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First results concerning the efficacy of entomopathogenic nematodes against *Hercinothrips femoralis* (Reuter)

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ABSTRACT

The efficacy of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora* against larvae and adults of banded greenhouse thrips, *Hercinothrips femoralis*, was studied under laboratory conditions. The activity of the biological agents under study was determined at three different temperatures (15, 20, and 25°C), with concentration of 200 infective juveniles per individual (larva or adult), a day:night ratio of 4:20 and relative humidity of 95 %. The experiment was conducted in plastic rearing vessels on French bean leaves with a slightly modified version of a method used for studying the bionomics of thrips. The mortality rate of the thrips was determined four days after the application of the nematode suspension. Temperature had significant influence on adult pest mortality, but no significant effects were found with nematode species. Neither temperature nor nematode species had significant effect on larval mortality, which ranged from 23 % (*S. feltiae* at 25°C) to approximately 50 % (*H. bacteriophora* at 15 and 25°C). Mortality of adults was significantly influenced only by temperature, with the nematodes being most efficient at 25°C (approximately 30 % mortality by *H. bateriophora*). The results of our research showed that foliar application of entomopathogenic nematodes might be a relatively efficient way for controlling *H. femoralis*, but the optimization of environmental factors would likely improve their efficacy further.

Key words: Thysanoptera, Terebrantia, Thripidae, Panchaetothripinae, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, efficacy

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IZVLEČEK

PRVI REZULTATI UČINKOVITOSTI ENTOMOPATOGENIH OGORČIC ZA ZATIRANJE RESARJA *Hercinothrips femoralis* (Reuter)

V laboratorijskih razmerah smo preučevali učinkovitost entomopatogenih ogorčic *Steinernema feltiae* in *Heterorhabditis bacteriophora* za zatiranje ličink in odraslih osebkov resarja *Hercinothrips femoralis*. Delovanje ogorčic na žuželko smo ugotavljali pri treh različnih temperaturah (15, 20 in 25°C), koncentraciji 200 infektivnih ličink/osebek, razmerjem med svetlobo in temo 4:20 in 95% relativni zračni vlagi. Poskus smo izvajali v plastičnih gojitvenih posodicah na fižolovih listih, z rahlo modificirano metodo, ki je bila primarno razvita za raziskave bionomije resarjev. Štiri dni po aplikaciji suspenzije ogorčic smo ugotavljali smrtnost izpostavljenih resarjev. Med vrstama ogorčic nismo ugotovili signifikantnih razlik v delovanju na škodljivca. Vrsta ogorčic in temperatura nista imeli signifikantnega vpliva na smrtnost ličink, ki je znašala od 23% (*S. feltiae* pri 25°C) do približno 50% (*H. bacteriophora* pri 15 in 25°C). Smrtnost odraslih osebkov je bila signifikantno determinirana le s temperaturo, pri čemer so bile ogorčice najbolj učinkovite pri 25°C (približno 30% smrtnost pri *H. bacteriophora*). Rezultati naše raziskave kažejo, da je lahko foliarna aplikacija preučevanih biotičnih agensov relativno učinkovit način zatiranja resarja *H. femoralis*, z optimiziranjem okoljskih dejavnikov pa je mogoče njihovo učinkovitost še izboljšati.

Ključne besede: Thysanoptera, Terebrantia, Thripidae, Panchaetothripinae, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, učinkovitost

1 INTRODUCTION

Banded greenhouse thrips, *Hercinothrips femoralis* (Reuter) (Thysanoptera: Thripidae), belong to a group of some ten Thysanoptera species which have been proven to be noxious to both cultivated and indigenous plants. Thrips are found across Africa, Central and North America and in Europe (Palmer *et al*., 1989; Simon, 1993). On the Old Continent and in other areas with moderate climate, these pests are particularly noted in greenhouses (Lacasa in Martinez, 1988; Tusnadi in Nemstothy, 1992; Vierbergen, 1995; Reiderne *et al*., 1997; Pintureau *et al*., 1999; Nedstam, 2001; Trdan, 2002).

Although *H. femoralis* is known under the common name of sugar beet thrips, it is found almost everywhere where bananas are grown (Lewis, 1997) and also causes economically important damage to a variety of ornamental plants. In fact, it can feed on more than 50 host plants including representatives of the genera *Amaryllis, Aralia, Begonia, Chrysanthemum, Croton, Dieffenbachia, Dracaena, Ficus, Gardenia, Hydrangea, Philodendron, Schefflera, Schlumbergera* and others (Beshear, 1979; Oetting and Beshear, 1980; Nedstam, 2001). The sucking of the larvae and adults results in the appearance of silver spots and the aesthetic appearances of the attacked plants are further diminished due to the presence of black excrement.

