580, *S. ionantha* compared to *P. vulgaris* P=0.863, *D. grandiflorum* compared to *P. vulgaris* P=0.713). Moreover, thrips mortality rates and thrips population densities were similar in all treatments (Tab. 3.1 and 3.2).



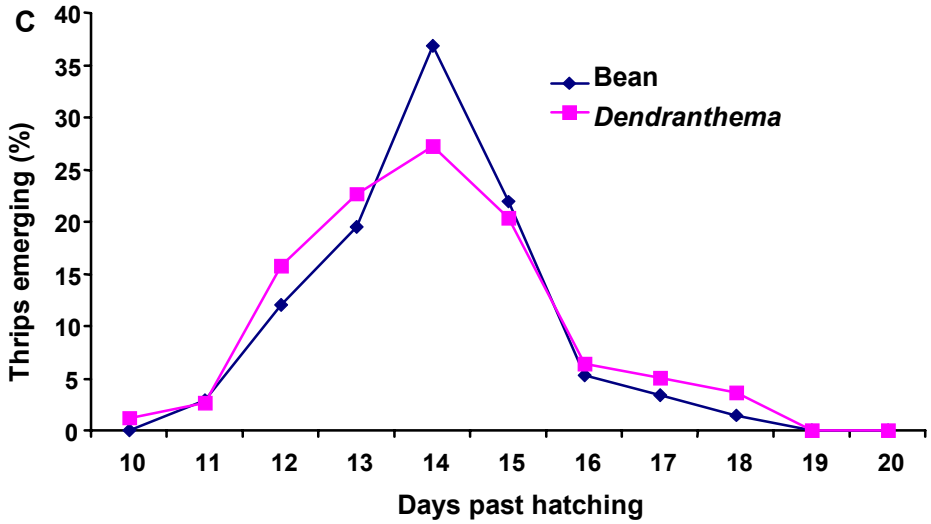




**Fig. 3.1A-C** Temporal pattern of adult thrips emerging from the soil beneath the host plant species. *Tagetes patula nana* (A), *Saintpaulia ionantha* (B) and *Dendranthema* x *grandiflorum* (C) was compared to the reference plant species *Phaseolus vulgaris* (bean). Graphs show the proportion of adult thrips emerging from the soil between the 10$^{th}$ and 20$^{th}$ day after hatching from the eggs. Data for treatments with initial populations of 25 thrips (N=10) and 50 thrips (N=10) were pooled.

**Tab. 3.1 Statistical analysis of the proportion of a thrips population pupating in the soil of ornamental and *P. vulgaris* plants. Varying *P. vulgaris* treatments and treatments with ornamental plants are compared to each other. Initial thrips population in all treatments was 25 and 50 thrips per plant. P-values are calculated by using exact test with binding correction.**

| Treatment 1 | Treatment 2 | P-value |
|---|---|---|
| *Phaseolus* (*S. ionantha* experiment) N=20 | *Phaseolus* (*D. grandiflorum* experiment) N=19 | 0.621 |
| *Phaseolus* (*D. grandiflorum* experiment) N=19 | *Phaseolus* (*T. patula nana* experiment) N=20 | 0.682 |
| *Phaseolus* (*S. ionantha* experiment) N=20 | *Phaseolus* (*T. patula nana* experiment) N=20 | 0.920 |
| *T. patula nana* N=20 | *S. ionantha* N=15 | 0.130 |
| *T. patula nana* N=20 | *D. grandiflorum* N=20 | 0.364 |
| *D. grandiflorum* N=20 | *S. ionantha* N=15 | 0.027* |

*according to Bonferroni the P-value has to be below 0.017 for a significant difference

In experiments using synchronised thrips populations the thrips density (25 or 50 thrips per plant) had neither an influence on mortality (Mann-Whitney U-test, Tab. 3.2) nor on the choice of the pupation site (Tab. 3.2), therefore data of the 25 thrips and the 50 thrips treatments were pooled.

The thrips mortality in the experiments with *S. ionantha* and *T. patula nana* ranged between 10.5% and 14% and did not differ significantly from the reference plant *P. vulgaris* (Tab. 3.3). Only with *D. grandiflorum* as host plant did mortality reach 20.5% and was significantly higher than on bean plants (Tab. 3.3).

The proportion of thrips pupating on the ornamentals above ground was at least 2fold higher compared to the bean plant. The highest pupation rates on the above ground plant parts were recorded for the ornamental plant *S. ionantha*. More than 5 times more thrips stayed on the plant for pupation compared to the reference plant, *P. vulgaris* (Fig. 3.2). However, the proportion of thrips leaving the plant to pupate in the soil was never below 91% (Fig. 3.2) and did not differ significantly among the ornamental plants (Tab. 3.1).

**Tab. 3.2 Analysis of the relationship between initial thrips population density (25 or 50 thrips per plant), mortality rates, and choice of the pupation site on different ornamental plant species (Mann-Whitney U-test).**

| Plant | | P-value for comparison of two thrips densities concerning: | | Number of replicates (N) in | |
|---|---|---|---|---|---|
| | | mortality rates | rates pupating on the plant | 25 thrips-treatment | 50 thrips-treatment |
| 1 | *S. ionantha* | 0.613 | 0.555 | 7 | 8 |
| | *P. vulgaris* | 0.204 | 0.204 | 10 | 10 |
| 2 | *D. grandiflorum* | 0.118 | 0.782 | 10 | 10 |
| | *P. vulgaris* | 0.589 | 0.638 | 10 | 9 |
| 3 | *T. patula nana* | 0.425 | 0.796 | 10 | 10 |
| | *P. vulgaris* | 0.956 | 0.509 | 10 | 10 |

**Tab. 3.3 Natural mortality of synchronised *F. occidentalis* in experiments on different ornamental plant species. P-values indicate significant difference in mortality rates on ornamentals and the reference plants *Phaseolus vulgaris* (Mann-Whitney U-test).**

| Host plant | Mortality [%] | P-Value | N |
|---|---|---|---|
| *S. ionantha* | 10.5 | 0.882 | 15 |
| *P. vulgaris* | 11.9 | | 20 |
| *D. grandiflorum* | 20.5 | 0.02 | 20 |
| *P. vulgaris* | 11.0 | | 19 |
| *T. patula nana* | 11.8 | 0.635 | 20 |
| *P. vulgaris* | 14.0 | | 20 |

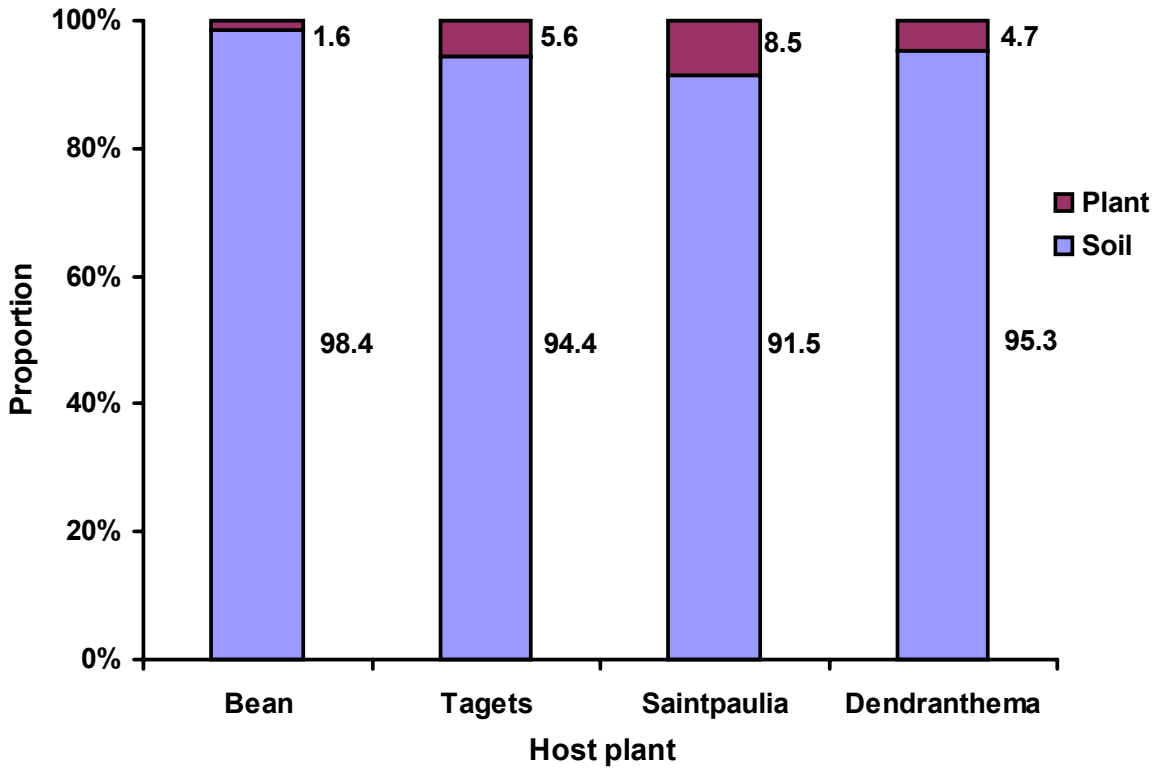



**Fig. 3.2 Proportions of the synchronised thrips population which pupate on the above ground plant parts (Plant) and in the soil (Soil). Data include intial thrips populations of 25 and 50 thrips per plant (N>15).**

Preferred pupation site of mixed thrips populations

In experiments with *D. grandiflorum* and *S. ionantha* as host plant a soil contamination with predatory mites of the genus *Hypoaspis* sp. (Acari: Laelapidae) and *Lasioseius* sp. (Acari: Podocinidae)) was observed. These predators were responsible for thrips population suppression in experiments with *D. grandiflorum* as host plant. Therefore, *D. grandiflorum* was excluded from the analysis and the number of replicates in the *S. ionantha* experiments had to be reduced. The origin of the mite contamination remains unclear. Most likely the young plants shipped by the breeder were already colonised with predatory mites.

However, population development on host plants was independent of the initial population size (Tab. 3.4). Thus, in order to analyse the impact of the population size on the choice of the pupation site, the final population size was categorised in either low infestation or high infestation. If thrips population was below the median it was defined as low infestation, if it was above the median it was defined as high infestation. Each class was analysed separately with regard to the proportion of thrips pupating on the plant (Tab. 3.4). In none of the experiments could a significant difference between the two infestation classes con-

cerning the proportion of thrips pupating on the plant be observed (Tab. 3.4). Thus, the thrips population density did not influence the choice of the pupation site. However, the results also suggest a tendency that an increasing infestation rate resulted in a decreasing number of thrips pupating on the plant.

**Tab. 3.4 Categorisation of thrips infestation on ornamentals and the reference bean plants after 20 days. Thrips numbers below the median were categorised as low and numbers above the median as high infestation. The P-value shows the significance of the difference between the proportions of thrips pupating on the plant at the two infestations degrees (T-test according to Welch).**

| | ***T. patula nana*** | | ***P. vulgaris*** | | ***S. ionantha*** | | ***P. vulgaris*** | |
|---|---|---|---|---|---|---|---|---|
| **Median** | 376 | | 375 | | 425.5 | | 369.5 | |
| **Total number of thrips per plant** | 225-373 | 379-764 | 280-374 | 376-599 | 258-403 | 448-588 | 275-367 | 372-572 |
| **Class of infestation** | Low | High | Low | High | Low | High | Low | High |
| **Mean number of prepupae and pupae** | 87.6 | 128.6 | 105.4 | 166.4 | 133.3 | 228.9 | 170.1 | 206.2 |
| **Mean number of prepupae and pupae on the plant (%)** | 7.5 (8.56) | 8.7 (6.77) | 2.5 (2.37) | 3.4 (2.04) | 9.8 (7.33) | 13.6 (5.92) | 1.9 (1.14) | 3.1 (1.48) |
| **P-value** | 0.544 | | 0.732 | | 0.166 | | 0.179 | |
| **Number of replicates** | 10 | 10 | 5 | 5 | 7 | 7 | 10 | 10 |

A comparison of the population proportions pupating on the different host plants showed a significant difference of the number of thrips pupating on the plant between *T. patula nana* and *S. ionantha* compared to the reference *P. vulgaris* (T-test according to Welch, *T. patula nana*: P<0.0001, *T. patula nana* N=20, bean N=10; *S. ionantha*: P<0.0001, *S. ionantha* N=14, bean N=20; for this analysis data of replicates with low and high population densities were pooled). More thrips remained on *T. patula nana* and *S. ionantha* than on *P. vulgaris* for pupation. Since there was no significant difference among the treatments in the thrips population density, data were pooled (Fig. 3.3). Additionally on *T. patula nana* more than three times more thrips pupated on the leaves than in the flowers and on *S. ionantha* little less than one third of the thrips staying on the plant pupated in the flowers (Fig. 3.3).

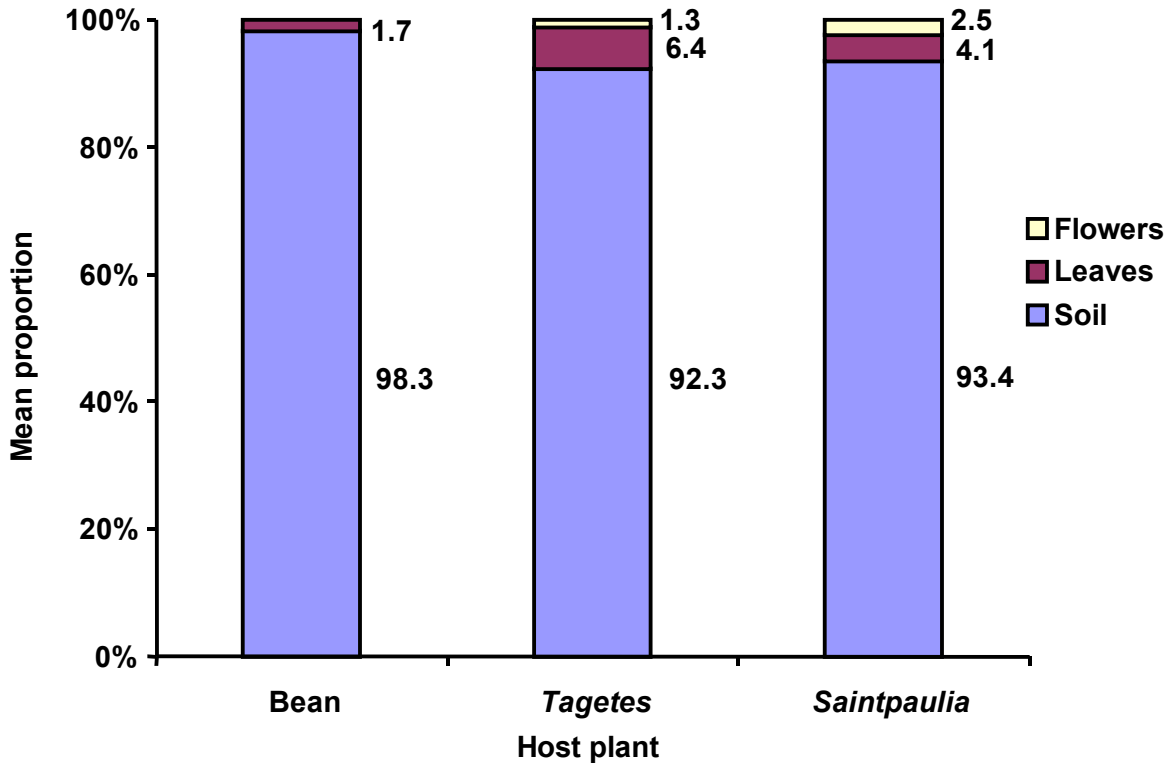



**Fig. 3.3 Mean proportion of thrips pupating in the flowers, on the leaves or in the soil beneath the host plant (Bean, *T. patula nana* and *S. ionantha*) 20 days after introduction of an initial thrips population.**

## 3.4 Discussion

Microcosm experiments were conducted to assess the proportion of a *F. occidentalis* population that leaves the host plant to complete the developmental cycle in the soil. The thrips behaviour was investigated at low and high population densities as well as for synchronised and mixed initial populations. Moreover intrinsic mortality rates were calculated and the temporal course of the emergence from the soil was recorded.

Unfortunately, the ornamental host plants were already infested with varying numbers of thrips at the beginning of the experiments. Therefore the infestation level with thrips was higher on ornamentals than on the reference plants, *P. vulgaris*. Most likely the plant age is the most important factor causing this infestation since the ornamentals were stored before the experimental use until they developed flowers, breeding of *T. patula nana* took two month and of *S. ionantha* around three month while in contrast bean plants were just ten days old. Therefore, the probability that ornamentals were infested with thrips in the glasshouse was much higher than for bean plants. All data had thus to be adjusted with

the natural infestation on the control plants. Unfortunately, the initial infestation was not balanced in all plants, some plants were more heavily infested than others, and therefore the correction resulted in high variation. Especially the high mortality rates in the experiments with *D. grandiflorum* as host plant reflect this variability.

The temporal pattern of emergence of adult thrips from the soil was similar for all tested plant species with synchronised thrips populations. Therefore the experimental conditions in all experiments and treatments were similar and allowed a comparison of the results.

In general the results show that the population size on the different plant species did not influence the behaviour of *F. occidentalis.* More than 90% of the population moved to the soil for pupation.

According to Tommasini and Maini (1995) the soil is the main pupation site but pupation on the plant may occur (Helyer *et al.* 1995; Kirk, 1997b). Glockemann (1994) speculated that far above the ground more thrips would pupate on plant parts above soil. In contrast, the current results show that more thrips pupated on plant parts above soil on *S. ionantha* as host plant than on the taller bean plants. Less than 2% of the thrips population completed the development on bean plants. With ornamentals as host plant the proportion of the thrips population pupating on the plant increased at most 4-fold (4.7% on *D. grandiflorum* up to 8.5% on *S. ionantha*). Nevertheless the largest part of the thrips population (at least 91%) completed its life cycle in the soil even with flowering ornamentals as host plants. Flowers did not seem to play an important role as pupation site; only in *S. ionantha* as host plant more than 50% of those thrips staying on the plant pupated in the flowers (flowers 2.5% leaves 4.1%, Fig. 3.3). Thus it can be concluded that the host plant species has a significant influence on the choice of the pupation site, but that the soil is the most important location to pupate.

Bailey (1933) reported that the immature stages of thrips stop crawling deeper in the substrate when the soil texture impedes a further penetration or when the physical or microclimatic conditions are suitable. Additionally, several authors mention a pronounced thigmotactic behaviour is characteristic for thrips (Tommasini & Maini, 1995; Ananthakrishnan, 1993, Merz, 1987). Possible locations on the plant accommodating these features are e.g. axils, bud scales, flowers or leaf sheath. Assuming that the choice of the pupation site is related to these factors, the ornamentals in the current study had far more suitable pupation sites for the thrips above ground compared to the beans. Especially the number of flowers on bean plants during the experimental period was very low and the few developing buds dropped to the ground early in the experiment. In contrast, *T. patula nana*

showed a strong ramification with high numbers of leaf axils and filled flowers. *D. grandiflorum* had three to five sprouts with a low buckling degree therefore the number of leaf axils was a bit lower compared to *T. patula nana* but still high. The morphology of *S. ionantha* heterogeneous: High number of leaf axils being close to each other in the shape of a rosette. Nevertheless, the number of possible pupation sites is not only dependent on the habit of the plant (e.g. rosette or upstanding) but also on the size of a plant. Since the *P. vulgaris* plants were only 10 days old compared to the few month old ornamentals, it is likely that the ornamental plants offered more pupations sites. This might be a reason for the low pupation rate of thrips on plant parts above ground on beans. Grout *et al.* (1986) showed that the pupation rate of *Scirtothrips citri* (Moulton) (Thysanoptera: Thripidae) on plant parts above ground is positive correlated with the size of the plant. Moreover Takrony (1973) quantified that a more intense light forced more *Hercinothrips femoralis* (Reuter) (Thysanoptera: Thripidae) to leave the plant and to pupate in the soil. Takrony (1973) therefore supposed that the larvae of thrips are negatively phototactic. Similarly Tommasini & Maini (1995) verified that *F. occidentalis* shows a negative phototactic behaviour. Based on this assumption it is possible that the ornamentals with a complex architecture used in the present study provided more protection against light than for example the *P. vulgaris* plants and thrips therefore more frequently remained on the plant for pupation. However, since in some replicates thrips pupae were found on the upper side of leaves exposed to high light intensities, it is likely that the phototactic behaviour is only one of many factors influencing the movements to the pupation sites.

Higgins (1992) speculated that the late second instar drops to the soil and pupates on the spot. In contrast, Tommasini and Maini (1995) stated that larvae actively leave the plant to find a pupation site. In both cases plants, much larger than the plants used in the presented experiments, with more foliage and leaf mass may represent a more difficult obstacle for the larvae on their way to the ground compared to young bean plants.

The microclimatic conditions also play an important role in choosing a pupation site. Prepupae and pupae are less susceptible than larvae and adult *F. occidentalis* towards a low humidity because of their low respiration rate. However, because of their impeded mobility they rely more on places with a constant humidity (Shipp & Gillespie, 1993). Since microclimatic conditions on plants show a high fluctuation, soil is preferred as pupation site (Shipp & Gillespie, 1993). It is also likely that those parts of the plant, which are accommodating to the thigmotaxis of thrips (axils, buds and sheaths), also show a consistent high relative humidity.

Conclusions

For a successful use of soil-dwelling antagonists, i.e. predatory mites, entomopathogenic nematodes or entomopathogenic fungi, the soil passage of thrips should be obligate independent of different thrips host plant species. A higher number of thrips that pupate on the plant reduces the control efficiency in the soil. The present study revealed that in all tested plant species with varying morphological features at least 91% of a thrips population leave the plant to pupate in the soil. It is concluded that a suitable antagonist foraging in the soil could considerably contribute to a control of *F. occidentalis*. Moreover, the varying morphological features of the plant species used in the present study influenced the choice of the pupation site to only a minor extent. The soil passage in the life cycle of *F. occidentalis* is most likely obligate. Soil-dwelling antagonist reveal the largest benefit when applied in advance (Van Driesche, 1999) A prophylactic use is expected to contribute substantially to keep pest levels beneath a damage threshold.

# 4 Cannibalism among soil-dwelling predatory mites: a reason for low predation efficiency of *Hypoaspis* spp. against thrips stages in the soil?

## 4.1 Introduction

*Hypoaspis aculeifer* (Canestrini) and *H. miles* (Berlese) (Acarina: Laelapidae) are soil-dwelling predatory mites. The prey spectrum of these polyphagous predators includes arthropods like Collembola, mites (Keith *et al.,* 1964, Barker, 1969, Ragusa *et al.,* 1986, Ragusa & Zedan, 1988), Sciaridae (Gillespie & Quiring, 1990, Chambers *et al.,* 1993) and other small Diptera, but also Nematodes and Enchytreae (Karg, 1982, Sardar & Murphy, 1987, Glockemann, 1992, Lesna *et al.,* 1995, 1996). Currently, *Hypoaspis* mites are successfully used to control mushroom flies and root mites especially in plant breeding and mushroom production. For example, *Geolaelaps* sp., a species closely related to *H. aculeifer*, reduced *Bradysia* sp. (Diptera: Sciaridae) by 80 % in cucumber production (Gillespie & Quiring, 1990). *Hypoaspis aculeifer* was able to reduce *Rhizoglyphus robini* Claprede (Acari: Acaridae) to very low numbers (Lesna *et al*., 1995), while *H. miles* suppressed sciarid population density in cyclamen and poinsettias (Chambers *et al*., 1993) and reduced sciarid emergence in mushroom production by 87 % (Jess & Kilpatrick, 2000). Moreover, several authors mention an impact of *Hypoaspis* spp. on thrips.

Despite this success, the efficiency of *Hypoaspis spp.* against sciarids and especially against the soil-dwelling thrips stages is sometimes limited (Wright & Chambers, 1994, Conijn *et al.,* 1997, Lesna *et al.,* 2000, Glockemann, 1992, see also Chapter 2) or can be achieved only with extremely high predator densities (Wright & Chambers, 1994, Ydergaard *et al.,* 1997). For example, Gillespie & Quiring (1990) found that a predatory mite closely related to *H. aculeifer* could reduce the emergence of adult *F. occidentalis* to about 30 % but was not able to keep thrips below the economic threshold. Similarly, *Hypoaspis* mites showed efficiencies of up to 80 % against *F. occidentalis* in microcosm experiments (Chapter 2). In contrast, Glockemann (1992) mentioned that *H. miles* had no effect on thrips population growth while *H. aculeifer* was at least able to slow down the population increase of *F. occidentalis*. The potential of *Hypoaspis* mites against western flower thrips is supported by laboratory studies, which show that the predatory mites are able to kill up to 3.5 thrips per day (Chapter 5).