Rare literature sources which deal with the chemical control of *H. femoralis* (Scarpelli and Bosio, 1999) show that these thrips are not tolerant to insecticides. Thus it is possible to use insecticides to reduce the number of the pest quickly and efficiently before they can spread to a greater extent. Recently, however, more and more importance has been assigned to finding environmentally acceptable ways of controlling plant pests (including thrips) – and a major line of research is the use of their natural enemies. Among these natural enemies are the entomopathogenic nematodes (EPNs); soil organisms which are mutually associated with bacteria of the family Enterobacteriaceae. The nematodes enter the prey through their body openings. After infection, the EPNs' symbiotic bacteria are released into the insect hemocoel, causing septicemia and death of the insect (Kaya and Gaugler, 1993).

Most previous research employing EPNs has been directed against soil pests (Kaya et al., 2006). Yet increasing research utilizes their application more broadly and in some cases, EPNs have already been used practically. One of these productive lines of research is the foliar application of EPNs with which a faster activity of nematodes can be attained. Amongst the Thysanoptera species, most previous research has been focussed on western flower thrips (*Frankliniella occidentalis* [Pergande]) (Arthurs and Heinz, 2006; Shapiro-Ilan *et al*., 2006), which is one of the four most harmful thrips species in the world (Kirk and Terry, 2003).

The aim of our research was to determine the efficacy of EPNs *Steinernema feltiae* and *Heterorhabditis bacteriophora* against the larvae and adults of *H. femoralis*. Differences in efficacy were expected between the temperatures and nematode species. Additionaly, we also expected differences in susceptibility of thrips according to their developmental stages.

2 MATERIAL AND METHODS

2.1 *Entomopathogenic nematodes and banded greenhouse thrips*

A laboratory investigation was carried out in the Entomological Laboratory of the Chair of Entomology and Phytopathology (University of Ljubljana, Biotechnical Faculty, Department of Agronomy) in Ljubljana, Slovenia. Commercial biopreparations of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) from Koppert B. V. (Berkel en Rodenrijs, The Netherlands) were used in the work. Both biopreparations, which were sent by air-mail, were used within 2 months of their receipt.

Hercinothrips femoralis was reared in the insectarium at room temperature and without artificial light. They were reared on young plants of *Mirabilis jalapa* and on the leaves of French bean (*Phaseolus vulgaris* L.). The suitability of the latter plant for rearing this thrips species has already been demonstrated (Takrony, 1973), but the rearing of the thrips on both hosts was relatively simple compared to the older rearing method of this insect (Laughlin, 1971). On both hosts, thrips had more numerous progeny than on a previous tested composition of *Chlorophytum comosum* and the orchids *Oncidium* Gower Ramsey and *Epidendrum* 'Ballerina yellow'. Adults and larvae of the pest were used for the laboratory research.

2.2 Laboratory bioassay

Suspensions of EPNs were prepared in glass jars. The efficacy of agents was tested using a concentration of 200 infective juveniles (IJs) per individual (larva or adult) or 1000 IJs in 1 ml of water. Transparent plastic vessels (10 x 10 x 3.5 cm) were used for the rearing of thrips. This rearing method has been used before to study the bionomics of thrips (Trdan, 2000). In contrast to the original method, no pollen was added to the bean leaves in the present study. Five thrips adults (or larvae) were transferred onto each leaf using a fine-tip brush. Following this, 1 ml of nematode suspension was added to each leaf, whereas in the control condition 1 ml of water without nematodes was added to the leaves. Suspensions were added using a pipette, with the tip being changed after every treatment. The plastic vessels were then closed.

The plastic vessels were put in a rearing chamber (RK-900 CH type from Kambič Laboratory equipment, Semič, Slovenia) with working capacity of 0,868 m^3 (width x height x depth = 1000 x 1400 x 620 mm), and with every treatment in 5 replications. Efficacy was tested at three different temperatures (15, 20, and 25°C), but with constant light:dark ratio of 4:20 and relative humidity of 95%. The number of dead individuals was determined 4 days after treatment.