Besides the possibility that certain prey species are not the preferred prey for *Hypoaspis* and the fact that *Hypoaspis* mites might have a limited searching efficiency, it is known

that cannibalism may limit the efficiency of predator populations (Leonardsson, 1991, Finke, 1994, Wagner & Wise, 1996, Wissinger *et al.,* 1996). Especially among predatory mites of the family Phytoseiidae, cannibalism is frequently found (Schausberger & Croft, 2000a). For example, the results of Croft *et al.* (1995) show that egg cannibalism is common even among well-fed adult *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae) females. In contrast, data on cannibalism for *Hypoaspis* mites are limited. Usher & Davis (1983) kept small groups of *H. aculeifer* at 16 °C in vessels observed them weekly and found indications that cannibalism only occurs after weeks of starvation in the absence of alternative prey.

In the current study, the cannibalistic behaviour of two *Hypoaspis* species commonly used for biological control is compared. It was examined if cannibalism might be responsible for a lowered efficacy of *Hypoaspis* spp. against target organisms in general and especially against soil-dwelling thrips stages. Specifically, we investigated the propensity of *H. miles* and *H. aculeifer* to cannibalism in the absence and in the presence of alternative prey in the laboratory.

## 4.2 Materials and methods

Rearing and synchronisation of predator species

*Hypoaspis* mites were reared as described above (Chapter 2.2). For synchronising ages of cohorts, gravid female and male *Hypoaspis* mites were chosen randomly from the laboratory rearing and placed in new rearing containers. After 48 h, they were again transferred into new rearing units. The eggs laid within this 48 h-period were kept under controlled conditions until the different developmental stages were used in the experiments. During the experimental run, synchronised mites were continuously reared as described to replace mites that have been killed by conspecifics. Synchronised mites of different developmental stage and sex (in case of adults) were transferred directly into the experimental arenas. The two nymphal stages – protonymph and deutonymph – were not separately treated in the cannibalism experiment.

Arenas and experimental design

All developmental stages were used as prey and those stages that ingest food (nymphs, adult females and males) were considered as predators. Overall, 11 predator prey combinations were examined. Each nymph was combined with either three eggs, three larvae or three nymphs. Each adult male was combined with either three eggs, three larvae,

three nymphs or three males and each adult female was combined with either three eggs, three larvae, three nymphs or three adult females. Additionally, combinations of two adult females and two adult males were tested. Test individuals and prey were taken from different colonies to avoid any effect caused by potentially close relatedness between predator and prey. The control consisted of the same treatments but additionally saprophytic nematodes were present as prey in each arena. Small translucent plastic vials with lid (diameter 36 mm and 45 mm high) were used as arena. The bottom of each arena was covered with a layer (5 mm) of plaster of Paris mixed with charcoal (7:1). For ventilation, a hole of 5 mm diameter was cut in the upper third of each vial and covered with Nylon tissue (mesh width 64 µm). Every other day seven drops of tap water were added to the plaster surface.

Mites killed or preyed upon were exchanged with new mites in the same developmental stage every day and eggs laid by the cannibals were removed. Each treatment was replicated nine times and the experiment lasted 14 days. Each experiment was terminated when a mite that was considered as predator died or reached the next developmental stage.

Statistical analysis

Normal distribution for all data was checked by Kolmogorov-Smirnov-adjustment-test. Levene-Test was used to verify homogeneity of variance. Differences in the cannibalistic behaviour of the tested developmental stages were investigated with ANOVA and T-test. Multiple comparisons were done with Scheffé-Test. To investigate the influence of the availability of prey on the fecundity of the females, the Pearsons correlation coefficient was calculated (Buehl & Zoefel, 2000).

## 4.3 Results

Adult females, males and nymphs of the two predatory mite species were tested for their cannibalistic behaviour against eggs, larvae, nymphs, females, and males as conspecific prey in presence and in absence of alternative prey. In presence of alternative prey, cannibalism among *Hypoaspis* mites never occurred. All tested predators either survived the experimental period or successfully completed their development. In contrast, a high number of cannibalism events were observed in the absence of alternative prey. In general, higher developmental stages were never killed by lower developmental stages i.e. nymphs never preyed on adults. Moreover, adult males never killed adult females. In the

following the results are analysed separately for each *Hypoaspis* species and finally the cannibalistic behaviour of both species and the influence of prey on female fecundity is compared.

Cannibalistic behaviour of *H. aculeifer*

Analysis of variance showed that the number of conspecifics killed was significantly influenced by the predator type (female, male, nymph) and the prey type (egg, larva, nymph, female, male) (Tab. 4.1). Therefore, the following multiple comparison analysis was done for each predator type separately. Female *H. aculeifer* were the only predator type that preyed on all other developmental stages on equal (Tab. 4.2) but overall low proportions (Fig. 4.1). Predation rates varied between 0.01 and 0.18 conspecifics per day (Fig. 4.1). In contrast, males and nymphs as predator showed an indifferent cannibalistic behaviour towards the different prey types. Male *H. aculeifer* killed significantly less conspecific males than eggs, larvae, or nymphs (Tab. 4.3). Females were never killed by males. Compared to larvae (0.2 per day) and nymphs (0.1 per day), conspecific eggs (0.35 per day) were destroyed significantly more frequently. Similarly, nymphs killed significantly more eggs (1.02 per day) than larvae (0.3 per day) or nymphs (0.04 per day) (Tab. 4.3), while females and males were never preyed upon (Fig. 4.1).

The three predatory developmental stages (female, male, nymph) fed on nearly the same number of larvae and nymphs. In contrast, females destroyed 0.06 eggs per day, while the number of destroyed eggs increased 6-fold for males and 17-fold for nymphs (Fig. 4.1).

**Tab. 4.1 Statistics for univariate analysis of variance on the influence of prey and predator on the number of killed conspecifics among *H. aculeifer* and *H. miles* (N=108)**

| | *H. aculeifer* | | | *H. miles* | | |
|---|---|---|---|---|---|---|
| | ***df*** | **F** | **P** | ***df*** | **F** | **P** |
| **Prey** | 4 | 41.291 | <0.001 | 4 | 7.992 | <0.001 |
| **Predator** | 2 | 58.7 | <0.001 | 2 | 7.101 | 0.001 |

**Tab. 4.2 Statistics for analysis of variance on the influence of prey on the number of cannibalism events with adult females (N=45), males (N=36) and nymphs (N=27) as predator**

| | *H. aculeifer* | | | *H. miles* | | |
|---|---|---|---|---|---|---|
| | ***df*** | **F** | **P*** | ***df*** | **F** | **P*** |
| **Female** | 4 | 3.379 | 0.02 | 4 | 11.581 | <0.001 |
| **Male** | 3 | 38.304 | <0.001 | 3 | 59.471 | <0.001 |
| **Nymph** | 2 | 81.097 | <0.001 | 2 | 4.895 | 0.02 |

*Because of heterogeneity of variance the level of significance is lowered to P≤0.01

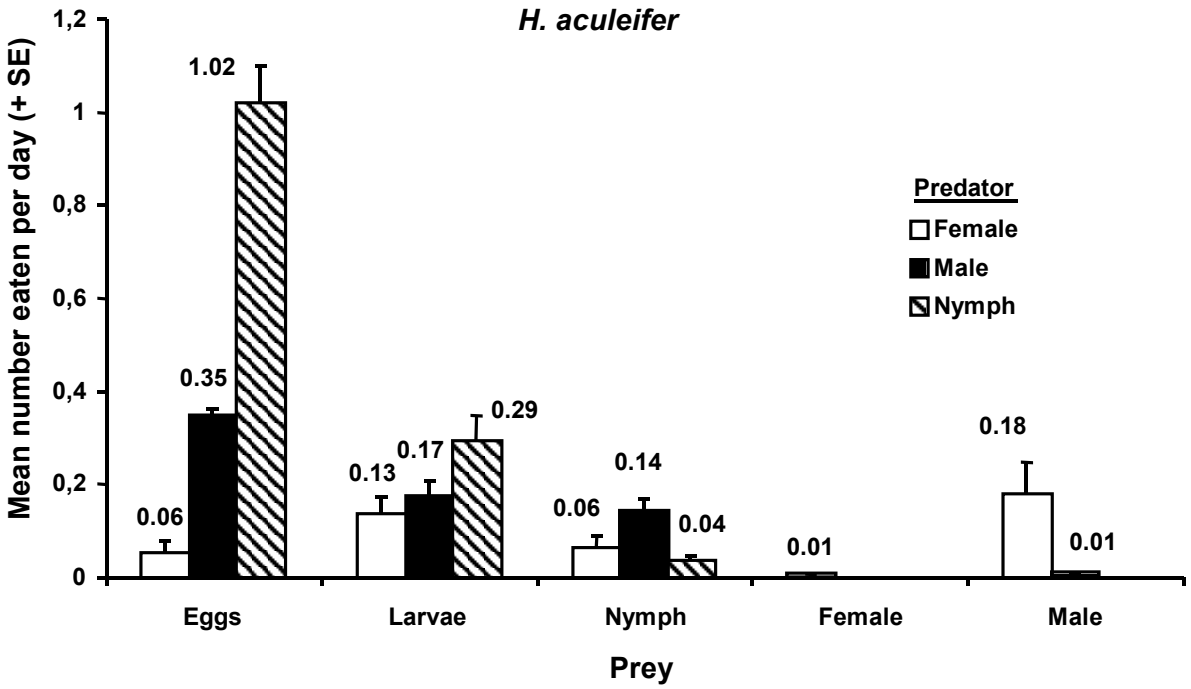



**Fig. 4.1 Cannibalism events on the part of a female, male or nymphal *H. aculeifer* as predator on conspecific eggs, larvae, nymphs, females and males as prey (N=9).**

Cannibalistic behaviour of *H. miles*

Similarly to *H. aculeifer* predator and prey stage influenced the cannibalistic behaviour of *H. miles* (Tab. 4.1). The statistical analysis was done separately for each predator stage. In contrast to *H. aculeifer*, female and male *H. miles* showed indifferent cannibalistic behaviour while no significant differences were found in case of nymphs preying on conspecific eggs, larvae or nymphs (Tab. 4.2). Moreover the results indicate that female *H. miles* mites destroyed significantly more nymphs (0.1 per day) and eggs (0.2 per day) than adult males (0.01 per day) (Fig. 4.2, Tab. 4.3). Male *H. miles* killed significantly more nymphs and larvae than males, Nymphs were more frequently prey for males than eggs or larvae (Fig. 4.2, Tab. 4.3). Most larvae were killed by nymphs while females and males killed nearly the same number of larvae (Fig. 4.2). The number of nymphs killed by nymphs was lower compared to the number of nymphs killed by males, but there was no significant difference compared to the number of nymphs killed by females (Fig. 4.2). *H. miles* males seemed to feed on less eggs compared to nymphs and females as predator, but this difference could not be confirmed statistically.

**Tab. 4.3 P-values of multiple comparison of cannibalism rate in varying treatments. The results of the treatments with females, males or nymphs of *H. aculeifer* and *H. miles* respectively as predators and conspecific eggs, larvae, nymphs, males or females as prey are compared in pairs. The less-than / greater-than-symbol in the 2nd and 3rd column shows the relation between the compared couple and the P-value in brackets shows the significance for this relation, significant values are underlined. Because of heterogeneity of variance the level of significance is lowered to P≤0.01 (N=9).**

| **Predator** | ***H. aculeifer*** | ***H. miles*** |
|---|---|---|
| | Comparison of cannibalism rate (P-value*) | Comparison of cannibalism rate (P-value*) |
| **Female** | Females < Males (0.045) | Females < Males (0.999) |
| | Females < Nymphs (0.881) | Females < Nymphs (<u>≤0.001</u>) |
| | Females < Larvae (0.228) | Females < Larvae (0.508) |
| | Females < Eggs (0.927) | Females < Eggs (<u>0.002</u>) |
| | Males > Nymphs (0.324) | Males < Nymphs (<u>0.001</u>) |
| | Males > Larvae (0.947) | Males < Larvae (0.677) |
| | Males > Eggs (0.260) | Males < Eggs (<u>0.005</u>) |
| | Nymphs < Larvae (0.770) | Nymphs > Larvae (0.030) |
| | Nymphs = Eggs (1) | Nymphs > Eggs (0.954) |
| | Larvae > Eggs (0.695) | Larvae < Eggs (0.158) |
| **Male** | Males < Nymphs (<u>0.002</u>) | Males < Nymphs (<u>≤0.001</u>) |
| | Males < Larvae (<u>≤0.001</u>) | Males < Larvae (<u>0.007</u>) |
| | Males < Eggs (<u>≤0.001</u>) | Males < Eggs (0.976) |
| | Nymphs < Larvae (0.809) | Nymphs > Larvae (<u>≤0.001</u>) |
| | Nymphs < Eggs (<u>≤0.001</u>) | Nymphs > Eggs (<u>≤0.001</u>) |
| | Larvae < Eggs (<u>≤0.001</u>) | Larvae > Eggs (0.021) |
| **Nymph** | Nymphs < Larvae (0.014) | Nymphs < Larvae (0.021) |
| | Nymphs < Eggs (<u>≤0.001</u>) | Nymphs < Eggs (0.101) |
| | Larvae < Eggs (<u>≤0.001</u>) | Larvae > Eggs (0.751) |

*Because of heterogeneity of variance the level of significance is lowered to P≤0.01

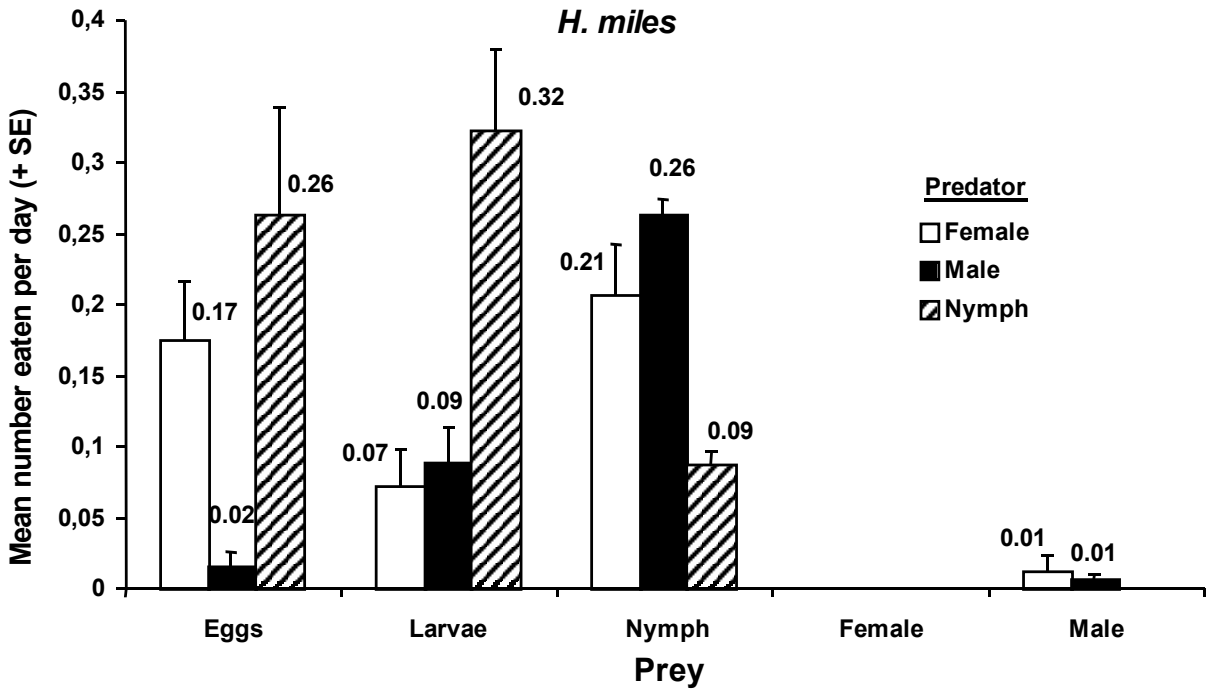



**Fig. 4.2 Cannibalism events on the part of a female, male or nymphal *H. miles* as predator on conspecific eggs, larvae, nymphs, females and males as prey (N=9).**

Comparison of cannibalism tendency of *Hypoaspis* species

In general, the various developmental stages of *H. aculeifer* and *H. miles* were prey to conspecifics at similar rates. For both species, adults were less frequently killed (0.018 per day) than either nymphs (0.31 per day), larvae (0.18 per day) or eggs (0.14 per day). Differences in the cannibalistic behaviour between the two species towards conspecifics were most pronounced for the juvenile developmental stages. While eggs were often prey to nymphs and males of *H. aculeifer*, all developmental stages of *H. miles* preyed on eggs at low rates. In contrast, nymphs of *H. aculeifer* were rarely prey to any of the developmental stages while nymphs of *H. miles* were killed frequently by adults of the same species. Additionally, cannibalism of females on males was observed more frequently for *H. aculeifer* than for *H. miles* and only *H. aculeifer* females preyed on conspecific females.

Overall, *H. aculeifer* nymphs and males showed a greater tendency to cannibalism than *H. miles* (Fig. 4.1 and 4.2). These differences were most pronounced in higher predation rates of *H. aculeifer* towards conspecific eggs and higher predation rates of *H. miles* towards conspecific nymphs. Moreover, only *H. aculeifer* females killed conspecific adults (males) at noticeable rates.

Impact of prey on life history parameters

Since the larvae of both mites are non-feeders, the nymphal stage is the only stage where it is possible to show any effect of cannibalism on development or the duration of this developmental stage. Only nymphs of *H. miles* were able to complete nymphal development and reach adulthood. In all other cases, no nymph reached the adult stage. The development of the nymphs on conspecific prey was delayed compared to the nematode prey (Tab. 4.4). Only nymphs of *H. miles* feeding on conspecific eggs were able to complete their life cycle within the experimental period of 14 days. Compared to the control (7.4 d) the nymphal stage lasted significantly longer with conspecific eggs as prey (11.1 d; Mann-Whitney-Test N=45 P<0.001). In all other cases nymphs did not reach adulthood.

**Tab. 4.4 Duration of the developmental stages of *H. miles* and *H. aculeifer* on *Turbatrix silusiae* as prey (control) and on conspecifics as the only food source at 24 °C. Asterisks following the values for duration of the nymphal stage in cannibalism treatments indicate a significant difference compared to the control with P<0.001 (Mann-Whitney-U-test). (For the control N=36, for the treatments N=9)**

| Prey | Duration of developmental stages [days (± SE)] | | | |
|---|---|---|---|---|
| | Egg | Larva | Nymph | Egg to adult |
| ***H. miles*** | | | | |
| *Turbatrix silusiae* (control) | 2.92 (±0.1) | 1.06 (±0.0) | 7.39 (±0.1) | 11 (±0.1) |
| Eggs | n.d. | n.d. | 11.1 (±0.3)* | > 14 |
| Larvae | n.d. | n.d. | > 14* | > 14 |
| Nymphs | n.d. | n.d. | > 14* | > 14 |
| ***H. aculeifer*** | | | | |
| *Turbatrix silusiae* (control) | 3.31 (±0.1) | 1.14 (±0.1) | 9.14 (±0.1) | 14 (±0.1) |
| Eggs | n.d. | n.d. | >14* | > 14 |
| Larvae | n.d. | n.d. | >14* | > 14 |
| Nymphs | n.d. | n.d. | >14* | > 14 |

Females of both species frequently laid eggs during the experiment. If females were allowed to prey only on conspecifics, the oviposition rate ranged from 0.09 to 0.13 eggs per day for *H. miles* and from 0.1 to 0.27 eggs per day for *H. aculeifer* (Fig. 4.3). In contrast, if saprophytic nematodes were available as prey the number of eggs laid by the females of both species increased more than 10fold (Fig. 4.3) (ANOVA, *df*=1, F=135.396, P<0.001; multiple comparison see Tab. 4.5).

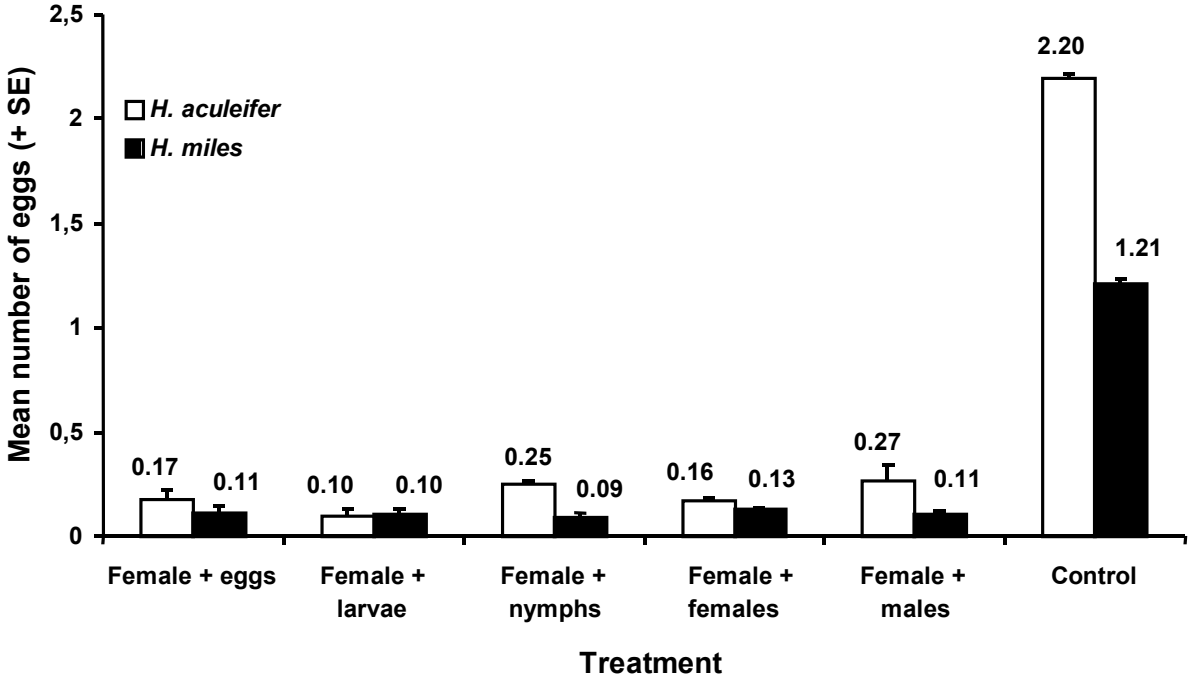



**Fig. 4.3 Mean number of eggs laid by female *H. aculeifer* and *H. miles* per day in different treatments of a cannibalism experiment compared to the control. In the control mites were fed with saprophytic nematodes (N=9).**

Most eggs were deposited during the first four to five days of the experimental period in most treatments, while in the control treatments eggs were laid continuously throughout the experimental period (Fig. 4.4). Only females of *H. aculeifer* preying on larvae and male conspecifics laid eggs continuously (Fig. 4.4). The total number of eggs laid was positive correlated with the number of cannibalism events for *H. aculeifer* ($r=0.532$, $N=45$, $P<0.001$) but not for *H. miles* ($r=0.102$, $N=45$, $P=0.506$). A detailed correlation analysis of the different treatments showed that the number of eggs laid was significantly influenced by cannibalism if *H. aculeifer* females preyed on male and larval conspecific prey (Tab. 4.6).

**Tab. 4.5 P-values of multiple comparison of oviposition rates in varying treatments of a cannibalism experiment on *H. miles* and *H. aculeifer*. All treatments are compared to each other. For both mite species the following treatments were used: A single female mite plus three eggs (f + egg), a single female plus three larvae (f + lar), a single female plus three nymphs (f + nym), four females (f + fem), a single female plus three males (f + mal) and a single female fed with saprophytic nematodes (f con) (N=9).**

| | | *H. aculeifer* | | | | | | *H. miles* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | f + egg | f + lar | f + nym | f + fem | f + mal | f con | f + egg | f + lar | f + nym | f + fem | f + mal | f con |
| *H. aculeifer* | f + egg | | 0.994 | 0.998 | 1 | 0.982 | <0.001 | 0.999 | 0.998 | 0.987 | 1 | 0.999 | <0.001 |
| | f + lar | | | 0.588 | 0.998 | 0.375 | <0.001 | 1 | 1 | 1 | 1 | 1 | <0.001 |
| | f + nym | | | | 0.993 | 1 | <0.001 | 0.751 | 0.673 | 0.501 | 0.877 | 0.713 | <0.001 |
| | f + fem | | | | | 0.959 | <0.001 | 1 | 0.999 | 0.995 | 1 | 1 | <0.001 |
| | f + mal | | | | | | <0.001 | 0.544 | 0.458 | 0.300 | 0.713 | 0.501 | <0.001 |
| | f con | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| *H. miles* | f + egg | | | | | | | | 1 | 1 | 1 | 1 | <0.001 |
| | f + lar | | | | | | | | | 1 | 1 | 1 | <0.001 |
| | f + nym | | | | | | | | | | 1 | 1 | <0.001 |
| | f + fem | | | | | | | | | | | 1 | <0.001 |
| | f + mal | | | | | | | | | | | | <0.001 |
| | f con | | | | | | | | | | | | |

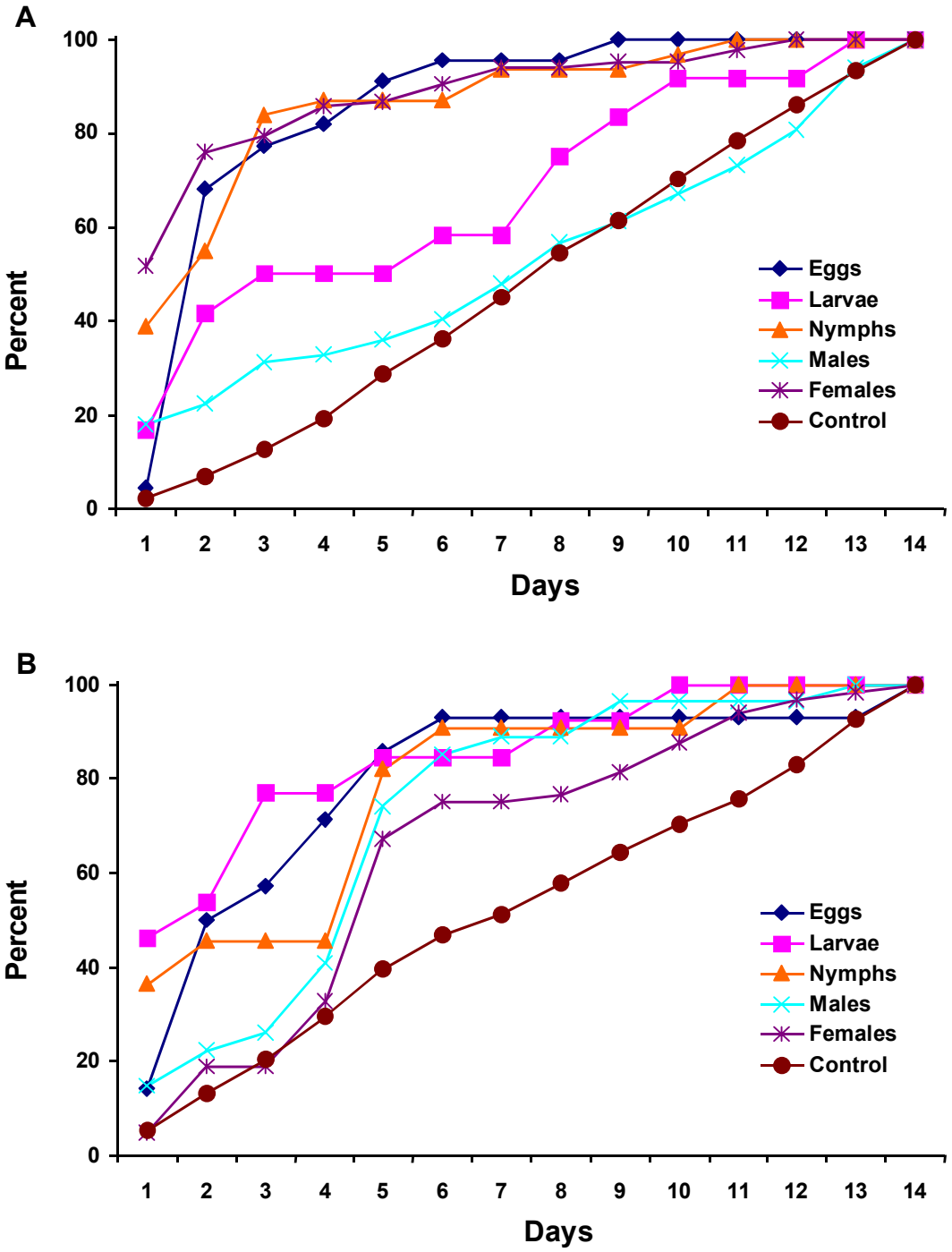



**Fig. 4.4 Cumulative oviposition rate of single females of *H. aculeifer* (A) and *H. miles* (B) during the experimental period in different treatments of a cannibalism experiment with different prey. Values are given in percent. The total number of eggs laid within the whole experiment corresponds to 100 % (N=9).**

**Tab. 4.6 Correlation coefficient according to Pearson (r) for oviposition rate and number of cannibalism events for *H. aculeifer* and *H. miles* calculated separately for treatments with females, males, nymphs, larvae and eggs as prey (N=9).**

| | *H. aculeifer* | | *H. miles* | |
|---|---|---|---|---|
| **Prey** | **r** | **P** | **r** | **P** |
| **Female** | 0.731 | 0.025 | -* | -* |
| **Male** | 0.884 | 0.002 | 0.577 | 0.104 |
| **Nymph** | 0.213 | 0.582 | 0.017 | 0.966 |
| **Larva** | 0.767 | 0.016 | 0.198 | 0.610 |
| **Eggs** | 0.318 | 0.405 | 0.745 | 0.021 |

* No cannibalism occurred in treatments with only females of *H. miles*

## 4.4 Discussion

*Hypoaspis* mites are frequently used as biocontrol agent against Sciaridae or root mites (Gillespie & Quiring, 1990, Glockemann, 1992, Chambers *et al.,* 1993, Lesna *et al.,* 1995, Jess & Kilpatrick, 2000). Some authors and especially suppliers proclaim at least a side effect of these predatory mites on developmental stages of thrips in the soil (Gillespie & Quiring, 1990, Glockemann, 1992, Chambers *et al.,* 1993, Wright & Chambers, 1994). Although they show high consumption rates on varying prey types in Petri dish experiments or in greenhouses, their efficiency sometimes seems to be limited. In the current study, it is speculated that intraspecific interactions, like cannibalism, could be responsible for the decreased efficiency observed in microcosm experiments (Chapter 2). Therefore, arena experiments were conducted to show the propensity of *H. aculeifer* and *H. miles* to cannibalism. Adult mites and nymphs were introduced as predators of conspecific eggs, larvae, nymphs, females or males as prey. In the following first the tendency of both *Hypoaspis* species to intraspecific interactions, i.e. cannibalism, and then the impact of conspecifics as food source on life history parameters is discussed.

Inclination to cannibalism

The number of cannibalism events was rather low compared to other arthropod predators, e.g. coccinellid beetles (*Harmonia axyridis* and *Coccinella septempunctata*, Coleoptera: Coccinellidae), which feed on up to 5 – 6 conspecific larvae per day (Yasuda & Ohnuma, 1999). The number of killed conspecifics in our treatments did not exceed one individual in three days, also all mites considered as predators survived, even though some of these predators did not show any cannibalism and did not feed during the entire

experimental period of 14 days. Only nymphs of *H. aculeifer* reached cannibalism rates of one egg per day. Moreover, the cannibalism rate did not increase towards the end of the experiment, although it is likely that predators became hungrier from day to day. Similar results were obtained by Usher and Davis (1983). They observed that *H. aculeifer* adults ate immature stages only after weeks of starvation and never showed cannibalism when alternative prey was available. Even though the overall tendency of cannibalism observed in the current study was rather low, it is obvious that *H. aculeifer* was more aggressive against conspecifics and showed a stronger propensity towards cannibalism compared to *H. miles*.

An advantage of cannibalism for generalist predators is to survive periods when prey is scarce (Schausberger & Croft, 2000b). Our results show a rather low tendency towards cannibalism for *Hypoaspis* mites. This could either be explained by the fact that the starvation period of 14 days in the current study was not long enough to increase cannibalism or that *Hypoaspis* species developed mechanisms to avoid cannibalism. According to Usher and Davis (1983), the latter is most likely for arrhenotokous species, since the occurrence of cannibalism among arrhenotokous species like *H. miles* or *H. aculeifer* increases the probability of females preying on their own male descendants. A circumstance that is more than unintentional. Additionally, the soil is a habitat in which dispersal is impeded. Developmental phases with a high dispersal activity are not known for *H. miles* and *H. aculeifer* and none of the two species is recorded to be phoretic. Therefore, it is likely that genetically related individuals frequently come in contact with each other. For this reason mechanisms to avoid kin cannibalism may be selected for and may be responsible for low kin cannibalism rates (Usher & Davis, 1983). Nevertheless, the cannibalistic behaviour of the two mite species is different and at the moment cannot be explained only by the mentioned mechanisms. As a result of the present experiments it is doubtful that the observed cannibalism has any impact on population development of one of the two *Hypoaspis* mites.

<u>Impact of cannibalism on nymphal development and reproduction</u>

Cannibalism occurs in many contexts across a wide spectrum of taxa and it can enable a population to remain viable when it would otherwise become extinct (Elgar & Crespi, 1992). When food resources for adults are too low to support a non-cannibalistic population, adults might use cannibalism to maintain egg production or to redirect reproductive efforts to survive periods of low resource availability (Elgar & Crespi, 1992). It was there-

fore expected that (1) adult *Hypoaspis* mites and especially gravid females frequently prey on conspecifics in order to survive and/or to ensure reproduction and (2) juvenile stages eat conspecifics to continue ontogenesis.

A literature review showed that the daily egg production of *H. miles* females preying on different prey species ranges between 1 and 3 and for *H. aculeifer* between 2 and 4 (Tab. 4.7). Corresponding to this, females of *H. miles* and *H. aculeifer* in the current study produced an average of 1.2 and 2.2 eggs per day respectively, in the control while the egg production never exceeded 2 and 4 eggs (*H. miles* and *H. aculeifer*) in the cannibalism treatments within the whole experimental period of 14 days.

**Tab. 4.7 Oviposition rate of *H. miles* and *H. aculeifer* at different rearing temperatures and on different prey species per day and per female. Data collected from different studies.**

| | Diet | Eggs per day and female | Source |
|---|---|---|---|
| ***H. miles*** | *Bradysia paupera* (Diptera: Sciaridae) | 1 | Ydergaard *et al.* 1997 |
| | *Lycoriella solani* (Diptera: Sciaridae) | 1-2 | Enkegaard *et al.* 1996, 1997 |
| | *Tyrophagus putrescentiae* (Acari: Acaridae) | 1-6 | Enkegaard *et al.* 1996, 1997 |
| | *Acarus siro* (Acari Acaridae) | 2-3 | Wright & Chambers 1994 |
| | *Bradysia paupera* (Diptera: Sciaridae) | 2-3 | Ydergaard *et al.* 1997 |
| | *Turbatrix silusiae* (Nematoda: Cephalobidae) | 1-2 | Present study |
| ***H. aculeifer*** | Enchytraeidae (Clitellata: Plesiopora) | 1-2 | Ignatowicz 1974 |
| | *Rhizoglyphus echinopus* (Acari: Acaridae) | 2-4 | Zedan 1988 |
| | *Isotoma* sp. (Collembola: Isotomidae) | 2-3 | Ragusa *et al.* 1986 |
| | *Histiostatum* sp. (Acari: Anoetidae) | 3-4 | Ragusa *et al.* 1986 |
| | *Tyrophagus* sp. (Acari: Acaridae) | 3-4 | Ragusa *et al.* 1986 |
| | *Rhizoglyphus echinopus* (Acari: Acaridae) | 4-5 | Lesna *et al.* 1996 |
| | *Turbatrix silusiae* (Nematoda: Cephalobidae) | 2-3 | Present study |

Nevertheless, only females of *H. aculeifer* feeding on conspecific larvae or males continuously laid eggs within the experimental period (Fig. 4.4) and egg production was positively influenced by cannibalism (Tab. 4.6). Although the cannibalism rate was very low, *H. aculeifer* seemed to profit more from preying on conspecifics than *H. miles*. Moreover, egg production of female in several treatments s decreased significantly after an initial period of approximately four days (Fig. 4.3 and 4.4). It is likely that egg production of both species was influenced by previous food uptake and not by cannibalism. It would

appear the food reserves were sufficient to maintain egg production for only four days. Similar results where observed for species of the family Phytoseiidae, i.e. conspecific prey did not provide sufficient nourishment for sustained reproduction (Walzer & Schausberger, 1999, Yao & Chant, 1989). Primarily starvation has a negative effect on the gonads and might lead to quiescence (Müller, 1992). Our results did not indicate quiescence but the oviposition rate clearly declined within a few days. Unfortunately, it remains unclear as to whether cannibalism could prolong survival of female mites, since all individuals survived the experimental period. It seems that *Hypoaspis* mites developed other mechanism to overcome shortage in prey availability, i.e. reduced metabolism rate, fat reserves for short time periods and quiescence or dormancy on the long run.

Although the cannibalism rate of the nymphal developmental stage was slightly higher compared to other life stages, only juveniles of *H. miles* feeding on conspecific eggs were able to complete their life cycle within the experimental period. On average, 0.26 eggs per day provided enough food to reach adulthood within 11.1 days, which is comparable to literature data on duration of the nymphal stage of 6.5 to 10.5 days (Tab. 4.8). In contrast, *H. aculeifer* nymphs did not reach adulthood within 14 days although they preyed on almost three times more conspecifics than *H. miles* (Tab. 4.4, Fig. 4.1 & 4.2). Since the nymphal stage lasted only 9.1 days with nematodes as prey it is likely that either the nutritious value of *H. miles* eggs is higher compared to *H. aculeifer* eggs or that *H. miles* is able to utilize eggs more efficiently as food. Additionally, the nutritional value of *H. miles* eggs seems to be higher compared to larvae since on average 0.26 eggs are enough for *H. miles* nymphs to develop to adults whereas 0.32 larvae per day are not. This hypothesis is supported by the results of Enkegaard *et al.* (1996 & 1997). They observed that a single protonymph of *H. miles* consumed on average 0.15 sciarid larvae (0.036 mg) or 8.8 *Tyrophagus* mites (Acari: Acaridae) (0.023 mg) per day and a single deutonymph 0.33 sciarid larvae (0.076 mg) or 12.8 *Tyrophagus* mites (0.033 mg) per day. With these diets, nymphal development of *H. miles* lasted 10.5 days on sciarid larvae and 12 days on *Tyrophagus* mites at a temperature of 20 °C. Although the biomass of the consumed prey is 10fold higher in the experiments of Enkegaard *et al.* (1996 & 1997) the mites did not develop faster on sciarid larvae or *Tyrophagus* mites compared to cannibalism on conspecific eggs in the current experiments (0.256 eggs equal 0.00352 mg). For *H. miles*, the nutritious quality of conspecific eggs is therefore most likely to be higher compared to sciarid larvae or *Tyrophagus* mites, when using the dura-

tion of the nymphal stage as a parameter for the quality or usability of a prey. On the other hand, *H. aculeifer* nymphs preyed on 1.02 conspecific eggs per day and did not reach adulthood. The nymphal phase of *H. miles* was prolonged by at least 3.7 days compared to the control and that of *H. aculeifer* was prolonged at least more than 4.9 days (Tab. 4.4), therefore the experimental period was long enough to conclude that *H. aculeifer* gains less benefit from feeding on conspecifics than *H. miles*. However, it should be mentioned that these comparisons remain at a speculative level, since the actual turn over of biomass is not measurable.

**Tab. 4.8 Duration of the developmental stages of *H. miles* and *H. aculeifer* at different rearing temperatures and on different prey species. Data collected from different studies.**

| | Temp. / °C | Duration of developmental stages / days | | | | Source |
|---|---|---|---|---|---|---|
| **Prey** | | **Egg** | **Larva** | **Nymph** | **Egg to adult** | |
| ***H. miles*** | | | | | | |
| *Lycoriella solani (Diptera: Sciaridae)* | 20 | 2.9 | 1.2 | 10.5 | 14.5 | Enkegaard *et al.* 1997 |
| *Tyrophagus* sp. *(Acari: Acaridae)* | 20 | 3.6 | 1.4 | 12 | 16.6 | Enkegaard *et al.* 1997 |
| *Acarus siro (Acari: Acaridae)* | 20 | 5.9 | 1.9 | 9.7 | 17.5 | Wright & Chambers 1994 |
| *Acarus siro (Acari: Acaridae)* | 24 | 4 | 1.0 | 6.5 | 11.4 | Wright & Chambers 1994 |
| ***H. aculeifer*** | | | | | | |
| *Tyrophagus* sp. (Acari: Acaridae) | 22.5 | 5.2 | 1.8 | 6.1 | n.d. | Lobbes & Schotten 1980 |
| *Tyrophagus* sp. (Acari: Acaridae) | 24.5 | 4.3 | 1.9 | 6.3 | n.d. | Lobbes & Schotten 1980 |
| *Histiostatum* sp. (Acari: Anoetidae) | 26 | n.d. | n.d. | 7.4 | 11 | Ragusa *et al.* 1986 |
| *Isotoma* sp. (Collembola: Isotomidae) | 26 | n.d. | n.d. | 8.5 | 12.5 | Ragusa *et al.* 1986 |
| *Rhizoglyphus echinopus* (Acari: Acaridae) | 26 | n.d. | n.d. | 7.5 | 11.9 | Ragusa & Zedan 1988 |
| *Tyrophagus* sp. (Acari: Acaridae) | 26 | n.d. | n.d. | 7.2 | 11.3 | Ragusa *et al.* 1986 |
| *Tyrophagus* sp. (Acari: Acaridae) | 26 | 1.8 | 1.1 | 8.6 | n.d. | Keith *et al.* 1964 |

The fact that the duration of the nymphal stage was significantly longer with conspecific prey than with *T. silusiae* or values given in literature (Tab. 4.4 and 4.8) shows that cannibalism alone did not lead to continued development within the experimental period. Schausberger and Croft (2000b) analysed cannibalistic behaviour of Phytoseiidae, and they mention that cannibalism prolonged development compared on optimal diet. Only a few nymphs reached adulthood when preying on conspecifics. Phytoseiid mites developed with conspecifics as prey two to three times slower than with their preferred prey. Our results show a similar tendency for both *Hypoaspis* mites, which were not able to complete their life cycle when exclusively preying on conspecifics within the experimental period. Only *H. miles* nymphs preying on conspecific eggs developed into adults. In addition, not only the quality of conspecific prey but also the low number of cannibalism events contributes to the prolonged development of juveniles.

Conclusions

Given the low number of cannibalism events in our study, it is difficult to assess the benefit of cannibalism for *Hypoaspis* mites. From the presented experiments, it is not known how long they are able to survive without any food so it is difficult to conclude whether individuals would rather starve to death before feeding on conspecifics. Additionally, there was no indication that conspecific prey provides sufficient nourishment for sustained reproduction and maintenance of a population. Nevertheless, our initial hypothesis for a reduced efficiency of *Hypoaspis* spp. as biocontrol agent against thrips due to cannibalism could not be confirmed. Additionally, it was shown in an other study that the soil-dwelling thrips instars larvae, prepupae and pupae were accepted as prey by both *Hypoaspis* species and ensured a sustained reproduction of the females (Chapter 5). Therefore, it is most likely that the foraging behaviour in the soil and/or the prey preference contribute to the low efficiency of predatory *Hypoaspis* mites. At the current level of knowledge it is also possible that availability and/or distribution of alternative food sources in the soil reduces the efficiency of the polyphagous *Hypoaspis* mites as biocontrol agent. Since in the greenhouse soil nematodes, Collembola, mites and often sciarid larvae are present, *Hypoaspis* finds a lavishly laid table. A combination of the mentioned factors is most likely responsible for the success of the application of *Hypoaspis* spp. as biocontrol agent. A polyphagous predator, like *H. aculeifer* or *H. miles*, will be more effective in controlling a designated target pest if no alternative prey is available, or the other way round when they do not have to search for prey because of non-target organisms

being available in a surplus compared to the target organism, it is not likely that they spend much resources on foraging for the target pest.

The low propensity towards cannibalism together with a broad host range and its longevity makes *Hypoaspis* spp. ideal candidates for a sustainable establishment in the greenhouse ground at least in order to complement common control strategies. Since *H. aculeifer* shows a higher fecundity on varying diets and a higher consumption rate especially on thrips (Chapter 2 and 5) it is assumed that even though it revealed to be more aggressive, especially against conspecific eggs, it is the better choice as biocontrol agent.

# 5 Predation capacity of predatory ground foraging *Hypoaspis* mites (Acari: Laelapidae): theory and practice in thrips control.

## 5.1 Introduction

*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is still one of the most important pests in protected cultures worldwide (Shipp *et al.*, 1991, Robb & Parrella, 1995, Tommasini & Maini, 1995, Lewis, 1997). As a generalist phytophagous species, *F. occidentalis* feeds on a wide range of crops of economic importance in the field and glasshouses (Yudin *et al.,* 1986, Brødsgaard, 1989, Tommasini & Maini, 1995).

In terms of biological control, mainly phytoseiid mites (Manjunatha *et al.*, 1998, Courcy Williams, 2001, Jacobson *et al.*, 2001b), anthocorid bugs (Wittmann & Leather, 1997, Riudavets & Castane, 1998, Meiracker & Sabelis, 1999) and sometimes chrysopid larvae (Riudavets, 1995) are utilized as natural antagonists against the feeding instars of *F. occidentalis* on the above ground plant parts. However, a large part of the *F. occidentalis* population pupates in the soil (Palmer, 1989, Childers *et al.*, 1994, Helyer *et al.*, 1995, Kirk, 1996, see also Chapter 3) and, therefore, at least two developmental stages of *F. occidentalis* escape from common biological control methods. Nearly one half of the development from egg to adult takes place in the soil; therefore, it is promising to search for efficient antagonists among organisms inhabiting the same environment. Besides entomopathogenic nematodes, which recently showed a control potential against *F. occidentalis* (Ebssa *et al.*, 2001a, b), soil-dwelling predatory mites of the genus *Hypoaspis* are most encouraging. These mites are found in soil throughout the world, both in greenhouses and outside, and they play an important role as regulators in soil ecosystems (Karg, 1998). Two mite species, *Hypoaspis aculeifer* (Canestrini) and *H. miles* (Berlese), (Acari: Laelapidae) are of special interest for biological control. Both are polyphagous predators and forage on the soil surface and in the upper soil layer for a wide spectrum of prey species and types like Nematodes, Enchytraeidae, Acari, Collembola and other small Arthropods (Karg, 1995). In experiments with eight different prey species, Brødsgaard *et al.* (1996) demonstrated that *H. miles* also fed on *F. occidentalis* pupae. However, specific information on their foraging behaviour, prey preference and efficacy as predators of thrips is still rare. Glockemann (1992) verified that *F. occidentalis* at least belonged to the prey spectrum of *Hypoaspis* mites in glasshouses. According to Glockemann (1992), *H. miles* had no influence on a thrips population and *H. aculeifer* could just slow down the population increase of *F. occidentalis* (Glockemann, 1992). In contrast, an

efficiency of up to 75% for *H. miles* and up to 80% for *H. aculeifer* against soil-dwelling instars of *F. occidentalis* with rather high predator densities of more than 700 mites per square meter was demonstrated in a recent study (Chapter 2). However, regardless of the predator densities the efficiency was limited, with small parts of the thrips population always escaping predation.

Although there are good results in controlling pests like sciarids (Chambers *et al.,* 1993, Wright & Chambers, 1994, Enkegaard *et al.,* 1997, Ali *et al.,* 1999, Jess & Kilpatrick, 2000) or bulb mites (Lesna *et al.,* 1995, 1996), the efficiency of *Hypoaspis* spp. even against the same pests and especially against thrips often is either limited (Wright & Chambers, 1994, Conijn *et al.,* 1997, Lesna *et al.,* 2000, Glockemann, 1992, Chapter 2) or the applied predator density required for a sufficient control of a pest is very high (Wright & Chambers, 1994, Ydergaard *et al.,* 1997).

The following aspects are conceivable, non-exclusive causes for the limited efficiency: (1) the target pest thrips might not be the preferred prey, (2) the presence of alternative food sources lowers the control effect on the target pest, (3) not all developmental stages inhabiting the soil e.g. larvae, prepupae and pupae in case of thrips, are equally vulnerable towards the attack by the predator, (4) the ability to locate the prey in the soil limits the predation rate, (5) intraspecific interactions, especially cannibalism, may constitute a further reason for a reduced efficiency, and (6) the acceptance and suitability of the varying prey species – in our case thrips instars – might be low.

To optimize biological control of thrips, all the mentioned factors should be investigated. In the current study, it was investigated whether a possibly low acceptance or predation capacity of *H. miles* and *H. aculeifer* might be a reason for the low control success of *F. occidentalis* sometimes observed. Additionally, we wanted to analyse the predatory mites' ability to reproduce on soil-inhabiting instars of *F. occidentalis* as prey. Finally, both species are compared in order to be able to give advice about which species is the better choice to control *F. occidentalis*.

## 5.2 Materials and Methods

Rearing and synchronization of *Hypoaspis* mites and thrips prey

*H. miles* and *H. aculeifer* were taken from synchronized laboratory cultures described above (Chapter 2.2 and 4.2). Two days after reaching adulthood, female and male mites were transferred into the experimental arenas.

Thrips were reared on *P. vulgaris* pods according to Bailey and Smith (1956) (Chapter 2.2).

Arenas and experimental design

Small translucent plastic vials with lid (diameter 36 mm and 45 mm high) were used as arenas. The bottom of each arena was covered with a layer (5 mm) of plaster of Paris mixed with charcoal (5:1). For ventilation, a hole of 5 mm diameter was cut in the upper third of each jar and covered with Nylon tissue (mesh width 64 µm). Every second day, seven drops of tap water were added to the plaster surface.

Five late L2 instars (five days past hatching), five prepupae or five pupae were transferred to each experimental unit to which either a single female or male of *H. miles* or *H. aculeifer* was added. The arenas were checked on a daily basis and dead thrips and those thrips, which had reached the next developmental stage, were replaced by fresh thrips. *Hypoaspis* eggs were counted and placed in separate rearing units to observe the hatching of the larvae. The number of dead thrips was documented. As a control for the oviposition, females were fed with a surplus of nematodes (*T. silusiae*) like in the rearing units. Each treatment was replicated six times and the experiment lasted 14 days.

Statistical Analysis

Normal distribution for all data was checked by Kolmogorov-Smirnov-adjustment-test. Levene-Test was used to verify homogeneity of variance. Univariate analysis of variance was calculated to analyze the influence of mite species, sex and developmental stage of *F. occidentalis* on the predation and oviposition rate. Significant differences were examined with ANOVA and T-test. Multiple comparisons were done by Scheffé-test.

## 5.3 Results

Predation of *H. miles* and *H. aculeifer* on soil-dwelling thrips instars

Univariate analysis of variance revealed that both the mite species and the sex of the mite had a significant influence on the predation rate, whereas the developmental stage of the prey had no influence (Tab. 5.1). There was no significant difference between the number of second instar larvae, prepupae and pupae killed by a single female or male, neither in *H. miles* nor in *H. aculeifer.* The number of killed thrips ranged between 1.45 and 2.09 for female *H. miles* and between 3.12 and 3.89 for female *H. aculeifer* (Fig. 5.1A). Male mites killed 0.48 to 0.65 (*H. miles*) and 0.54 to 0.61 (*H. aculeifer*) thrips per

day (Fig. 5.1B). The number of killed thrips was pooled for all developmental stages and mean values of both species (Tab. 5.2) were compared to each other by analysis of variance. *H. aculeifer* females preyed on significantly more thrips compared to *H. miles* (ANOVA N=35, F=108.80, P<0.001) (Tab. 5.2).

As already mentioned, no difference was found between the number of thrips killed by a single male of one of the mite species, additionally analysis of variance showed that in case of males there was no difference between the two *Hypoaspis*-species (ANOVA N=37, F=0.098, P=0.756) (Fig. 5.1B).

An accumulated depiction of the predation rate over the experimental period showed that in both *Hypoaspis* species, as well as in both sexes, the predation proceeds nearly linear (Fig. 5.2), thus nearly the same number of thrips was killed each day. Additionally, Fig. 5.2 B indicates that there is no difference between the predation rate of male *Hypoaspis* mites.

**Tab. 5.1 Statistical analysis for predation efficiency of *Hypoaspis miles* and *H. aculeifer* on different developmental thrips stages. Results of univariate analysis of variance with tests of effects between the examined subjects (mite species, mite sex, developmental thrips stage).**

| Source of variation | *df* | Mean square | F-value | P-value |
|---|---|---|---|---|
| Mite species | 1 | 3091.87 | 125.19 | <0.001 |
| Mite sex | 1 | 13971.17 | 565.70 | <0.001 |
| Thrips developmental stage | 2 | 18.59 | 0.75 | 0.475 |
| Mite species * Mite sex | 1 | 2907.91 | 117.74 | <0.001 |
| Mite species * Thrips developmental stage | 2 | 215.12 | 8.71 | <0.001 |
| Mite sex * Thrips developmental stage | 2 | 24.15 | 0.98 | 0.382 |
| Mite species * Mite sex * Thrips developmental stage | 2 | 104.91 | 4.25 | 0.019 |

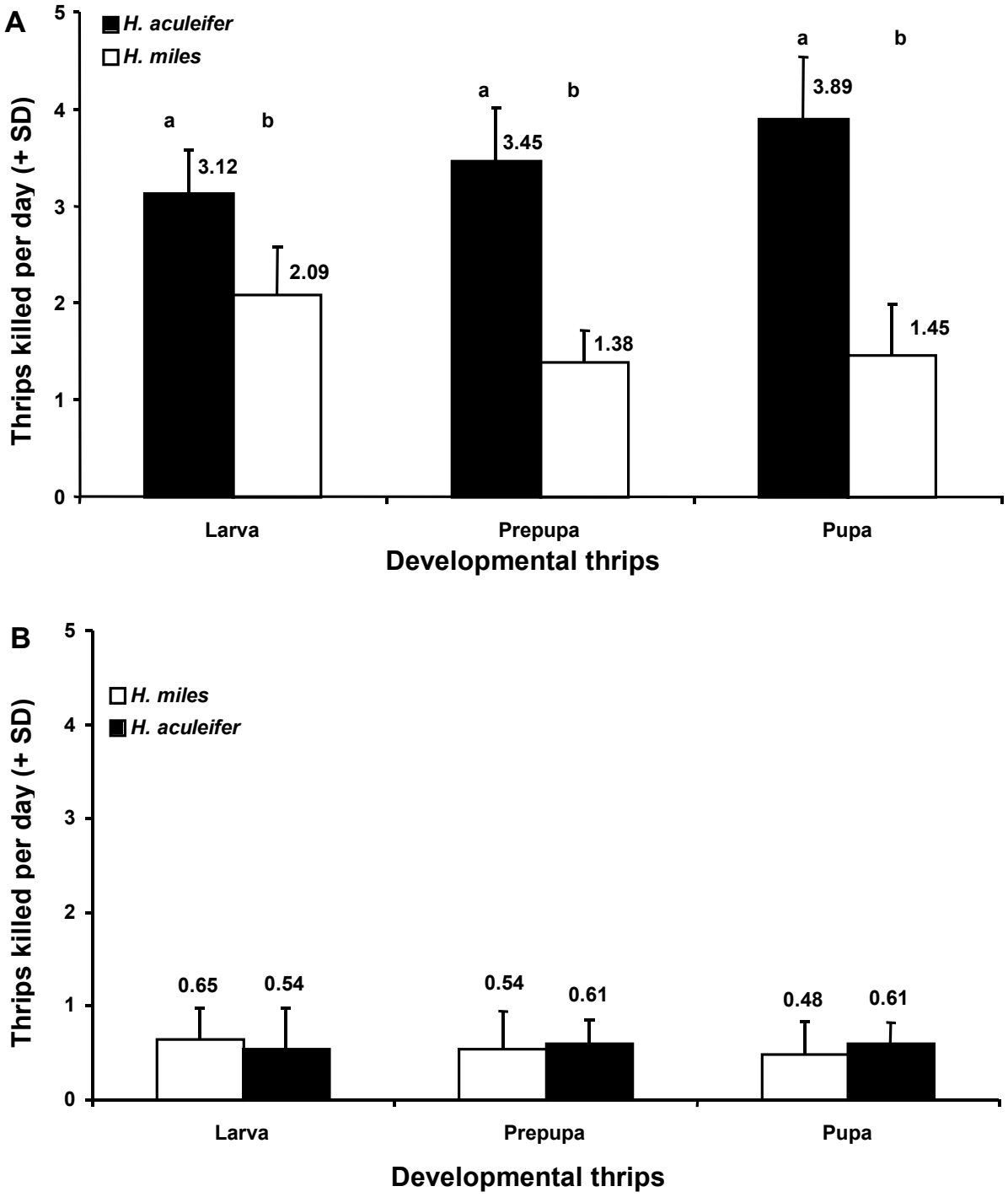



**Fig. 5.1 Mean number of thrips larvae, prepupae and pupae killed by a single female (A) or male (B) *Hypoaspis* mite within a day. Letters above the bars indicate significant differences between treatments. Male mites showed no significant differences in the number of prey killed per day. (N=6)**

**Tab. 5.2 Number of soil-dwelling instars of *F. occidentalis* killed by a single female or male of *H. miles* or *H. aculeifer* per day (N=6 for each treatment).**

| Species | Sex | L2 (±SD) | Prepupae (±SD) | Pupae (±SD) | Mean (±SD) |
|---|---|---|---|---|---|
| ***H. miles*** | Female | 2.09 (± 0.48) | 1.38 (± 0.33) | 1.45 (± 0.54) | 1.61 (±0.34) |
| | Male | 0.65 (± 0.32) | 0.54 (± 0.41) | 0.48 (± 0.36) | 0.56 (±0.3) |
| ***H. aculeifer*** | Female | 3.12 (± 0.45) | 3.45 (± 0.55) | 3.89 (± 0.64) | 3.49 (±0.45) |
| | Male | 0.54 (± 0.44) | 0.61 (± 0.25) | 0.61 (± 0.22) | 0.58 (±0.24) |

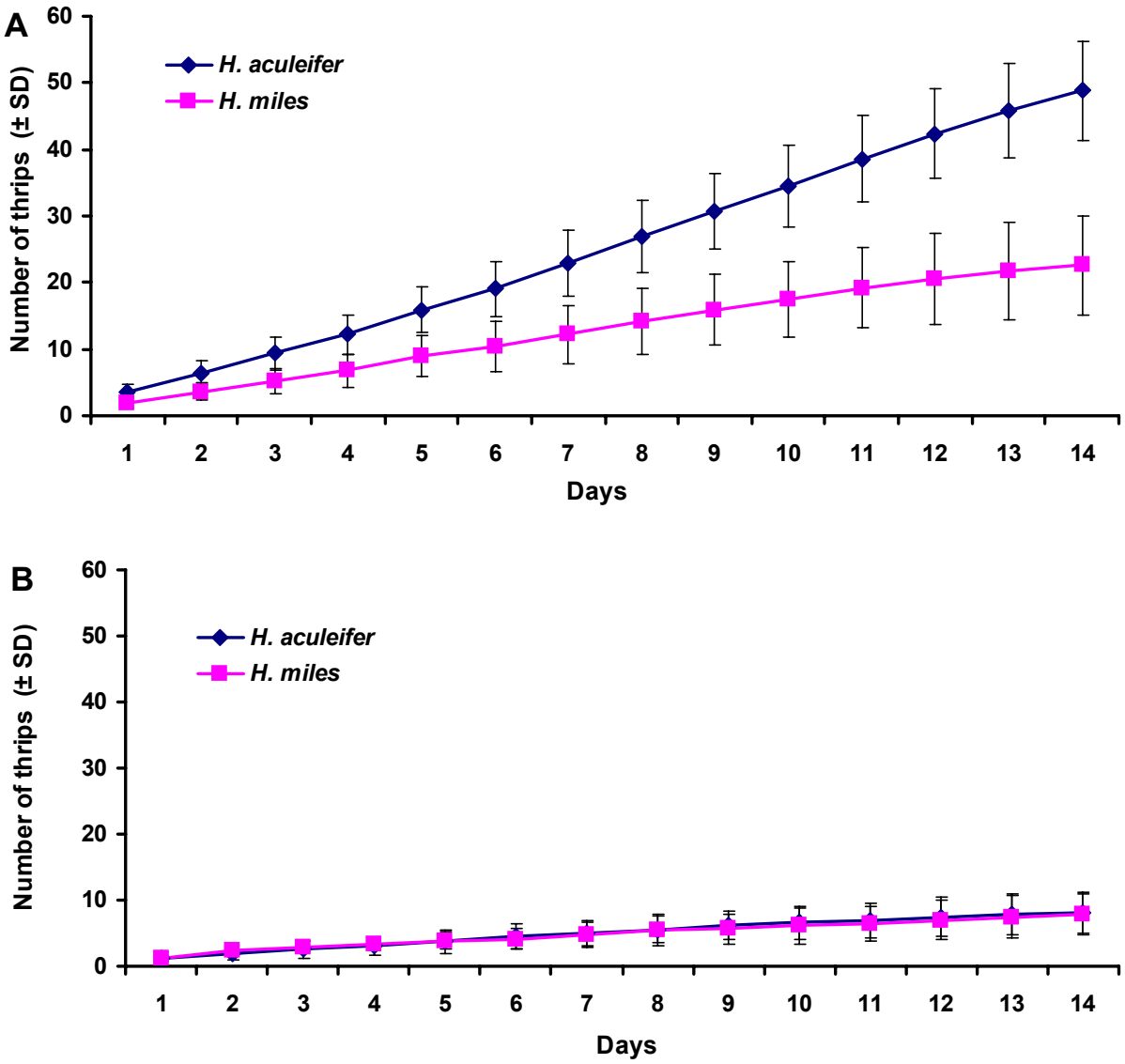



**Fig. 5.2 Cumulative average number of soil-dwelling instars of *F. occidentalis* killed by single female (A) and male (B) predatory *Hypoaspis* mites during the 14 d experimental period. (N=18).**

Oviposition on soil-dwelling thrips instars as prey

Neither the developmental stage of the thrips nor the nematode prey in the control has a significant influence on the oviposition rate (Tab. 5.3). With all thrips stages as prey nearly the same number of eggs was laid by each *Hypoaspis* species as in the control (Tab. 5.4). Therefore, the mean value for egg production was calculated over all treatments with thrips instars as prey (Tab. 5.4) and data were analysed by ANOVA. The analysis revealed a significant difference between the two *Hypoaspis* species: *H. aculeifer* produced at least two times more eggs than *H. miles* when both used *F. occidentalis* as prey (Fig. 5.3).

**Tab. 5.3 Statistical analysis for oviposition of *Hypoaspis miles* and *H. aculeifer* on different developmental thrips stages and a nematode (*Turbatrix silusiae*) as prey in the control. Results of univariate analysis of variance with tests of effects between the examined subjects (mite species, prey).**

| Source of variation | *df* | Mean square | F-value | P-value |
|---|---|---|---|---|
| Mite species | 1 | 30.18 | 419.63 | <0.001 |
| Prey | 3 | 0.23 | 3.20 | 0.04[#] |
| Mite species * Prey | 3 | 0.67 | 9.35 | <0.001 |

[#] Because of heterogeneity of variance the level of significance is lowered to P≤0.01

**Table 5.4 Oviposition rate of *H. miles* and *H. aculeifer* per day within the experimental period on different developmental stages of *F. occidentalis* as prey and mean value over all thrips stages. In the control treatment mites are fed with nematodes (treatments N=6, control N=9).**

| Species | L2 (±SD) | Prepupae (±SD) | Pupae (±SD) | Mean (±SD) | Control (±SD) |
|---|---|---|---|---|---|
| ***H. miles*** | 1.1 (± 0.71) | 0.51 (± 0.42) | 0.71 (± 0.64) | 0.76 (±0.53) | 1.21 (± 1.17) |
| ***H. aculeifer*** | 2.52 (± 0.91) | 2.45 (± 0.97) | 2.51 (± 0.90) | 2.50 (±0.87) | 2.20 (± 0.83) |

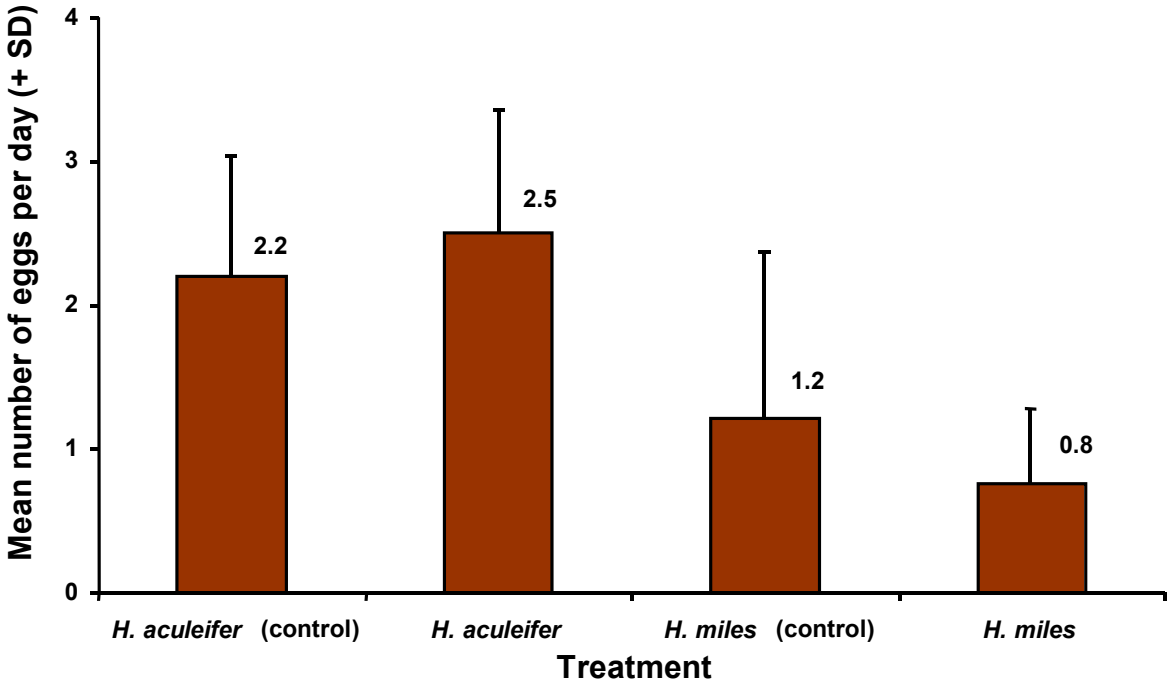



**Fig. 5.3 Mean number of eggs laid by *H. aculeifer* and *H. miles* preying on saprophytic nematodes, *Turbatrix silusiae* (control) and soil-dwelling instars of *F. occidentalis* per day (N=18).**

The oviposition of *H. aculeifer* proceeded nearly linear (Fig. 5.4); the daily egg production remained constant. However, in *H. miles*, a slight increase in egg production between the second and the fifth day could be observed. Thereafter it remained constant and from the 11$^{th}$ to the 14$^{th}$ day egg production decreased (Fig. 5.4). Neither in the case of *H. aculeifer* nor *H. miles* did the egg production in the thrips treatments differ from the control with nematodes. Larvae hatched from all eggs, which had been removed from the arenas during the experimental period.

In the control treatments of each *Hypoaspis* species, the daily egg production remained nearly constant.

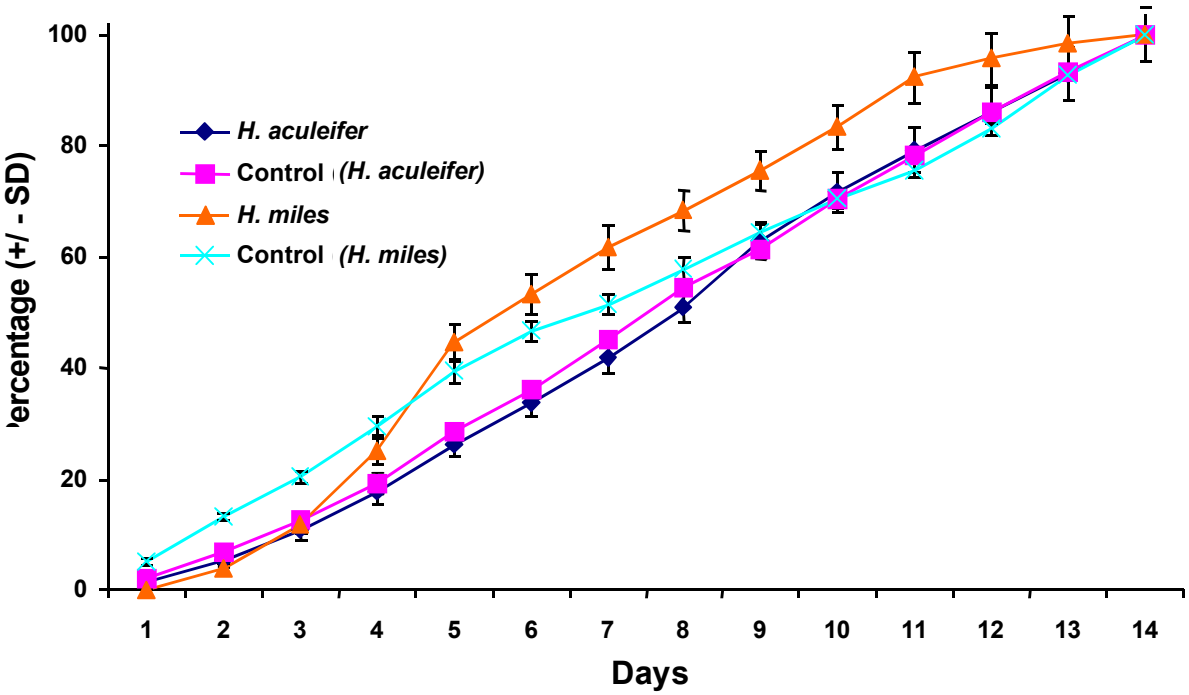



**Fig. 5.4 Cumulative average number of eggs laid by single *Hypoaspis* mites preying on soil-dwelling instars of *F. occidentalis* and saprophytic nematodes, *Turbatrix silusiae* (control) during the 14 d experimental period (N=18).**

## 5.4 Discussion

*Hypoaspis* mites are polyphagous, soil-dwelling predators with a broad range of prey taxa (Karg 1995). At least two species of this genus are frequently used as biocontrol agent against Sciaridae, Collembola or bulb mites like *Rhizoglyphus* sp. (Lesna *et al.*, 1996, Enkegaard *et al.*, 1996, 1997, Ydergaard *et al.*, 1997, Folker-Hansen & Krogh, 1998, Ali *et al.*, 1999, Lesna *et al.*, 2000). Because of their broad host range, these predatory mites are encouraging beneficials to control thrips instars in the soil. In Chapter 2 was shown that *H. miles* and *H. aculeifer* are able to reduce a population of *Frankliniella occidentalis*. However, the potential to control *F. occidentalis* in microcosms, as well as in greenhouses (Glockemann, 1992, Wiethoff personal communication), was limited. To characterize factors that are responsible for this reduced efficiency, we investigated in an arena experiment the predatory behavior of individual *Hypoaspis* mites in presence of thrips larvae, prepupae or pupae. We first discuss acceptance of different prey by *Hypoaspis* mites and then focus on the nutritional value for female reproduction. Finally our current knowledge will be set into the general framework for the use of *Hypoaspis* mites in biological control.

Acceptance of soil-dwelling thrips instars and predation capacity of *H. miles* and *H. aculeifer*.

Even though there are several publications on the use of *Hypoaspis* mites against Sciaridae and on *H. aculeifer* against *Rhizoglyphus* sp. (see above), data on the predation capacity of the predatory mites on any prey species are scarce (Tab. 5.5).

Brødsgaard *et al.* (1996) examined prey preferences of *H. miles* and found this predator in a two-choice experiment feeding on the same number of sciarid larvae as thrips pupae. They observed females feeding on 1.2 to 2.1 thrips per day, an observation that could be confirmed by our results. Moreover, Glockemann (1992) conducted several experiments on the benefit of *H. miles* and *H. aculeifer* as biocontrol agents against *F. occidentalis*, but did not give exact data on the daily predation rate. No data are available on *H. aculeifer* consuming *F. occidentalis*. Furthermore, there are no data on the preferences for or the avoidance of certain thrips instars. Since the pharate pupae of Thripidae develop inside the prepupae (Moritz, 1997), it was expected that the pupae might be less susceptible to predators compared to the larvae or prepupae, but this could not be confirmed by our data. Instead it was found that all thrips instars that are present in the soil, are consumed in equal numbers by both *Hypoaspis* species (Fig. 5.1).

Compared to *Amblyseius* mites (one of the most important commonly-used beneficials against thrips) the *Hypoaspis* mites in our experiments preyed on a slightly smaller number of thrips: *H. miles* ate one to two thrips instars per day, *H. aculeifer* three to four, while *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) showed a predation capacity of four to six thrips and *A. barkeri* (Hughes) two to three larvae of *F. occidentalis* (Brødsgaard, 1989, Houten *et al.*, 1995). However, when comparing predatory mites of the family Phytoseiidae with *Hypoaspis* mites one has to consider that the phytoseiids only prey on the very small first instar larvae of thrips, whereas *Hypoaspis* spp. prey on two times larger instars, the biomass consumed by a single mite may therefore be similar.

**Tab. 5.5 Predation rate of *H. miles* (Part A) and *H. aculeifer* (Part B) at different rearing temperatures and on different prey species per day and per female. Data collected from different studies.**

**Part A**

| | Prey species and stage | Sex of predator | Consumed per day | °C | Source |
|---|---|---|---|---|---|
| *H. miles* | *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) | Female | 21.7 | 20 | Enkegaard *et al.* 1997, 1996 |
| | *Isotomorus* sp. (Collembola: Isotomidae) | Female | 1.5 | 20 | Brødsgaard *et al.* 1996 |
| | *Lycoriella solani* (Winnertz) (Diptera: Siaridae) L2-L4 | Female | 1 | 20 | Enkegaard *et al.* 1997, 1996 |
| | *L. solani* | Female | 1.7-2.9 | 20 | Brødsgaard *et al.* 1996 |
| | *Bradysia paupera* Tuomikoski (Diptera: Siaridae) L2 | Female | 4 | 20 | Wright & Chambers 1994 |
| | *B. paupera* L3 | Female | 1.5 | 20 | Wright & Chambers 1994 |
| | *B. paupera* L4 | Female | 0.6 | 20 | Wright & Chambers 1994 |
| | *F. occidentalis* pupae | Female | 1.2-2.1 | 20 | Brødsgaard *et al.* 1996 |
| | *F. occidentalis* L2, prepupae or pupae | Female<br>Male | 1.3-1.9<br>0.3-0.8 | 24 | Present study |

**Part B**

| | Prey species and stage | Sex of predator | Consumed per day | °C | Source |
|---|---|---|---|---|---|
| *H. aculeifer* | *Rhizoglyphus echinopus* (Fum. & Rob.) (Acari: Acaridae) | Female<br>Male | 3.8-5.2<br>0.2-0.4 | 25 | Ragusa & Zedan 1988, Zedan 1988 |
| | *Sinella coeca* (Schott) (Collembola) | Female | 0.1-0.8 | 24 | Usher & Davis 1983 |
| | *Hypogastrura denticulate* (Bagnell) (Collembola) | Female | 0.1-1.3 | 24 | Usher & Davis 1983 |
| | *F. occidentalis* L2, prepupa or pupa | Female<br>Male | 3-4<br>0.4-0.8 | 24 | Present study |

Almost all data given in the literature refer to females. Practically nothing is known about the predation rates of male mites. Only Ragusa and Zedan (1988) examined the differences in consumption of female and male *H. aculeifer* mites on different developmental stages of *Rhizoglyphus echinopus*. They observed females consuming at least three times more *R. echinopus* than males. Whereas, Wright and Chambers (1994) only mentioned that male mites showed a lower consumption rate. Our data support the conclusions from Wright and Chambers (1994). Females of *H. miles* prey on 2.7 times more thrips than males, and females of *H. aculeifer* killed even 5.8 times more thrips than males. Therefore, it was concluded that the sex ratio of mass released *Hypoaspis* mites can be an important factor for the overall efficiency of the predatory mites.

Reproduction on thrips instars as prey

Overall, the oviposition rate of *H. miles* was lower than that of *H. aculeifer*. A daily egg production of 2.5 ± 0.87 of *H. aculeifer* on thrips instars as prey and 2.2 ± 0.83 on nematodes as prey in our experiments (Tab. 5.4) verifies the data from the literature (Tab. 5.6). Ragusa *et al.* (1986) and Lesna *et al.* (1996) found a higher egg production on *Rhizoglyphus* sp. (Acari: Acaridae) and *Tyrophagus* sp. (Acari: Acaridae), but their experiments were conducted at higher temperatures, which probably explains the higher oviposition rates observed in these studies. In the case of *H. miles*, the oviposition rate in our experiments (1.2 ± 1.2 in the control with the nematode *T. silusiae* and 0.8 ± 0.5 with thrips instars as prey) was a little lower compared to the data given in the literature (Tab. 5.6). When using the oviposition rate as an indication for the suitability of a prey, both *F. occidentalis* and *T. silusiae* seem to be as suitable as *Rhizoglyphus* mites or Collembola for *H. aculeifer* whereas mites like *Tyrophagus* sp. and *Acarus* sp. and Sciarid larvae (*Bradysia paupera* Tuomikoski (Diptera: Sciaridae)) appear to be more suitable for *H. miles.* This theory is supported by the temporal pattern of egg production in our experiments: While the egg production was constantly high in the control treatment from the first to the 14th day for both predator species it slightly increased in case of *H. miles* from the second to the 5th day and decreased from the 11th to the 14th day (Fig. 5.4). A similar trend was not observed with *H. aculeifer*. Since predation was constant during the whole experimental period the increased oviposition activity of *H. miles* is possibly due to an adaptation to thrips prey. It seems that *H. miles*, in contrast to *H. aculeifer*, needed a small time span in the beginning of the experiments to utilize thrips instars for egg pro-

duction. This adaptation, together with the decrease in the egg production at the end of the experiment, might indicate a lower suitability of thrips instars for *H. miles*.

Since larvae emerged from all eggs collected in the control and in the thrips treatments, reproduction and propagation exclusively on thrips larvae, prepupae and pupae as prey appears to be possible for both *Hypoaspis* species, even though the oviposition activity of *H. miles* was slightly lower compared to other prey species mentioned in the literature. Long term experiments are needed to verify the behaviour of the predatory mites. Nevertheless, *H. aculeifer* shows a higher reproduction on soil-dwelling thrips instars than *H. miles*.

**Tab. 5.6 Oviposition rate of *H. miles* and *H. aculeifer* at different rearing temperatures and on different prey species per day and per female. Data collected from different studies.**

| | Diet | Temp. °C | Eggs per day and female | Source |
|---|---|---|---|---|
| ***H. miles*** | *Bradysia paupera* Tuomikoski (Diptera: Sciaridae) | 20 | 1 | Ydergaard *et al.*, 1997 |
| | *Lycoriella solani* (Winnertz) (Diptera: Sciaridae) | 20 | 1-2 | Enkegaard *et al.*, 1996, 1997 |
| | *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) | 20 | 1-6 | Enkegaard *et al.*, 1996, 1997 |
| | *Acarus siro* L. (Acari Acaridae) | 20 | 2-3 | Wright & Chambers, 1994 |
| | *Bradysia paupera* (Diptera: Sciaridae) | 25 | 2-3 | Ydergaard *et al.*, 1997 |
| | *F. occidentalis* | 24 | 1 | Present study |
| | *Turbatrix silusiae* (de Man) (Nematoda: Cephalobidae) | 24 | 1-2 | Present study |
| ***H. aculeifer*** | Enchytraeidae (Clitellata: Plesiopora) | 24 | 1-2 | Ignatowicz, 1974 |
| | *Rhizoglyphus echinopus* (Fum. & Rob.) (Acari: Acaridae) | 25 | 2-4 | Zedan, 1988 |
| | *Isotoma* sp. (Collembola: Isotomidae) | 26 | 2-3 | Ragusa *et al.*, 1986 |
| | *Histiostatum* sp. (Acari: Anoetidae) | 26 | 3-4 | Ragusa *et al.*, 1986 |
| | *Tyrophagus* sp. (Acari: Acaridae) | 26 | 3-4 | Ragusa *et al.*, 1986 |
| | *R. echinopus* | 26 | 4-5 | Lesna *et al.*, 1996 |
| | *F. occidentalis* | 24 | 2-3 | Present study |
| | *T. silusiae* | 24 | 2-3 | Present study |

*Hypoaspis* mites and biological control of thrips

Our results show that all thrips instars in the soil are equally suitable as prey for both *Hypoaspis* species and that the predatory mites are able to reproduce on a thrips diet. Therefore, *Hypoaspis* mites should actually be able to substantially reduce a thrips population as far as the acceptance of soil-dwelling thrips instars and the predation capacity are concerned. Nevertheless, as mentioned above, the literature shows that even though they are indeed able to decimate a thrips population, the control success is limited. If the acceptance and the predation capacity are not responsible for the sometimes reduced control efficiency, which reasons are conceivable?

In microcosm experiments it was found that five individuals of *H. miles* killed 22.5 and five *H. aculeifer* mites killed 28.8 soil-dwelling thrips instars within eight days (Chapter 2). On average the daily consumption rate for *H. aculeifer* was 25% higher compared to *H. miles*. However, this does not necessarily indicate that the mites in the enclosed microcosms preyed on just 0.6 thrips each day. More likely predation rates are much higher in the beginning of the experiment and after prey had become scarce the predation rates decreased and the ability to locate thrips prey in the soil can be a limiting factor. This hypothesis is supported by the experiments of Wright and Chambers (1994). They found that *H. miles* was not feeding on sciarid pupae and speculated that pupae are not attacked because they are immobile and, therefore, not recognized as prey. Similarly, Shereef *et al.* (1980) observed that *H. miles* fed only on the moving developmental stages of its prey. Thus prey movement appears to be an important stimulus for prey location. Prepupae and pupae of thrips of the family Thripidae are resting stages, which only move when they are disturbed. In the small, two-dimensional arenas in our study, where thrips had no possibility to hide, it is assumed that mites frequently contacted thrips. If movement really plays an important role during foraging, it is most likely that they are more often recognized as prey in the experimental arenas than in the more complex structure of natural environments, where foraging mites less often contact the resting thrips stages.

Considering the broad host range of *Hypoaspis* spp. and the results of Brødsgaard *et al.* (1996) the presence of preferred alternative food sources, i.e. sciarid larvae, might reduce the efficiency against thrips. Moreover, saprophytic or parasitic nematodes, Collembola, mites and often sciarid larvae are available in the soil and *Hypoaspis* spp. often find a surplus of prey. The numerical ratio among all suitable prey species may also play an important role for thrips control. A polyphagous predator, like *H. aculeifer* or *H. miles*,

will be more effective in controlling a designated target pest when no alternative prey is available.

Since it has been shown that cannibalism, which inevitably leads to reduced effectiveness, directly limits a predator population (Finke, 1994, Leonardsson, 1991, Wagner & Wise, 1996, Wissinger *et al.* 1996) it is speculated that cannibalism might be a feasible reason for a reduced effectiveness. However, our own results showed that cannibalism among the examined *Hypoaspis* mites plays a minor role for their effectiveness in the biocontrol of thrips (Chapter4).

*H. miles* or *H. aculeifer* as biocontrol agent against thrips

The broad host range, a low propensity towards cannibalism and their longevity make *Hypoaspis* spp. ideal candidates for use as beneficials in the glasshouse soil. Nevertheless *H. aculeifer* was found to be more aggressive (Chapter 2 and 4) and the current results show higher consumption rates for *H. aculeifer* compared to *H. miles*. Moreover, *H. aculeifer* seems to be capable of reproducing faster and establishing higher population densities than *H. miles.* In conclusion, *H. aculeifer* should be preferred for biological control of thrips. Additionally, the extreme differences in predation rates of female and male predatory mites indicate that the use of *Hypoaspis* mites in biological control programs can be enhanced by the release of female biased populations. However, since the efficiency of *Hypoaspis* mites against *F. occidentalis* is limited, the use of beneficials foraging on the upper plant parts should be complemented by application of *Hypoaspis* mites to the soil to optimise thrips control.

# 6 Impact of entomopathogenic nematodes on different soil-dwelling stages of Western Flower Thrips*, Frankliniella occidentalis* (Thysanoptera: Thripidae), in arena and microcosm experiments

## 6.1 Introduction

Among phytophagous thrips Western Flower Thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most important pests and most problematic to control (Chapter 1).

A limited range of natural enemies, including predatory bugs of the genus *Orius* (Heteroptera: Anthocoridae) as well as predatory mites of the genus *Amblyseius* (Acari: Phytoseiidae), preying on foliage feeding life stages of WFT, is available (Loomans & Lenteren, 1995, Riudavets, 1995). However, these predators do not control the soil-dwelling late L2, prepupae and pupae. WFT spends about one third of its life cycle in the soil. At present no single control option of WFT provides satisfactory control (e.g., Parrella *et al*., 1999). Therefore, identifying biocontrol agents that additionally control the soil-dwelling life stages is of paramount importance for the development of successful biological control against WFT.

Entomopathogenic nematodes (EPN) (Rhabditida: Steinernematidae and Heterorhabditidae) are obligate parasites of a large number of insect species. EPN transmit bacteria that are lethal to their host. Over the last two to three decades, EPN have become increasingly popular as biocontrol agents, especially against soil inhabiting pests. The two genera in these families, *Steinernema* and *Heterorhabditis*, consist of over 20 species among which many are effective in controlling insect pests of different orders. Only limited research has been conducted for the control of WFT by EPN, but virulence of some EPN species has been reported (Tomalak, 1994, Helyer *et al*., 1995, Chyzik *et al.*, 1996). Moreover, in a previous study susceptibility of soil-dwelling developmental stages of WFT to different strains of EPN was investigated (Ebssa, 2000). However, no data are available on the susceptibility of the soil-dwelling development stages of WFT to EPN. Moreover, little is known regarding efficacy of virulent EPN strains against WFT populations composed of different soil-dwelling developmental stages of WFT. Strain selection for the present study was based on results of previous experiments (Ebssa, 2000). The selected strains were subsequently tested against mixed population structures of soil-dwelling developmental stages of WFT both in arena (one strain) and microcosm experiments (three

strains). Moreover, the relative susceptibility of the different soil-dwelling development stages of WFT to EPN was investigated.

## 6.2 Materials and methods

Nematode culture

EPN used in this study, i.e. *Steinernema feltiae* (Filipjev) strain Sylt, *S. carpocapsae* (Weiser) strain DD136 and *Heterorhabditis bacteriophora* Poinar strain HK3, were obtained from the Institute of Phytopathology, Kiel University, Germany. Following the protocol of Woodring and Kaya (1988), all strains were reared in the laboratory at 23 ± 2°C in last instars of the wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Infective juveniles (IJ) were stored in a cold room at 4°C. The nematodes were allowed to acclimate at room temperature for at least 6 h before they were used in the subsequent bioassays. Prior to application, the concentration of the EPN suspension (in number of IJ/ml suspension) was determined by counting (Woodring & Kaya, 1988). To avoid any efficiency difference among the strains due to their shelf life, only two- to three-week old EPN strains were used in the experiments.

Western Flower Thrips rearing

Following the protocol according to Bailey and Smith (1956) WFT were reared on pods of green beans (*Phaseolus vulgaris* L.) (Chapter 4.2).

Assay Arena

For the laboratory experiments, the following assay arena was used: Non-sterilised 2.5 g soil (Fruhstorfer Erde Type P, moisture content (MC) of about 38.4% (determined by oven drying)), sieved by 2 × 2.5 mm sieve pore size, was added to a plastic Petri dish (35 × 10 mm). The soil is commercially available (Archut GmbH, Lauterbach – Wallenrod, Germany) and used in many German greenhouses. It is composed of humus, clay and peat in the proportion of 15:35:50, respectively. 400 IJ/cm$^2$ of *S. feltiae* in 1 ml water or distilled water (thus, final MC about 67%) was pipetted on the top of the soil after introducing the immature stages of WFT. The inner edge of the lid of the Petri dish was lined with modelling clay so that the Petri dish could be tightly closed to avoid any escape of emerging adult WFT. A small hole (∅ 7 mm) was drilled in the centre of the lid of the Petri dish onto which nylon tissue (pore size ∅ 64 µm) was glued to allow ventilation but preventing WFT from escaping. The inner part of the lid of the Petri dish, except the hole, was painted with

insect glue (Temmen GmbH, Hattersheim, Germany) so that emerging adult WFT could get stuck to it (subsequently referred to as 'sticky traps').

The late L2, prepupae and pupae used in the experiments were collected 14, 16 and 17 days after emergence of neonate larvae, respectively. All insects were first individually examined under the binocular and then transferred to the arena using a fine Kolinsky hairbrush. Thereafter the EPN suspension or distilled water was pipetted to the top of the soil in the Petri dish. Then, the Petri dish was tightly closed and kept for one week in a climate chamber at 23 ± 2°C, 18:6 h L:D photo period and 60–90 % RH. At the end of the experiments (i.e., seven days later), all WFT adults (dead or alive) and alive prepupae and pupae from the soil in the assay arena (on the surface of the soil and inside the soil) and adults that got stuck in the sticky traps were counted. Thus, corrected mortality was calculated based on the total number of adults and alive immature stages counted at the end of the experiment.

Influence of population structure of soil-dwelling life stages on EPN efficacy

Different proportions of late L2, prepupae and pupae were added to the assay arena (for the different proportions tested, refer to table 6.1) and subsequently treated with EPN. The total number of immature WFT transferred to each arena was 21. These proportions represent different population structures of soil-dwelling developmental stages of WFT that can be present in the soil at different times. Each treatment with different proportion of immature stages had its own control.

Effect of WFT position in the arena on efficacy of EPN

In a previous study using the same bioassay it was observed that, after transferring the immature WFT to the arena, the L2 penetrated into the soil, whereas the majority of the prepupae and pupae stayed on the top of the soil and hence, the EPN attacked the insects at their respective position (Ebssa, 2000). To assess the effect of the position of the soil-dwelling stages of WFT on the efficacy of the EPN, an additional experiment was carried out. The same assay arena as described above was used. Ten WFT pupae were placed at the bottom, middle, or top of the Petri dishes, i.e. at 8, 4 and 0 mm soil depths, respectively. Finally, in all three treatments a *S. feltiae* suspension at a concentration of 400 IJ/cm² was pipetted to the top of the soil and the arena was tightly closed as described above. Each position had its own control treatment using distilled water instead of

EPN. Each treatment was replicated five times and numbers of WFT were assessed as described above.

**Tab. 6.1 The number of larva (L), prepupa (B) and pupa (P) of WFT applied to assay arena.**

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

EPN efficacy in microcosm experiments

Seeds of *P. vulgaris* were sown and at cotyledon stage, the seedlings were transplanted to a pot (∅ 11 cm) filled with soil (similar to laboratory experiments), forming a top area of approximately 78 $cm^2$. At the two-leaves stage, the plants were caged using acryl-glass tubes (Chapter 4.2). Twenty adult females and two males of WFT of the same age were released into the cage, using the same sex ratio as observed in the stock culture. Two similar sized side-holes in the lower portion of the acryl-glass tubes were used as 'windows' for releasing the adult WFT to the seedlings and for pipetting the EPN suspensions. Under these conditions, in preliminary experiments, pupation of WFT usually occurred eight to ten days after introduction of the adult thrips. Hence, either EPN suspension (*H. bacteriophora, S. feltiae* and *S. carpocapsae* at concentrations of 400 and 1,000 IJ/$cm^2$) or distilled water (as control) was applied to the pots 12 days after thrips introduction. Fifteen minutes after the EPN application, the soil was irrigated with 50 ml of distilled water to rinse the EPN on the top of the soil so that the nematodes could reach the pupating WFT in the soil. Completely randomised block design with five replications per treatment was used. To provide sufficient time for pupation, the seedlings were kept for

two additional days after EPN application until the 14th day after introduction of the WFT. Thereafter, the acryl-glass tube and the shoot part of the seedling with all foliage feeding stages of WFT were removed. Then, the pots were covered with the lid of a Petri dish (∅ 100 mm) that had been painted with insect glue to serve as sticking trap for emerging adults.

The number of WFT offspring for a period of 15 days (including the day of introduction) was assessed. For this, control pots were sampled and all foliar stages of WFT (L1/L2, prepupae, and pupae) were counted under microscope. WFT that were in the soil for pupation were collected later as emerged adults in the sticky traps. The emerging adults in all treatments were counted daily for ten consecutive days, starting one day after the removal of the cage. At the end of the experiment, all adults remaining on the top of the soil in the pots were also counted. Efficacy of the different EPN strains was assessed using corrected mortality values.

Statistical analysis

Mortality data were corrected for control mortality following Abbott's formula (1925) and analysed using analysis of variance (ANOVA) (SAS, 1996). Data uniformity was checked and in case of non-uniform data distribution the non-parametric Wilcoxon rank test was used (Sokal & Rohlf, 1995). The corrected mortality means were compared to zero (corrected mortality of control) using the Dunnett test (SAS, 1996). When significant factor effects were detected by means of ANOVA, corrected mortality means at different levels of the respective factor were compared using Tukey's multiple means comparison procedure. A significance level of $\alpha$=0.05 was used in all analyses.

## 6.3 Results

EPN efficacy under laboratory conditions

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

Applying EPN to any of the tested populations of immature WFT resulted in a significant reduction of the number of emerging adult WFT (N=78, Z=8.1 and P=0.0001). EPN-

induced mortality significantly varied in the tested populations of the immature WFT (*df*=12, 24; F=3.53, P=0.004) and was significantly lower in treatments with a high larval proportion (Fig. 6.1). Maximum mortality was recorded with high proportions of prepupae and/or pupae.

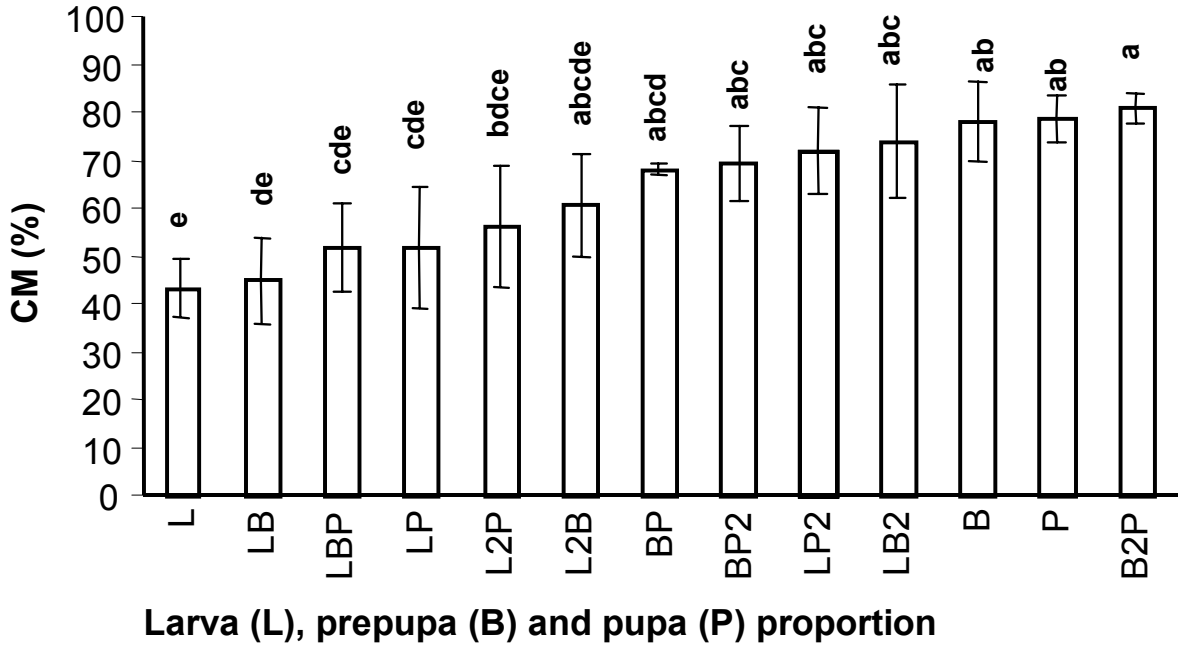



**Fig. 6.1 Corrected mortality (±SE) (%) of WFT by *Steinernema feltiae* as affected by the proportions (%) of second instar larvae (L), prepupae (B) and pupae (P) in the population structure. Refer to table 1 for the acronyms of proportion of immature stages. Bars with the same letters are not significantly different at P=0.05.**

The proportion of late L2 in the population negatively affected the mortality of WFT by EPN ($R^2$=0.69, P=0.0001). The proportion of prepupa and pupa did not significantly influence mortality ($R^2$=0.19, P=0.054 for prepupa, and $R^2$=0.15, P=0.088 for pupa). Moreover, the slopes of the regression lines (Fig. 6.2) of prepupa and pupa proportions were not significantly different (*df* =1, 37; F=0.07; P=0.80). Since under natural conditions all immature stages of WFT exist together in varying proportions, a multiple regression analysis was performed. The results of the analysis indicated that unlike that of prepupa and pupa partial regression coefficient was significant for larvae (t=3.50, P=0.0013) thus EPN-induced mortality was determined only by the proportion of late L2.

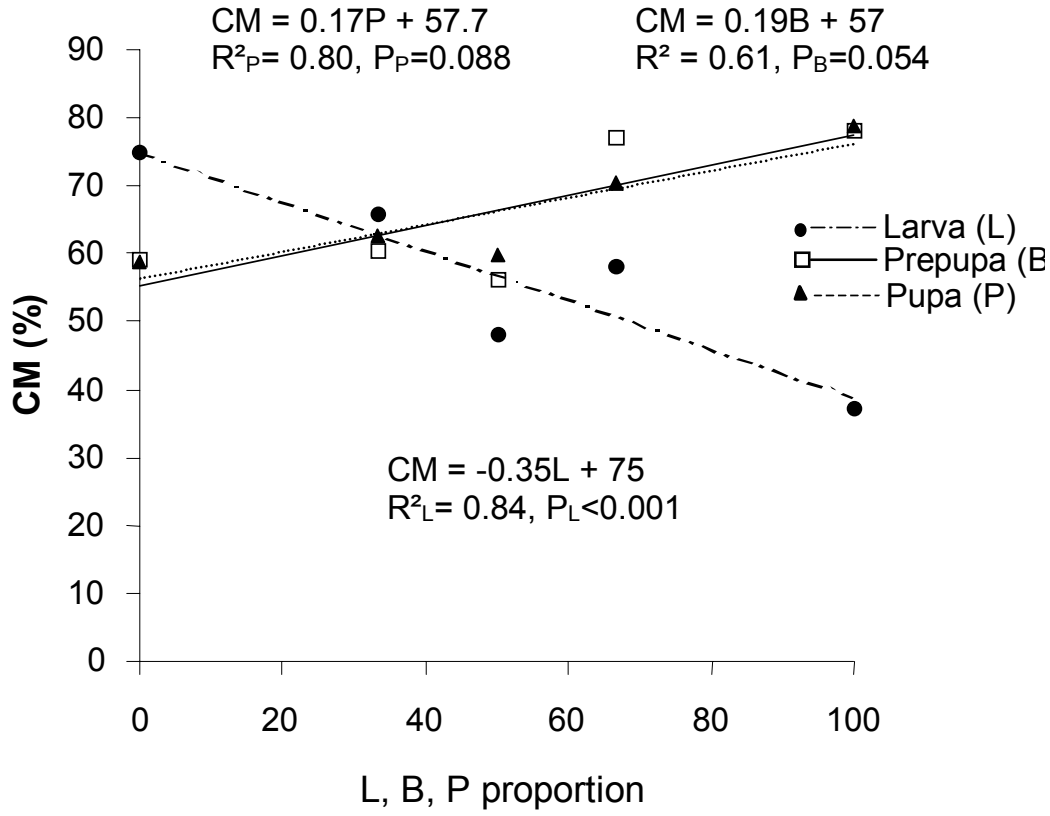



**Fig. 6.2 Relationship between the proportions of larvae (L), prepupae (B), or pupae (P) in the population composition and EPN (*Steinernema feltiae*) induced corrected mortality (CM) (%) in WFT. $R^2_L$ and $P_L$, $R^2_B$ and $P_B$, $R^2_P$ and $P_P$ indicate the correlation coefficients and P-values of larva, prepupa, and pupae, respectively.**

Results of the additional experiment carried out to study the effect of position of immature WFT in the arena on the efficacy of *S. feltiae* are presented in table 6.2. In the control treatment a significantly higher proportion of adults emerged from the top compared to the two treatments in the soil. However, EPN-induced mortality was not significantly influenced by the position of the pupae (*df*=2, 14; F=1.65; P=0.232).

**Tab. 6.2 WFT adult emergence in control treatment and corrected mortality of pupae at different position in the arena after application of EPN (*Steinernema feltiae* Sylt).** [a]

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

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

EPN efficacy in microcosm experiments

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

WFT Population development and distribution on plant

Fifteen days after WFT adults were introduced to the bean seedlings, 73.2% of the total F1 WFT in the control pot was on the plant where most of them (94.7%) were L1/L2. However, the remaining proportion (i.e., 26.8% of the total F1 WFT in the pot) was in the soil for pupation and was counted as emerged adults. The F1 adults started to emerge from the soil 17 days after WFT introduction thus, all adult WFT on the plant at the time of data collection were considered members of the parent population. At the end of the experiment, i.e. 25 days after adult introduction, no more emerging adults were observed. On the 25$^{th}$ day after adult introduction, dead adults, which presumably died due to starvation, were counted from the top of the soil in each pot. In the control, they account for 31.6% of the total number of emerged adults. The total number of F1 WFT per pot in the control treatment was 229.60 ± (SD=58.57); this figure includes numbers of L1/L2, prepupae, and pupae (counted from plant leaves 15 days after adult introduction) and adults (counted from the sticky traps 17 to 24 days after adult introduction and counted from the top of the soil in the pot at the end of the experiment). Out of the total WFT that pupated within a fortnight, 86% and 14% of the WFT pupated in the soil and on the leaves, respectively.

Adult emergence

Considering only those adults that got stuck to the sticky trap, emergence peaked around 20 days after the initial introduction of WFT adults (Fig. 6.3). The number of adults counted only from the sticky trap and those counted both from the sticky traps and the top of the soil differed significantly (Tab. 6.3). However, the difference did not depend on the different treatments (i.e. control, and EPN strains at higher and lower dose rates) (*df*=6, 52; F=0.07; P=0.998, data analysed after square root transformation). In general, 28.7% ± 13.8 of the total adult WFT stayed on the top of the soil after emergence while the remaining 71.3% ± 13.8 left the soil and subsequently were caught in the sticky traps. The proportion of adults that stayed on the soil was not influenced by the application of different EPN strains or distilled water (*df*=6, 28; F=0.54; P=0.774). Hence, corrected mortality

values did not differ significantly whether they were based on adult counts in sticky traps or both from sticky traps and soil counts (Tab. 6.3).

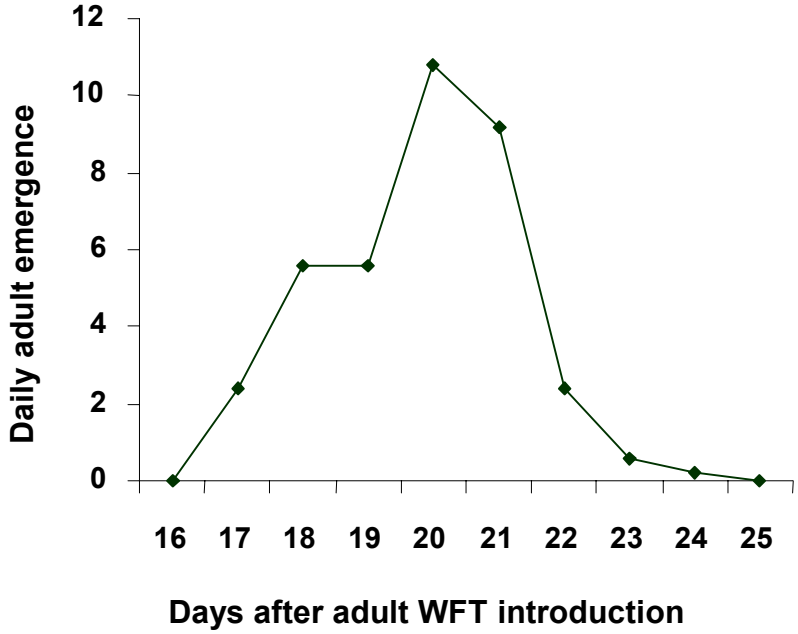



**Fig. 6.3 Daily WFT adult emergence counted from the sticky traps in control treatment.**

**Tab. 6.3 Number of adult WFT counted only in sticky traps and from both sticky traps and the top of the soil. Corrected mortality (CM in %, ± SE) of WFT was calculated based on the number of adults counted using both methods. [a]**

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

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

[b] Data analysed after square root transformation

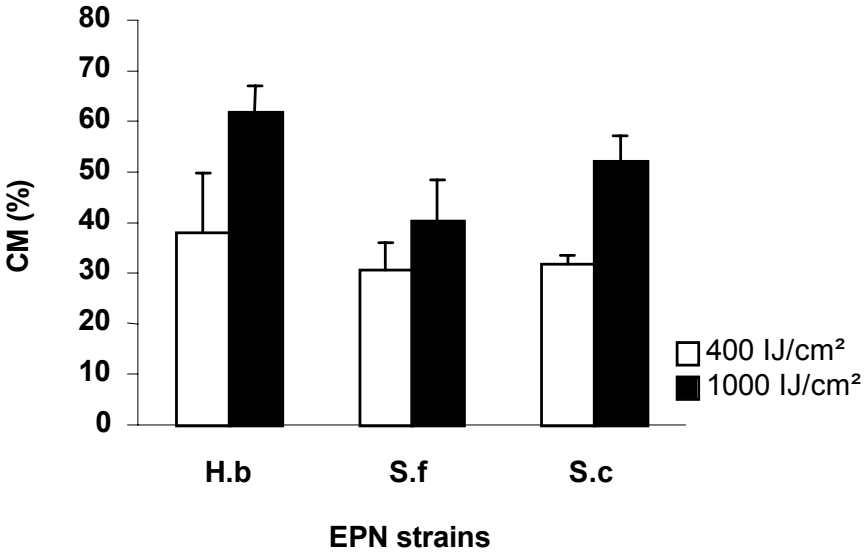



**Fig. 6.4 Corrected mortality (CM in %, ± SE) of WFT caused by EPN strains (*Steinernema feltiae* (S.f), *S. carpocapsae* (S.c) and *Heterorhabditis bacteriophora* (H.b)) at 400 and 1,000 IJ/cm$^2$.**

Mortality

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

Increasing the dose rate from 400 to 1,000 IJ/cm$^2$ significantly increased mortality in WFT (*df*=1, 20; F=10.28; P=0.004). For the higher concentration maximum mortality was recorded for *H. bacteriophora* (61.8%) and lowest mortality in *S. feltiae* (< 50%) (Fig. 6.4). However, for the two dose rates together EPN-induced mortality did not differ significantly among the three tested strains (*df*=2, 20; F=2.05; P=0.155).

## 6.4 Discussion

In the laboratory experiments, late L2 descended into the soil in the arena while the majority of the prepupae and pupae remained on the top of the soil, confirming results of a previous study (Ebssa, 2000). However, data from the experiment on spatial distribution of WFT pupae showed that the position of the pupae in the arena did not affect mortality induced by *S. feltiae*. Moreover, since mortality by *S. feltiae* in prepupae and pupae did not differ significantly, the lower mortality in treatments with high proportions of late L2 in

the population structure was most likely not influenced by the position of the immatures in the arena but reflects a higher susceptibility of WFT prepupae and pupae to EPN.

Differences in susceptibility to EPN exist among different developmental stages of insects. Early L1 of *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) are the most susceptible stages (Glazer & Navon, 1990). In contrast, later larval stages of *Maladera martida* Argaman (Coleoptera: Scarabaeidae) were reported to be the most susceptible stage (Glazer & Gol'berg, 1989). In *Tipula paludosa* Meigen (Diptera: Tipulidae) and *T. oleracea* L. (Diptera: Tipulidae) highest mortality was recorded for the L1 approaching the first moult while young L1 were less susceptible (Peters & Ehlers, 1994).

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

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

In our microcosm experiments, no host plants were provided as a food source for the emerging adults, and about 30% of the adults that emerged from pupae did not leave the soil but stayed on the top of the surface. However, the remaining 70% of the F1 showed negative geotaxis and consequently were caught in the sticky traps. Ten days after first adult emergence, most of the adults that were counted from the top of the soil were dead, indicating that they might have been the first F1 adults, which emerged from the pupae and died presumably due to starvation. IJ might still have been active at the time of adult emergence, as it was observed for *S. carpocapsae* even at the end of the experiment (data not presented). However, the proportion of adults on the soil and on the sticky traps did not differ between control and EPN treatments. Most likely, adult WFT either did not recognise the presence of IJ in the soil or were not bothered by the presence of EPN.

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

Very often EPN-induced mortality in laboratory assays cannot be reproduced in the field (Georgis & Gaugler, 1991). However, correct choice of the bioassay method in the laboratory can substantially enhance the likelihood of later success in the field (Griffin, 1993). In previous laboratory assays, up to 97% mortality of WFT pupae was recorded using a dose rate of 1,000 IJ/cm$^2$ (Ebssa, 2000). In the experiments under laboratory conditions, EPN were directly applied to the pupa and prepupa, or reached L2 within 0.8 cm soil depth. In the microcosm experiments, however, nematodes reached the soil-dwelling life stages of WFT, either by passive movement with percolating water (e.g. Selvan *et al*. 1994), or through active downward migration to a limited depth as reported by Grewal *et al*. (1994) for *H. bacteriophora*. This means that under field conditions, the concentration of IJ that reaches the resting WFT in the soil is lower than the applied concentration. Therefore, the amount of post EPN release irrigation water is crucial with respect to the depth of WFT pupation if too much the majority of IJ go down with the drain if too little the IJ remain on the surface only. Hence, in ongoing studies we are investigating the effect of varying amounts of irrigation water on the efficacy of EPN against WFT.

Under natural conditions, late L2 crawl in a continuous manner from the foliage to the ground even after applications of EPN, thus increasing the density of WFT in the soil. Moreover, persistence of EPN in the soil type used in our experiments (i.e. Fruhstorfer Erde) is not known. All these factors might affect the efficacy of EPN. Therefore in ongoing studies, we are investigating the effects of prey density and EPN persistency on WFT control.

In a study by Chyzik *et al*. (1996) all EPN strains tested, except for *H. bacteriophora* HP88, failed to control WFT in pots at a concentration of 400 IJ/cm$^2$. In our experiments, the water used to rinse nematodes down helped most of the IJ to reach the resting WFT. Gaugler *et al*. (1997) suggested that a pre-EPN-application irrigation is required. Prior to the EPN applications the soil in the microcosm experiments was moist enough and hence, for all strains EPN-induced mortality in WFT even at a concentration of 400 IJ/cm$^2$ was significantly higher than the control mortality.

In the microcosm experiments EPN-induced mortality in WFT significantly increased with increasing the dose rate from 400 to 1,000 IJ/cm$^2$. Hence, in greenhouses higher EPN concentrations can result in enhanced WFT control. At present, a dose rate of 1,000 IJ/cm$^2$ is very high compared to EPN concentrations used for control of other insects. However, high concentrations of EPN are required for sufficient control of WFT because (I) EPN do not perpetuate in WFT as hosts (data not presented), most likely due to the small body size of the thrips, and (II) upon disturbance, soil-dwelling development stages of WFT become mobile, thereby possibly escaping an EPN attack. Sulistyanto and Ehlers (1996) reported that *H. bacteriophora* (strain EN0043) applied at 1.5 million IJ/m$^2$ (i.e. 150 IJ/cm$^2$) successfully controlled grubs *Phyllopertha horticola* (L.) (Coleoptera: Scarabaeidae) in a golf course with population reductions of up to 83%. WFT is economically a very important pest and already developed resistance to many insecticides (e.g. Broadbent & Peer, 1997). Moreover, at present there is no single control technique available that assures a high control level of WFT (Parrella *et al.*, 1999). Our results indicate, that EPN could become an important and promising component in future biocontrol strategies against WFT, targeting the pest from different angles through combined releases of predators against life stages on the foliage and EPN applications against soil-dwelling life stages. In ongoing studies we are studying the combined effects of predator releases and EPN applications on the population dynamics of WFT under greenhouse conditions.

# 7 Efficacy of entomopathogenic nematodes against soil-dwelling life stages of Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae)

## 7.1 Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important cosmopolitan pest of a wide range of economically important crops. This pest is – as mentioned repeatedly – extremely problematic to manage (Chapter 1).

Chemical control of WFT is difficult because of its cryptic feeding behaviour (flowers and leaf axis) and life strategy (i.e. pupation in the soil) (Palmer, 1989, Helyer & Brobyn, 1992). Moreover, the high frequency of insecticide applications for WFT control, coupled with the short generation time in *F. occidentalis* has led to an increasing incidence of insecticide resistance in WFT in recent years (Immaraju *et al*., 1992, Brodsgaard, 1994, Zhao *et al*., 1995, Broadbent & Pree, 1997). For biological control, a limited range of natural enemies against the foliage life stages of WFT is available, including several species of predatory bugs of the genus *Orius* (Heteroptera: Anthocoridae) as well as predaceous mites like *Amblyseius barkeri* (Hughes) and *A. cucumeris* (Oudemans) (Acari: Phytoseiidae) (Loomans & Lenteren, 1995, Riudavets, 1995, Tommasini & Maini, 1995). Because WFT spends about one third of its life cycle in the ground, these predators cannot sufficiently control thrips populations (Loomans & Lenteren, 1995, Riudavets, 1995). Therefore, identification of natural enemies targeting the soil-inhabiting life stages of WFT could substantially improve biological control of *F. occidentalis*.

Entomopathogenic nematodes (EPN) (Rhabditida: Steinernematidae and Heterorhabditidae) are suitable biological control agents for soil-inhabiting insects. Even though only limited research has been conducted on the control of WFT by EPN, virulence of some EPN species was reported (Tomalak, 1994, Helyer *et al*., 1995, Chyzik *et al*., 1996). However, data on the susceptibility of the soil inhabiting thrips instars being available is scarce and the knowledge about differences among the EPN strains with regard to thrips control is small (Chapter 6). Thus, the objective of this study was to screen a further number of strains of different EPN species with special regard to various concentrations for control of the soil-dwelling life stages of WFT.

## 7.2 Materials and methods

Nematode culture

Six different strains of *Steinernema feltiae* (Filipjev) and *S. carpocapsae* (Weiser), *Heterorhabditis bacteriophora* Poinar (Tab. 7.1), kindly provided by R.-U. Ehlers, Institute of Phytopathology, Kiel University, Germany, were tested. The nematodes had been previously reared for several generations in the laboratories of the Institute of Phytopathology in Kiel. Following the nematode rearing protocol of Woodring and Kaya (1988), all strains were reared in the laboratory at 23 ± 2°C in last instar larvae of the wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). According to the rearing protocol, infective juveniles (IJ) were harvested from white traps (Woodring & Kaya, 1988) and stored in a cold room at 4°C. The nematodes were allowed to acclimate at room temperature for at least 6 h prior to subsequent testing in the bioassays. Before the application, the concentration of the EPN suspension (in number of IJ/ml suspension) was determined by counting (Woodring & Kaya, 1988). To avoid any difference in efficacy due to varying shelf life, in all experiments only two- to three-week old EPN strains were used.

**Tab. 7.1 Entomopathogenic nematode strains tested.**

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

Western Flower Thrips rearing

For the rearing procedure of *F. occidentalis* see Chapter 2.2.

Assay Arena

Non-sterilized 2.5 g soil (Fruhstorfer Erde Type P, moisture content (MC) of about 38.4% (weight by weight, determined by oven drying), sieved by 2 x 2.5 mm pore sized sieve, was added to a plastic Petri dish (3.5 cm in diameter and 1.0 cm in height). The soil is commercially available (Archut GmbH, Lauterbach – Wallenrod, Germany) and used in

many greenhouses for plant production. It is composed of humus, clay and peat in the proportion of 15:35:50 percent, respectively. According to the treatment, 1 ml of the nematode suspension or distilled water (thus, final moisture content (MC) about 67%) was pipetted on the top of the soil after application of immature stages of WFT.

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

The late second instar larvae (L2), prepupae and pupae used in the experiments were collected from the synchronized stock culture 14, 16 and 17 days after emergence of neonate larvae, respectively. All insects were first individually examined under the binocular and then transferred to the top of the soil in the arena using a fine Kolinsky hairbrush. Thereafter the nematodes were applied and the Petri dish was tightly closed and kept for one week in a climate chamber at 23 ± 2°C, 18:6 h L:D photo period and 60–90 % RH. At least, four replications per treatment were used. At the end of the experiments, all WFT adults (dead or alive) and alive prepupae and pupae from the soil in the assay arena (on the surface of the soil and inside the soil) and adults that got stuck on the sticky traps were counted. At the end of each experiment samples of alive development stages of the thrips were controlled for parasitisation by EPN. Corrected mortality was calculated based on the total number of adults and alive immature stages counted at the end of the experiment.

Two sets of experiments were carried out.

<u>Set A - Screening of EPN strains against immature stages of WFT</u>

All EPN strains listed in table 7.1 were tested against L2, prepupae and pupae of WFT. For experiments with late L2 and prepupae, the previously described assay arena was used.

Ten late L2 were transferred to each arena. The late L2 immediately showed a positive geotaxis and went into the soil just after the transfer to the arena. A nematode suspension at a concentration of 400 IJ/cm$^2$ or distilled water (for the control treatment) was pipetted on the soil when all larvae had descended into the soil. Experiments with prepu-

pae were conducted in a similar manner except that in the experimental set-up used the prepupae stayed on the surface of the soil and the nematode suspension or distilled water was pipetted directly on the immatures. The experiments with prepupae were repeated over time.

One of the limiting factors for the use of EPN for insect control is desiccation of the media where the nematodes are applied to (Gaugler, 1988). To screen EPN strains under such conditions for efficacy against WFT pupae, an experiment was carried out at comparatively lower soil moisture content. A plastic pot (∅ 5.5 cm) was filled with 70 g non-sterilized soil (Fruhstorfer Erde Type P) so that it formed a top area of approximately 20 $cm^2$. Similar to the experiments with late L2 and prepupae, EPN strains were applied at a concentration of 400 IJ/$cm^2$ rate just after 16 pupae had been transferred to the top of the soil of each pot. To adjust the soil MC to approximately 45% (w/w), the concentration of the EPN was adjusted so that only 5 ml of the nematode suspension was applied to each pot. The inner part of a lid of a 100-mm Petri dish was painted with insect glue and used as cover for the experimental pot, hence serving as a sticky trap. On daily bases, emerging WFT adults were counted from the sticky traps. The adults were counted for seven consecutive dates until no more adults emerged from the soil.

Set B - Dose rate study of EPN strains against immature stages of WFT

Based on the results of the screening experiments in Set A, four EPN strains, i.e. H.b H, S.f S, S.c D and S.f O, against late L2, and three EPN strains, i.e. H.b H, S.f S, and S.c D, against prepupae and pupae were all farther tested at 0 (control), and concentrations of 100, 200, 400, and 1000 IJ/$cm^2$. Each dose rate of a given strain was tested with four replicates. To investigate the dispersing behaviour of EPN strains in the arena, for experiment on late L2, IJ were counted from the sticky trap and from the bottom of the arena after removing the soil in the Petri dishes.

Statistical analysis

Mortality data were corrected for control mortality following Abbott's formula (1925) and analysed by analysis of variance (ANOVA) (SAS, 1996). Whenever two factors exist (e.g. EPN strains and dose rates) and significantly interacted, means of the levels of one factor were compared at each level of the other factor. Otherwise, when the factors' interaction was not significant, means of the levels of one factor were compared irrespective of the levels of the other (Sokal & Rohlf, 1995).

Data of the two experiments on EPN-induced mortality in WFT prepupae was checked for homogeneity of variance using the HOVTEST=LEVENE option of SAS (SAS, 1996). Effect of time was analysed according to Sokal and Rohlf (1995).

The corrected mortality means were compared to zero (corrected mortality of control) using Dunnett's two-sided test. When significant factor effects were detected by means of ANOVA, corrected mean mortality of the different levels of the respective factor was compared using Tukey's multiple means comparison procedure (SAS, 1996).

Correlation and regression analyses were performed to investigate the relationships between corrected mortality and the varying concentrations of IJ/cm$^2$ tested. $LD_{50}$, $LD_{75}$ and $LD_{90}$ were calculated by means of probity analysis (SAS, 1996). To normalise variance for all analyses, the number of IJ counted from the different parts of the assay arena were square root transformed. A significance level of $\alpha$=0.05 was used in all analyses.

## 7.3 Results

### Set A - Screening of EPN against WFT

Larvae

The proportion of adult emergence in the control was relatively low (68% (SE=6.3)) with 28% natural mortality. However, all EPN strains tested caused significantly higher larval mortality than natural mortality in the control (P<0.001). Significant differences in larval mortality were observed between the EPN strains tested, with highest mortality recorded in the H.b H, S.f S, and S.f O and lowest in the S.f C treatment, respectively (Fig. 7.1). However, in all EPN strains larval mortality exceeded 50%.

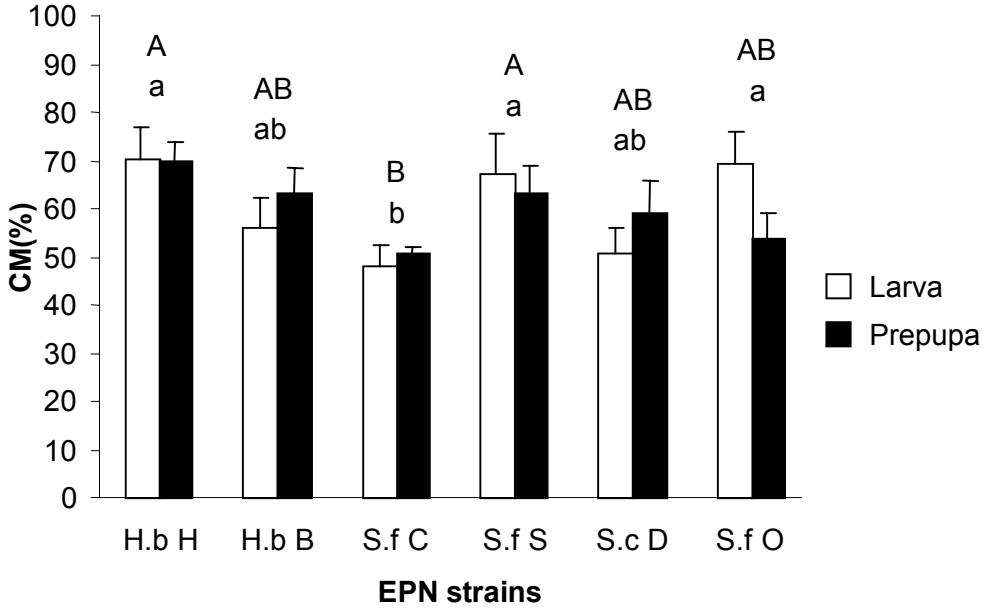



**Fig. 7.1 Mean corrected mortality (CM (% ± SE)) of Western Flower Thrips late second instar larvae and prepupae caused by different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *H. bacteriophora* HB Brecan (H.b B), *Steinernema feltiae* CR (S.f C), *S. feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O)). Bars followed by the same letter (lower case for larvae, upper case for prepupae) do not differ significantly (P=0.05).**

Prepupae

No variance heterogeneity in EPN-induced prepupal mortality was recorded between the two experiments (*df*=1, 46; F=1.18; P=0.28). Moreover, the interaction of time and EPN was not significant (*df*=5, 36; F=2.01; P=0.10). Hence, the data of the two experiments was pooled.

In the control 78% (SE=4.8) of the applied prepupae developed to adults with 20% natural mortality. All EPN strains tested caused significantly higher mortality than in the control treatment (P<0.001). In S.f C significantly lower prepupal mortality was recorded compared to H.b H and S.f S but to the other strains tested. In this experiment H.b H and S.f S caused the overall highest mortality in WFT prepupae (Fig. 7.1).

Pupae

In the control 89% (SE=4.7) of the pupae developed to adults with 11% natural mortality. Mortality of pupae in the S.f C (P=0.208) and in the S.f O treatment (P=0.07) did not differ significantly from the natural mortality in the control. However, in the four other EPN

strains tested, significantly higher pupal mortality was recorded compared to the control (P<0.001). Moreover, significant differences in pupal mortality were recorded between the strains, with highest mortality in the S.f S (54.5%) and lowest in the H.b H (29.4%) treatment (Fig. 7.2).

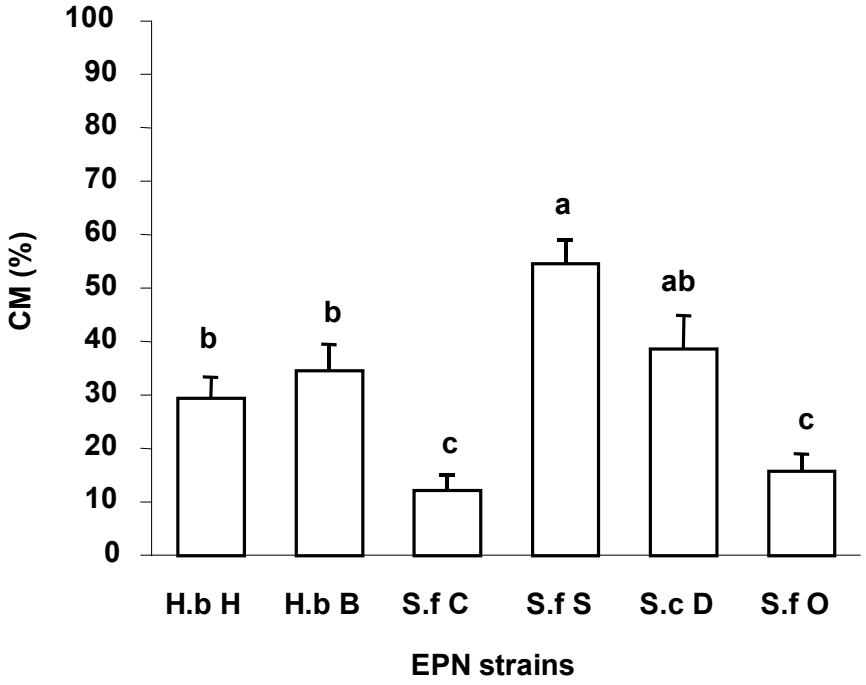



**Fig. 7.2 Mean corrected mortality (CM (% ± SE)) of Western Flower Thrips pupae caused by different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *H. bacteriophora* HB Brecan (H.b B), *Steinernema feltiae* CR (S.f C), *S. feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O)). Bars with the same letters are not significantly different at P=0.05.**

### Set B - Dose rate study of EPN on WFT

Larvae

In the control 78% (SE=3.4) of the applied larvae developed to adults with 21% natural mortality. All EPN at all dose rates caused a significantly higher mortality than recorded in the control treatments (P<0.001). No significant interaction between dose rates and EPN strains was found (*df*=9, 45; F=0.9; P=0.48). Therefore, EPN strains were compared irrespective of the different concentrations, and similarly, the dose rates were directly compared irrespective of the EPN strains. Significant differences in mean larval mortality between the four tested EPN strains were recorded, with highest mortality (69%) in the S.f O and lowest (41%) in the S.c D treatment (Fig. 7.3). Mortality of WFT larvae significantly increased with increasing dose rates up to a concentration of 400 IJ/cm$^2$ (Fig. 7.4). A fur-

ther increase in the concentration to 1,000 IJ/cm$^2$ did not yield a significantly higher mortality. Except for S.f O, the dose rates significantly correlated with larval mortality (Tab. 7.2). Moreover, fiducial limits for the $LD_{50}$ and $LD_{75}$ values could not be estimated for S.f O strain, indicating that mortality did not depend on dose rate for this strain (Tab. 7.2). The IJ applied to the top of the soil in the assay arena migrated into the soil until the bottom of the arena or left the soil and moved to the sticky trap. The number of IJ of the different EPN strains counted on the sticky traps differed significantly between the various concentrations tested (*df*=9, 45; F=2.46; P=0.02). Thus, the difference of EPN strains in their ability to move and search for their hosts was compared at each dose level. At all concentration levels, significantly higher numbers of S.c D IJ were recorded on the sticky traps (Tab. 7.3). The other three strains did not differ significantly in this respect. Across all strains tested, increasing the concentrations lead to higher IJ trap catches on the sticky traps, with significantly highest numbers of IJ recorded in the 1,000 IJ/cm$^2$ treatments (Tab. 7.3). Significantly higher numbers of IJ at the bottom of the Petri dishes were found in the H.b H ($P<0.05$) compared to the S.f S and S.c D treatments, with mean number of IJ/cm$^2$ of 2.5 (SE=0.33), 1.4 (SE=0.25), and 1.3 (SE=0.30), respectively. The mean number of IJ/cm$^2$ in the S.f O treatment ($1.6 \pm 0.25$) did not differ significantly from the other three strains. Moreover, the downward movement of the IJ was not affected by the varying concentrations tested (*df*=3, 45; F =2.65; P=0.06).

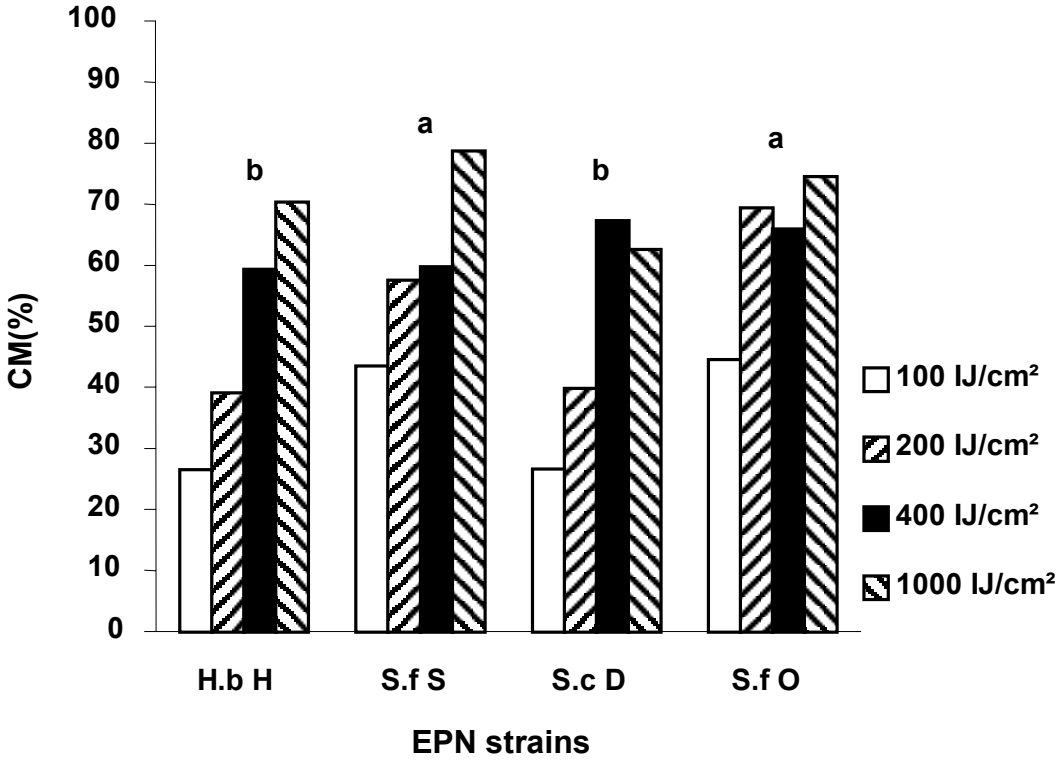



**Fig. 7.3 Mean corrected mortality (CM (%)) of Western Flower Thrips late second instar larvae caused by different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O)) applied at 100, 200, 400 and 1,000 IJ/cm$^2$. The interaction of EPN strains and dose rates was non-significant. Consequently, the EPN strains were compared regardless of the dose rates. Different letters above the four bars of a strain (as means of bars of different dose rates for a given strain) indicate significant differences among the strains at P=0.05.**

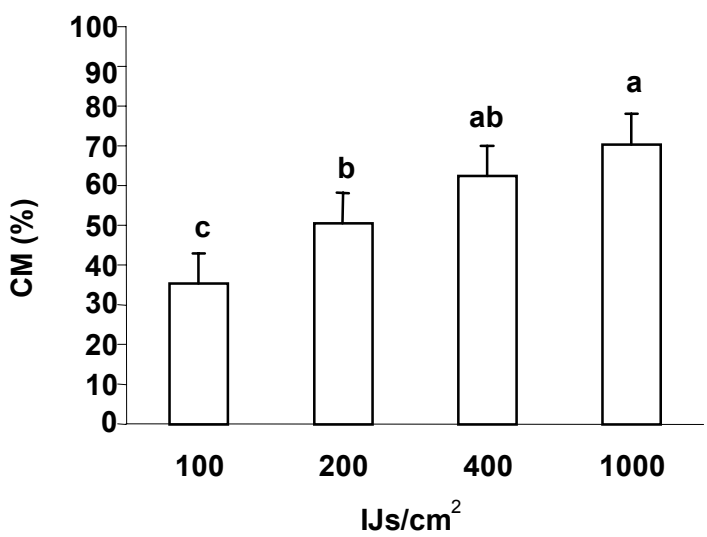



**Fig. 7.4 Corrected mortality (CM (% ± SE)) of Western Flower Thrips late second instar larvae caused by EPN strains applied at 100, 200, 400 and 1,000 IJs/cm$^2$. The data were pulled from four EPN strains (i.e. *Heterorhabditis bacteriophora* HK3, *Steinernema feltiae* Sylt, *S. carpocapsae* DD136 and *S. feltiae* OBSIII). Mortality values at different dose rates followed by the same letters are not significantly different at P=0.05.**

**Tab. 7.2 Dose effects of *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S), *S. carpocapsae* DD136 (S.c D) and *S. feltiae* OBSIII (S.f O) on the mortality of Western Flower Thrips late second instar larvae.**

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

[a] B=slope of regression equation: CM(%)=Log(Dose) + C.
[b] P=probability that the slope b is not different from zero.
[c] FL=lower and upper fiducial limits
NE=not estimated

**Tab. 7.3 Number of IJ/cm² counted on the sticky traps.**

| | Dose rate (IJ/cm²)[a, b] | | | |
|---|---|---|---|---|
| **Strain** | 100 | 200 | 400 | 1000 |
| H.b H | 2.50 Bb | 6.17 Bab | 9.75 Bab | 16.25 Ba |
| S.f S | 4.17 Bb | 12.33 Ba | 17.33 Ba | 14.00 Ba |
| S.c D | 43.58 Ab | 50.92 Ab | 83.92 Aab | 140.08 Aa |
| S.f O | 6.83 Bb | 9.08 Bb | 22.92 Bab | 44.17 Ba |

[a] The data were analysed after square root (x+0.5) transformation.
[b] Means within the same column (upper case) and row (lower case) followed by the same letters are not significantly different at P=0.05 (Tukey multiple means comparison).

Prepupae

In the control 74.2% (SE=4.2) of the applied prepupae developed to adults with 25% natural mortality. In all EPN strains tested at all dose rates studied mortality of prepupae was significantly higher than in the control (P<0.001). No significant interactions between the three EPN strains tested and the four different concentrations were recorded (*df*=6, 33; F=1.26; P=2.91). Therefore, the EPN strains were compared irrespective of the dosage. Likewise, the dose rates were compared regardless of the EPN strains. Application of both H.b H and S.f S resulted in a significantly higher mortality of WFT prepupae compared to the S.c D treatment (Fig. 7.5). For all strains, at the lowest dose rate of 100 IJ/cm$^2$ already 50% of mortality in WFT prepupae was achieved. Increasing the concentration to 400 IJ/cm$^2$ lead to a significant increase in prepupal mortality but a higher dose

rate did not yield significantly higher mortality (Fig. 7.6). Since the corrected mortality of prepupa even at the lowest dose rate was more than 50%, $LD_{50}$ values could not be estimated for all EPN strains used in this experiment (Tab. 7.4). However, except for S.f S ($r^2$=0.4, P=0.136) mortality correlated significantly with dose rates of H.b H ($r^2$=0.79, P=0.004) and S.c D ($r^2$=0.96, P=0.024).

**Tab. 7.4 Dose effects of *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D) on the mortality of Western Flower Thrips prepupae.**

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

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

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

[c] FL=lower and upper fiducial limits.

NE=not estimated, ns=nonsignificant.

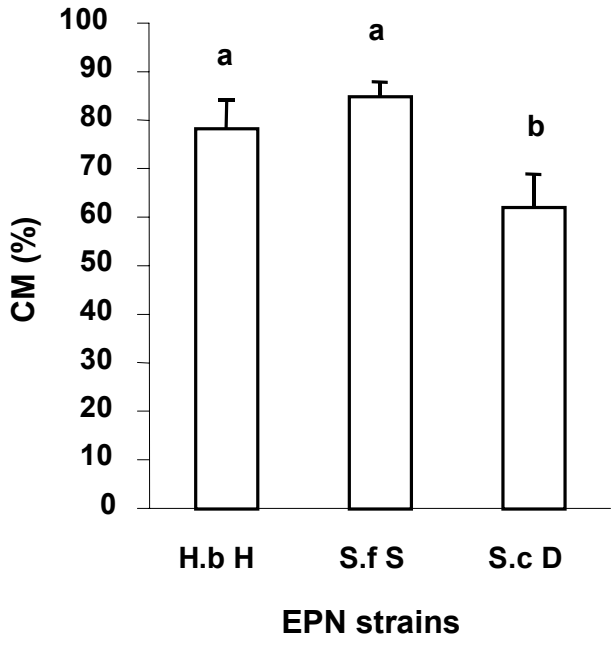



**Fig. 7.5 Mean corrected mortality (CM (% ± SE)) of Western Flower Thrips prepupae as affected by three different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D)). The data were pulled from four different dose rates (i.e. 100, 200, 400, and 1000 IJs/cm$^2$). Bars with the same letters are not significantly different at P=0.05.**

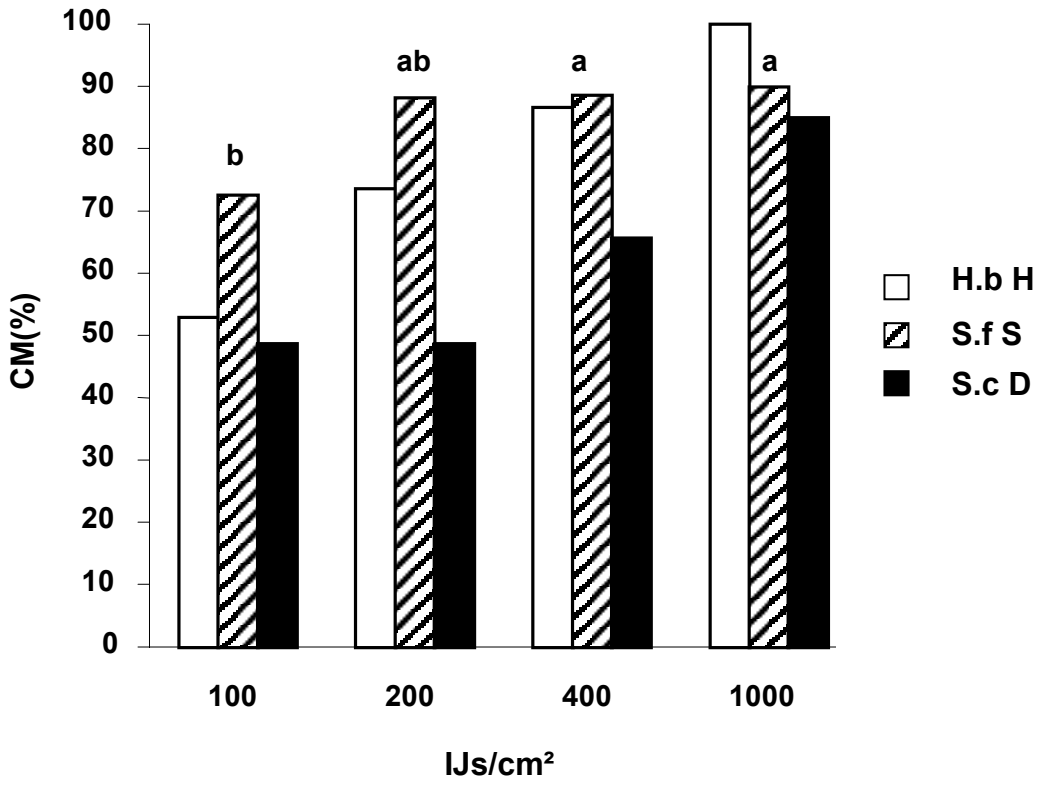



**Fig. 7.6 Corrected mortality (CM%) of Western Flower Thrips prepupae caused by EPN strains applied at 100, 200, 400 and 1,000 IJ/cm$^2$. The interaction of EPN strain and dose rate was non-significant. Hence, dose rates were compared regardless of EPN strains. Different letters above the three bars of one concentration (as means of bars of different EPN strains, i.e., *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D) for a given dose) indicate significant differences among the dose rates (P=0.05).**

Pupae

In the control 81.8% (SE=3.3) of the applied pupae developed to adults with 18% natural mortality. In all EPN strains tested at all dose rates studied mortality of pupae was significantly higher than in the control (P<0.001). No significant interactions between the three EPN strains tested and the four different concentrations were recorded (*df*=6, 29; F=0.24; P=0.960). Therefore, the EPN strains were compared irrespective of the dosage. Likewise, the dose rates were compared regardless of the EPN strains. No significant differences in pupal mortality were found between the three different EPN strains tested (*df*=2, 29; F=1.58; P=0.222). However, mortality in WFT pupae was significantly affected by the EPN concentrations (Fig. 7.7). Across the three EPN strains tested, the lowest concentration of 100 IJ/cm$^2$ resulted in approximately 50% mortality of WFT pupae. The significantly highest mortality was recorded at a concentration of 400 IJ/cm$^2$ and a further increase in the dose rate did yield significantly higher mortality rates in pupae (Fig. 7.7).

The slopes of the regression of mortality-dose rate of H.b H (0.05), S.f S (0.04) and S.c D (0.03) did not significantly differ from each other (P<0.05). Thus, the data for the three strains was pooled. The subsequent regression analysis revealed a strong increase in mortality to increasing dosages of IJ (Fig. 7.8).

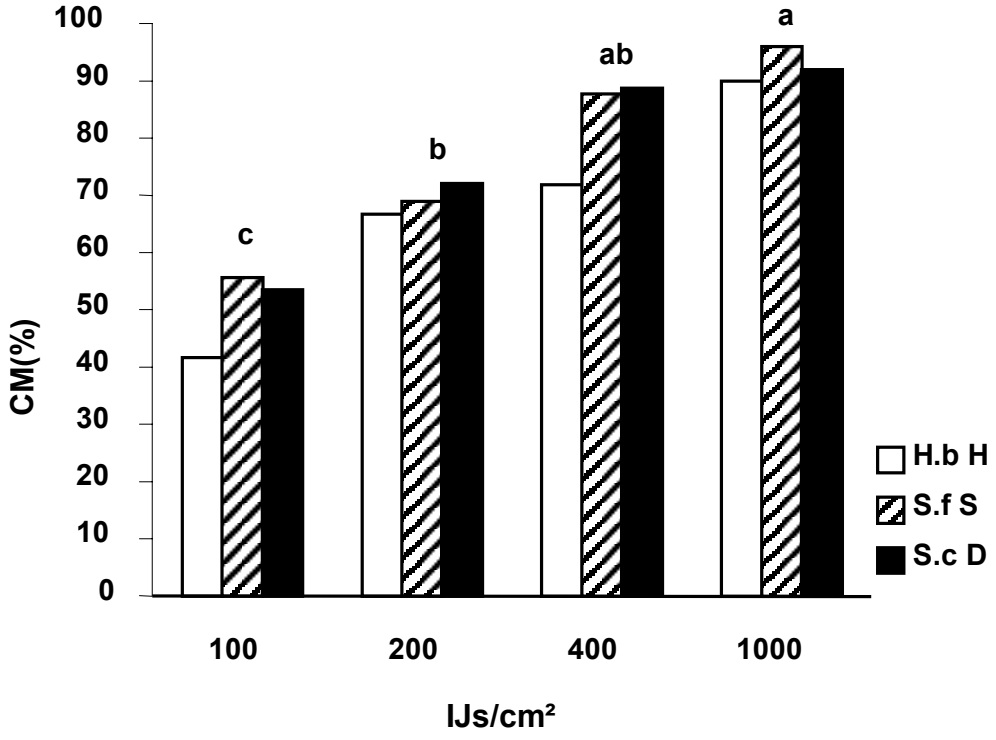



**Fig. 7.7 Mean corrected mortality (CM%) of Western Flower Thrips pupae caused by EPN strains applied at 100, 200, 400 and 1,000 IJ/cm$^2$. The interaction of EPN strains and dose rate was non-significant. Hence, dose rates were compared regardless of EPN strains. Different letters above the three bars of one concentration (as means of bars of different EPN strains, i.e., *Heterorhabditis bacteriophora* HK3 (H.b H), *Steinernema feltiae* Sylt (S.f S) and *S. carpocapsae* DD136 (S.c D) for a given dose) indicate significant differences among the dose rates (P=0.05).**

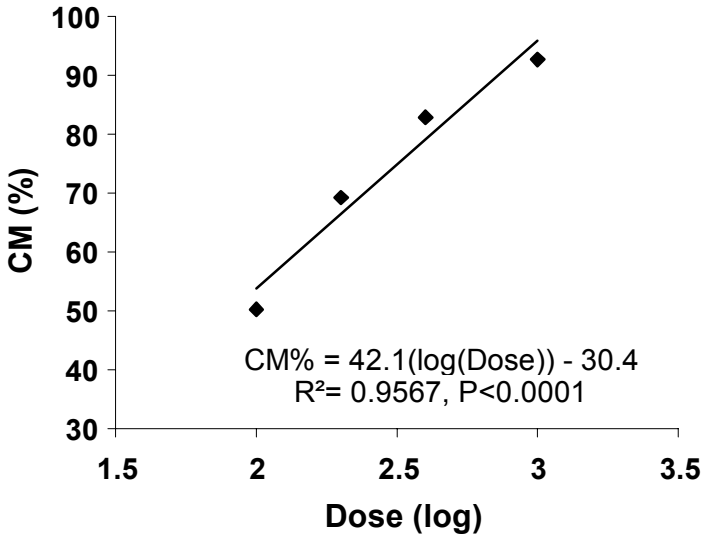



**Fig. 7.8 Functional relationship between corrected mortality values (CM%) of Western Flower Thrips pupae caused by three different EPN strains (i.e. *Heterorhabditis bacteriophora* HK3, *Steinernema feltiae* Sylt and *S. carpocapsae* DD136) and the logarithms of four different dose rates of IJ (i.e. 100, 200, 400 and 1,000 IJs/cm$^2$).**

## 7.4 Discussion

In all experiments the number of WFT adults and alive immature stages recovered after one week in the control was about 70–90%. During the development of WFT mortality is highest in the L2 stage (Soria & Mollema, 1995) and Helyer *et al.* (1995) also reported 68% adult emergence from WFT pupae in control treatment in compost. Hence, the low control mortality enabled us to study the effects of EPN on the different developmental stages of WFT. Furthermore, at the end of the experiments none of the surviving WFT were found to be parasitised by EPN, indicating that EPN virulence in our study can be expressed in terms of EPN-induced mortality.

Immature WFT developmental stage comparison

The commercial soil substrate utilized in our experiments, is commonly used in German greenhouses. In such types of soils, the water potential reduces relatively fast. During preliminary experiments we observed in the climate chamber with a RH of 60-90% a 25% MC reduction of the soil substrate in the assay arena (data not presented). At low soil moisture contents EPN are less infective because they lack a sufficient water film for effective movement to their hosts (Gaugler, 1988). Movement is considered optimal when the soils are near their field capacity at which IJ are able to initiate host seeking and can cause high rates of infection. Thus, to select EPN species/strains against WFT under such lower water potential, we screened EPN in Set A against WFT pupae at a compara-

tively lower MC of the soil. This additional factor enabled us to select EPN strains that could still parasitise WFT under comparatively lower MC. For a given EPN strain, mortality of pupae at the lower soil MC in Set A was inferior to pupal mortality at the comparatively higher MC used in Set B.

In the experimental set-up used in this study the late L2 WFT readily descended into the soil, thus reflecting natural conditions. Consequently, the IJ mainly attacked their hosts in the soil, and most of the EPN strains tested showed high virulence against WFT, indicating that the IJ percolated into the soil with the suspension applied and successfully infected the thrips. However, the soil depth in our bioassays was only approximately 0.8 cm, whereas under field conditions WFT pupation occurs at a depth of 1.5–2.0 cm (Tommasini & Maini, 1995, Moritz, 1997). Contrary to the late L2, the majority of the prepupae and pupae introduced into the bioassay arena stayed on the top of the soil. Mortality of late L2 and prepupae in Set A differed only slightly. However, lower mortality in late L2 compared to prepupae and pupae was recorded in Set B, corroborating results of an earlier study by Ebssa *et al.* (2001b), using a similar methodological set-up. Possibly, in WFT late L2 are less susceptible to EPN than prepupae and pupae.

Differences in susceptibility to EPN can exist among different developmental stages of insects. For instance, early L1 are the most susceptible development stages in *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) (Glazer & Navon, 1990). In contrast, later larval stages were reported to be the most susceptible development stages in *Maladera martida* Argaman (Coleoptera: Scarabaeidae) (Glazer & Gol'berg, 1989). In *Tipula paludosa* Meigen (Diptera: Tipulidae) and *T. oleracea* L. highest mortality was recorded for the L1 approaching the first moult while young L1 were less susceptible (Peters & Ehlers, 1994).

EPN strain comparison

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

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

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

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

We recorded significantly higher IJ numbers of the *H. bacteriophora* strain H.b H from the bottom of the Petri dish, which reflects the cruiser behaviour in foraging of *H. bacteriophora* (i.e. actively searching for its host) (Campbell & Gaugler, 1993). However, at lower concentrations, i.e. 100 and 200 IJ/cm$^2$, H.b H caused significantly lower mortality in WFT L2 compared to the two *S. feltiae* strains S.f S and S.f O. IJ of *S. feltiae* posses some characteristics of ambushing and some of cruisers foraging behaviour. They are equally effective at finding mobile and non-mobile hosts on a two-dimensional nictation surface substrate (Grewal *et al*., 1995). Our results indicate, that IJ of the *S. feltiae* strain S.f S used this foraging advantage on soil-dwelling stages of WFT that are mobile upon disturbance. If some of the IJ search and disturb the resting prepupae and pupae, the other juveniles, which are sitting and waiting for bypassing hosts, could attach themselves to the moving immatures of WFT. Thus, in most of the experiments, IJ of S.f S were constantly superior to that of the other EPN strains tested for control of WFT.

Dose comparison

Results from the dose rate experiments show that irrespective of the tested EPN strains, a comparatively high concentration of 400 IJ/cm$^2$ was needed for a high control of the soil-dwelling stages of WFT. In the experimental set-up used in our study, such a dose rate resulted in approximately 60% mortality of late L2 in the soil and around 80% mortality of prepupae and pupae on the soil surface. Lower concentrations of IJ yielded a significantly lower mortality in WFT. Most likely, the small body size of WFT immatures is the reason why such high concentrations of IJ are required to obtain high mortality. In a similar study, Chyzik *et al*. (1996) also recorded highest mortality of WFT prepupae and pupae at a dose rate of 400 IJ/cm$^2$. Contrary to these results, Helyer *et al.* (1995) observed low mortality in WFT pupae in compost irrespective of the EPN dose rate used. However, other small-bodied insect pests like fungus gnats, *Bradysia* spp. (Diptera: Sciaridae), can be efficiently controlled by EPN at comparatively lower concentrations (Harris *et al*., 1995, Gouge & Hague, 1995). Our results also indicate that lower dose rates i.e., 100–200 IJ/cm$^2$ already caused 30–50% mortality in soil-dwelling life stages of WFT. At present the efficacy of the biological control strategies used against WFT is considered rather low (Parrella *et al*., 1999). Hence, any biological alternatives are potentially of great interest for WFT control. We believe that EPN can possibly become part of a rather elaborated biological control approach, attacking WFT from several angles, by using predatory bugs and predatory mites against the foliar feeding life stages of the thrips, and EPN against the soil-dwelling immatures. Moreover, recent findings show that soil-dwelling life stages of WFT can also be efficiently controlled through releases of predatory mites of the genus *Hypoaspis* (Acari: Laelapidae) (Berndt & Poehling, 1999). Several studies indicate that EPN can be successfully combined with other biological control agents (e.g. Choo *et al*., 1996). In ongoing experiments we are evaluating the combined impact of EPN and inundative releases of *Hypoaspis* spp. for control of soil-dwelling life stages of WFT.

# 8 General discussion

*F. occidentalis* is still one of the most important pests in protected vegetable as well as ornamental plant production (e.g. Lewis, 1998, Parrella *et al.*, 1999). Although countless approaches are conducted, the control problem is still unsolved. The hidden life strategy, sheltered eggs and soil passage impede the effectiveness of insecticides and require repeated insecticide applications which cause residue problems and destroy many attempts of integrated pest management programmes (Riudavets, 1995). However, the intensive and often prophylactic sprayings, combined with the short generation time of thrips, contributed to the appearance of resistances (Kiers *et al.*, 2000, Jensen, 2000a, Jacobson *et al.*, 2001a). Resistance against various active compounds of pesticides is wide spread and especially cross-resistances make the situation worse (Jensen, 2000b). To combat these difficulties, biological control strategies have gained increasing attention within the last two decades. Anthocorid bugs (e.g. *Orius* spp.) or phytoseiid mites (*Amblyseius* spp. and *Neoseiulus* sp.) are the most popular beneficials, are commercially available and have been introduced frequently (Castane *et al.*, 1996, Manjunatha *et al.*, 1998, Meiracker & Sabelis, 1999, Courcy Williams, 2001, Jacobson *et al.*, 2001a). In the literature, authors repeatedly mention that efficiency of the predators is not sufficient, especially in ornamentals and if virus diseases emerge within the crop (Riudavets, 1995, Albajes & Alomar, 1999, Montserrat *et al.*, 2000). In both cases, the damage threshold is very low. Additional biocontrol strategies and further antagonists are needed. The commonly utilised thrips antagonists target exclusively the thrips instars, which inhabit the above soil plant parts, whereas a considerable part of the thrips developmental cycle takes place in the litter and soil beneath the host plant. Although the soil passage is mentioned repeatedly in publications (Palmer, 1989, Childers *et al.*, 1994, Helyer *et al.*, 1995, Kirk, 1996), the proportion of a thrips population withdrawing to the soil for pupation has never been quantified. The results presented in Chapter 2 demonstrate the importance of the soil passage in the thrips life cycle with *P. vulgaris* as model plant. Since more than 97% of the thrips population in these microcosm experiments pupated in the soil, it was concluded that an efficient thrips antagonist foraging in the soil could conceivably contribute to manage *F. occidentalis* in protected plant cultures.

Experiments described in Chapter 3 exhibited that even on plants with a more complex structure (rosette like habitus of *S. ionantha*, composite flowers and slightly ramified growth of *D. grandiflorum*, bushy, richly ramified shoots and filled flowers of *T. patula nana*), obviously offering much more niches for pupation on the plant, the majority of a

thrips population left the plant for pupation in the soil. In fact, significantly more thrips completed their life cycle on ornamentals compared to bean plants but in all experiments at least 90% pupated in the soil. The WFT population size had no impact on the number of thrips pupating in the soil.

In Kirk's (1996) opinion the soil passage is - as far as flower infesting thrips like *F. occidentalis* are concerned - an adoption to the short life of flowers. He speculated that thrips possibly could not complete the life cycle if blossoms fall and thrips larvae inside the blossoms are still immature and not ready for pupation. However, a further advantage of withdrawing to the soil or leaf litter for pupation is a protection factor towards unfavourable abiotic conditions (fluctuating temperature and humidity) and predation. From the evolutionary point of view, the soil passage was possibly the result of thrips feeding on mature flowers. If thrips gained more profit from pupating in the soil after accidentally falling to the ground there might have been a high selective pressure to hide in crevices in the soil for pupation instead of climbing up a plant again. Maybe within many generations the soil passage because of its benefit might have become part of the life cycle.

However, in the presented experiments it was possible to significantly reduce the thrips population with soil foraging predatory mites (*H. miles* and *H. aculeifer*) or entomopathogenic nematodes (a number of strains from different species). *Hypoaspis* mites caused a thrips mortality of at least 45% or 58% and up to maximum of 76% or 81% (*H. miles* and *H. aculeifer*, respectively). Entomopathogenic nematodes also caused mortality rates of 31% (*Steinernema feltiae*) to 53%, or even 62% (*S. carpocapsae* or *Heterorhabditis bacteriophora*, respectively). Even comparatively low EPN densities of 100 to 200 IJ/cm² in arena experiments caused 30 to 50% thrips mortality.

However, the experiments presented also show that in all attempts the control success in the microcosms was limited; a small proportion of thrips always escaped from control. Possible reasons for these findings are discussed above; they are briefly summarised in the following and further on discussed in context of *Hypoaspis* species use in practise.

## 8.1 *H. miles* and *H. aculeifer* as thrips antagonists

Results of the microcosm experiment (Chapter 2) suggest on the one hand that *H. miles* and *H. aculeifer* are obviously able to reduce a thrips population but on the other hand thrips seem not be the preferred prey of *Hypoaspis* and it is speculated, that alternative prey being available in the soil (nematodes, mites, Collembola etc.) and the number of these prey organisms in relation to the target pest (WFT) may have an influence on the

efficiency towards *F. occidentalis* instars. Prey preference and switching behaviour to feeding on the most abundant prey is a character to be considered when evaluating polyphagous predators like *Hypoaspis* mites for biological control in glasshouse environments (Montserrat *et al.*, 2000). On the one hand, it is very likely that alternative prey species being available lessens the number of captured thrips instars but on the other hand these alternative prey species support a sustained desired establishment of *Hypoaspis* mites in the soil of the glasshouse area. However, the ability of *Hypoaspis* spp. to locate thrips in the soil might be a limiting factor, which would result in a low encounter rate. However, this important aspect needs to be analysed and will be subject of further investigations.

The predation experiment (Chapter 5) clarified that thrips larvae, prepupae and pupae are accepted as prey and that the handling time and the daily consumption rate of *Hypoaspis* sp. is not a limiting factor, since *H. miles* killed up to 2 and *H. aculeifer* up to 3.9 instars of *F. occidentalis* within 24 h. Furthermore, predation exclusively on thrips instars ensured a sustained reproduction of both species. The possibility that cannibalism could lead to a reduced thrips predation was excluded because both species revealed a very low propensity towards feeding conspecifics (Chapter 4), despite this, other intraspecific interactions may be responsible for an impeded thrips predation.

In addition to the reasons mentioned, there are various features which make a prediction of the effect of any biocontrol measurement more difficult and which reflect the complexity of the soil as ecosystem, even in glasshouse areas. Especially in soil culture, within a short period of time populations of various organisms, such as nematodes, Collembola, Sciaridae, Psychodidae, Myriapoda, Isopoda and mites, develop. Most members of these groups are saprophytic and are included in the prey spectrum of *H. miles* and *H. aculeifer*, but they also include numerous predators. *Hypoaspis* mites, as a part of a multitrophic system, are predators and prey (Eisenbeis & Wichard, 1985, Karg, 1993a, b, 1994). Often before application of any beneficial, predatory arthropods are already present in a glasshouse. Wiethoff (pers. comm.) found beside diverse Collembola species and insect larvae, a number of *Hypoaspis* species in soil samples of glasshouse soil of a commercial vegetable grower. Similarly, in pot culture of ornamentals several predatory arthropod populations develop spontaneously. For example, Denecke (pers. comm.) found *Hypoaspis* and *Lasioseius* species in the pots of *D. grandiflorum* and *S. ionantha*. Most predators in the soil are polyphagous or at least oligophagous (Karg, 1993b) and may thus, as competitors, complement the thrips control. However, since knowledge about prey preferences of the abundant predator species is scarce, it is also considered

to be very likely that they prey on the introduced *Hypoaspis* species. Currently the entire soil subsystem is far from being completely understood. There is also a lack of knowledge concerning intraguild predation among predators and multitrophic interactions between the organisms in the soil.

However, it is not only the acceptance or avoidance of a designated target prey, intra- or interspecific interactions or abiotic conditions that are crucial for the control success. Equally important are life history parameters of predator and prey. If a predator consumes many prey individuals, but has a low propagation rate, the contributor has to apply the predator frequently to optimise the control. Similarly, if the predator feeds on many pest individuals but the pest reproduces more rapidly, the predator cannot control the pest. A comparison of life history parameters reveals the theoretical development of the populations of predator and prey.

At a temperature of 24 °C, the development of *H. miles* from egg to adult takes 11.4 (Wright & Chambers, 1994) or at 20 °C 11.6 to 17.5 days (depending on the diet) (Enkegaard *et al.*, 1997). *H. aculeifer* requires 11 to 12.5 days at 26 °C (Ragusa *et al.*, 1986, Ragusa & Zedan, 1988). In contrast, the developmental time of *F. occidentalis* is 17.9 days at 23 °C (Brødsgaard, 1994a). *H. miles* produces one egg per day and *H. aculeifer* 2 to 3 eggs exclusively feeding on thrips instars (Chapter 5) while *F. occidentalis* shows an oviposition rate of 1 to 2 eggs per day (Lewis, 1997a). The net reproductive rate ($R_0$) of WFT on beans (at 23 °C) is 12.2, on *D. grandiflorum* (at 25 °C) 99.5 and on cucumber (at 25 °C) 22.1 (Kirk, 1997b and articles mentioned there) (Tab. 8.1), whereas *H. aculeifer* for example has been stated to produce 70 to 145 eggs per female on mould mites, bulb mites and Collembola ($R_0 \approx 20$) (Lobbes & Schotten, 1980, Chi, 1981, Shereef *et al.*, 1980, Zedan, 1988). This data shows that the reproduction rate of the supposed predators – at least of *H. aculeifer* - fits the reproduction of WFT, thus *H. aculeifer* should theoretically be able to considerably reduce thrips in the glasshouse. However, comparing the intrinsic rates of natural increase (calculated for comparable temperatures) the value given for WFT is slightly higher than that for *H. miles* or *H. aculeifer* (Tab. 8.1). Thus, *Hypoaspis* sp. would have difficulties to control a population of WFT even when both population sizes are equal. This impression is supported by the observation that the portion of thrips control held by *Hypoaspis* mites in a glasshouse surrounding is limited (Wiethoff pers. comm.). The lower generation time (T) of WFT compared to *Hypoaspis* spp. is most likely responsible for this finding.

**Tab. 8.1 Estimates of population parameters of *Hypoaspis miles*, *H. aculeifer* and *Frankliniella occidentalis* on varying food sources and at varying temperatures. Data collected from the literature**

| Species | Prey / host | Temperature (°C) | Net reproductive rate ($R_0$) | Generation time (T) (Days) | Intrinsic rate of natural increase ($r_m$) ($Day^{-1}$) | Source |
|---|---|---|---|---|---|---|
| ***H. miles*** | *Bradysia paupera* | 20 | 37.8 | 50.3 | 0.072 | Ydergaard *et al.*, 1997 |
| | *Lycoriella solani* | 20 | 27.4 | 44.3 | 0.0747 | Enkegaard, *et al.*, 1996 |
| | *Tyrophagus putrescentiae* (Acari: Acaridae) | 20 | 9.10 | 40.7 | 0.0543 | Enkegaard, *et al.*, 1996 |
| | *B. paupera* | 25 | 49.7 | 29.4 | 0.133 | Ydergaard *et al.*, 1997 |
| ***H. aculeifer*** | *Glycyphus domesticus* (Acari: Acaridae) | 24 | 10.8 | 20.5 | 0.1161 | Barker, 1969 |
| | *T. putrescentiae* | 22.5 | 21.3 | 23.9 | 0.128 | Lobbes & Schotten, 1980 |
| | *T. putrescentiae* | 24 | 17.2 | 26.7 | 0.107 | Barker, 1969 |
| | *T. putrescentiae* | 24.5 | 20.1 | 24.8 | 0.121 | Lobbes & Schotten, 1980 |
| | *Drosophila melanogaster* (dead) | 24.5 | 14.9 | 37.0 | 0.073 | Lobbes & Schotten, 1980 |
| | *Tetranychus urticae* (frozen) | 24.5 | 15.6 | 38.7 | 0.071 | Lobbes & Schotten, 1980 |
| | *T. urticae* (alive) | 24.5 | 5.6 | 37.5 | 0.046 | Lobbes & Schotten, 1980 |
| | *Vicia faba* pollen | 24.5 | 11.7 | 39.6 | 0.062 | Lobbes & Schotten, 1980 |
| ***F. occidentalis*** | *P. vulgaris* | 23 | 12.2 | 17.9 | 0.140 | Brødsgaard, 1994b |
| | *Dendranthema sp.* | 25 | 99.5 | 26.9 | 0.171 | Robb & Parrella, 1991 |
| | Cucumber | 25 | 22.1 | n.d. | 0.166 | Rijn *et al.*, 1995 |

## 8.2 EPN as thrips antagonists

As described above, roughly speaking EPN show two host locating strategies: 'Ambushers', following a sit-and-wait strategy, stay where they are and wait for hosts to pass by. 'Hunters' or 'cruisers', actively migrate through the substrate searching for a host. EPN strains showing the first strategy only come in contact with thrips instars when the L2 passes the waiting nematode IJ while seeking for a suitable pupation site or when the IJ are rinsed to the thrips instars during irrigation. Cruiser EPN meet thrips instars while migrating through the soil.

Actually some strains mix both strategies. Nevertheless, the minute size of thrips lowers the likelihood of any encounter. For this reason, relatively high IJ densities are needed in practise for a considerable thrips control.

However, thrips are frequently infested by IJ but a development or even completion of the life cycle inside larvae, prepupae or pupae was never observed (Chapter 6 and 7). The size of thrips instars in the soil excludes any propagation of EPN and their efficiency as BCA is reduced. It is concluded that the body content of a single thrips is not enough to ensure development or life of EPN. Consecutively, repeated applications are necessary to yield benefit from EPN as biocontrol agent against WFT, the time span between two applications depends on the activity and longevity of the IJ which for its' part depends extremely on the abiotic conditions. One has to keep in mind that the water content of the soil and the irrigation extent after IJ release substantially influences the control success of the EPN. Since not all species and strains of EPN are - as being "hunters" - actively migrating towards a possible host but have to be passively transported to their supposed target host, e.g. by percolating irrigation water. For all IJ, high moisture content of the soil is needed but just the same IJ are not aquatic, meaning that an excess of water also reduces nematodes' efficiency because they drown. On the other hand, the defence behaviour, even of thrips prepupae and pupae (moving upon disturbance and jerking the abdomen), impedes the infestation process of IJ trying to penetrate into the thrips' body.

It is also speculated that the characteristic of thrips pupae being pharate may impede the infestation success of IJ. However, no significant difference concerning the susceptibility of prepupae and pupae was observed, and prepupae and pupae are more often killed than larvae (Chapter 6).

## 8.3 EPN and *Hypoaspis* mites in practise

The most important variable with regard to the control success seems to be the water content of the soil and the application method as far as virulent EPN are concerned. A short-term efficiency of *Hypoaspis* mites from the current state of knowledge seems to be influenced by the availability of alternative food sources. For an enhanced control of WFT by the usage of nematodes or *Hypoaspis* mites, more than one application is needed. Nevertheless, it is considered that *Hypoaspis* mites or EPN could be valuable parts of an integrated pest management programme, and both beneficials could complement thrips control efforts above soil. Besides the shown possibilities to utilise *Hypoaspis* mites or EPN for thrips control, also attempts to use entomopathogenic fungi were recently conducted (Vestergaard *et al.*, 1995, Castineiras *et al.*, 1996, Butt & Brownbridge, 1997, Ekesi *et al.*, 1998, Brownbridge *et al.*, 1999, Jacobson *et al.*, 2001a) and may become more important in the future. Any attempt that supports thrips control in favour of biological control treatments is a further step to manage the Western Flower Thrips. However, when asked which beneficial acting in the soil might be preferred at the current state of knowledge, *Hypoaspis* mites are favoured: The high densities of the tested EPN strains that are required to result in a considerable thrips control is not economic. Beneficial producers advise to apply an EPN density of 0.5 Mio IJ per square meter (50 IJ/cm²) for control of Sciaridae or the black vine weevil (*Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae)). In contrast, the experiments presented suggest the use of at least 400 IJ per square centimetre as far as the strains are concerned which were screened for thrips control. This amount of IJ equals 4 Mio per square meter indicating that the costs for a regular use in glasshouses are out of all proportions to the profit. Moreover EPN cannot be established in the glasshouse and have to be applied frequently. In contrast, *Hypoaspis* mites usually reveal no difficulties to establish in a glasshouse environment since they have a very wide host range. Additionally they show a very high starvation tolerance and stay active even after weeks of starvation whereas the persistence of IJ of EPN under glasshouse conditions is a maximum of a fortnight. It cannot be excluded that EPN strains will be available in the future that show a very high virulence towards thrips instars in the soil since numerous nematode strains are available just waiting for bioassays on their virulence towards thrips. Moreover not only screening of the available strains but also breeding and selection programmes are reported to lead to more effective nematodes (Schirocki & Hague, 1997, Grewal *et al.*, 1996). The results from the experiments presented suggest that, in principle, thrips larvae, prepupae and pupae are susceptible to

EPN infestation. Possibly the EPN production may becomes reasonable, which would make EPN an economic thrips control agent. Similarly the application of *Hypoaspis* mites – especially of *H. aculeifer* – is expensive: Producers of beneficials advise to use densities of 250 mites per square meter to control *Bradysia paupera* (Diptera: Sciaridae). In the experiments presented at least the two to threefold number of mites was necessary, but because of the mentioned advantages of *Hypoaspis* mites the costs in relation to usefulness is evaluated to be much more favourable.

Of course, one has to keep in mind that the findings presented here at first display the interrelations of soil passage and host plant in a complete artificial microcosm system and the results have to be verified in a more practical situation. Kirk (1997b) mentions that the relevance of such laboratory figures to the field is uncertain because the experiments may have omitted some factor that is critical out of doors or even in a glasshouse environment. Nevertheless, several soil samples from glasshouses of vegetable growers (Wiethoff, pers. comm.) and from bean plants cultivated in glasshouse for pod production (as food for the *F. occidentalis* stock culture) (unpublished data) confirmed at least the importance of the soil passage as part of the thrips' life cycle in the glasshouse. As far as the control effects presented here are concerned one has to bear in mind that further research is needed to prove the presented findings under glasshouse conditions.

## 8.4 Conclusion

It is obvious that ground dwelling predators can substantially contribute to reduce a source population of thrips in the well-protected refuge soil, but they are not able to reduce the pest population below the economic threshold. It is suggested that combinations of different antagonists, attacking different thrips instars living in the soil as well as on the leaves above ground, may result in a more successful strategy to optimise biological control of thrips. From combinations of leaf and soil-dwelling predators, desired additive effects could be expected. Compared to combinations of antagonists acting above ground, the interspecific competition (i.e. intraguild predation) should be neglectable due to the different foraging habitats.

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