2.3 Statistical analysis

A multifactor analysis of variance (ANOVA) was conducted to determine the differences in mortality rates (%) between the larvae and adults of banded greenhouse thrips, reared in three different treatments (two species of EPNs and untreaed control) at three different temperatures. Before the analysis, each variable was tested for homogeneity of treatment variances. The mortality rate data were corrected for control mortality according to Abbott's formula (Abbott, 1925) and the data were normalized by an arcsine square-root transformation before the analysis. Duncan's multiple range test ($P \leq 0.05$) was used to separate mean differences among the parameters in all the treatments. All statistical analyses were performed with Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Maryland, USA) and figures were created with MS Office Excel 2003 (MS Corporation). The data are presented as untransformed means ± SE.

3 RESULTS

With general analysis of variance it was determined that temperature (P < 0.0498) and developmental stage of the pest (P < 0.0002) had significant effects on mortality rate of *H. femoralis,* while no significant effects were seen with nematode species (P < 0.1337), interaction between EPN species and temperature (P < 0.3412), interaction between EPN species and developmental stage (P < 0.2202), interaction between temperature and developmental stage of the pest (P < 0.3821) and interaction between all three factors (P < 0.8940). With average mortality of 37.68 ± 4.44%, larvae were more susceptible to nematode attack than were adults, which showed a mortality rate of 15.40 ± 3.31%.Significantly the lowest mortality of thrips was seen at 20°C (16.88 ± 4.65), and between 15 (30.89 ± 6.12%) and 25°C (31.85 ± 4.86%) no respective significant differences were ascertained. *H. bacteriophora* showed the highest efficacy rates against thrips (30.71 ± 4.75%), but this was not significantly higher than the mortality rate caused by *S. feltiae* (22.37 ± 3.94).

Individual analysis of variance did not confirm any influence of EPN species (P < 0.1151), temperature (P < 0.5276), or their interaction (P < 0.5220) on the mortality of thrips larvae. Larval mortality ranged from just over 23 % (*S. feltiae* at 25°C) to approximately 50 % (*H. bacteriophora* at 15 and 25°C) (Figure 1).

Temperature had significant influence (P < 0.0049) on adult pest mortality, but no significant effects were found with EPN species (P < 0.7693) and its interaction with temperature (P < 0.5696). The lowest efficacy of both biological agents was found to occur at 15°C (about 17 % mortality of adults) and at 20°C (about 5 % mortality of adults), beside that between 15°C (about 15 % mortality of adults at both nematode species) and 25°C (about 30 % mortality of adults treated with *H. bacteriophora*) no significant differences were determined (Figure 2).

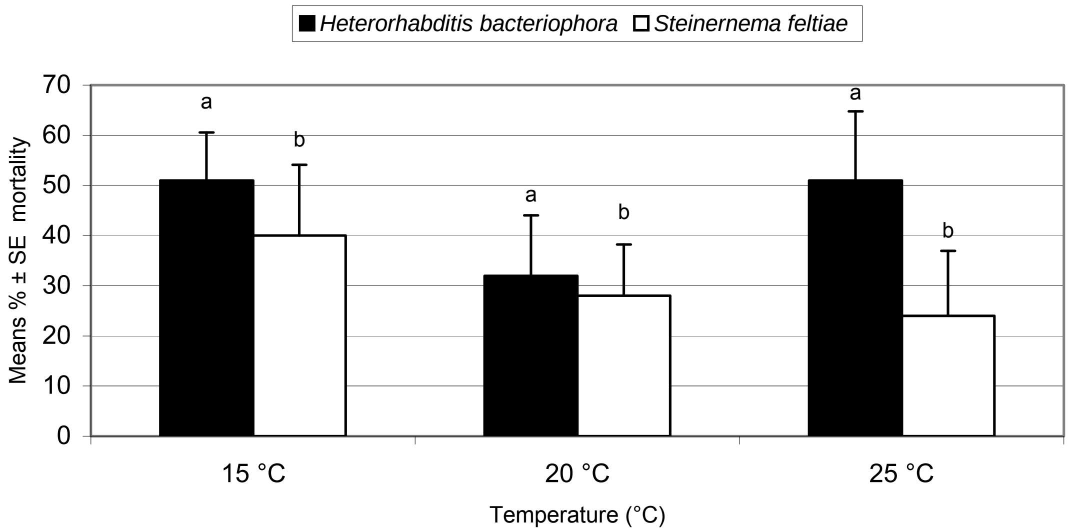

Figure 1: Mean larval mortality (±SE) of *Hercinothrips femoralis* treated with two different species of entomopathogenic nematodes (200 IJs/individual) depending on rearing temperature. Data shown are corrected for control mortality and analyzed by multifactor ANOVA. Mean values followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test. The letters correspond to the grouping of means for temperature, while for EPN species no signifficant diferences were established.

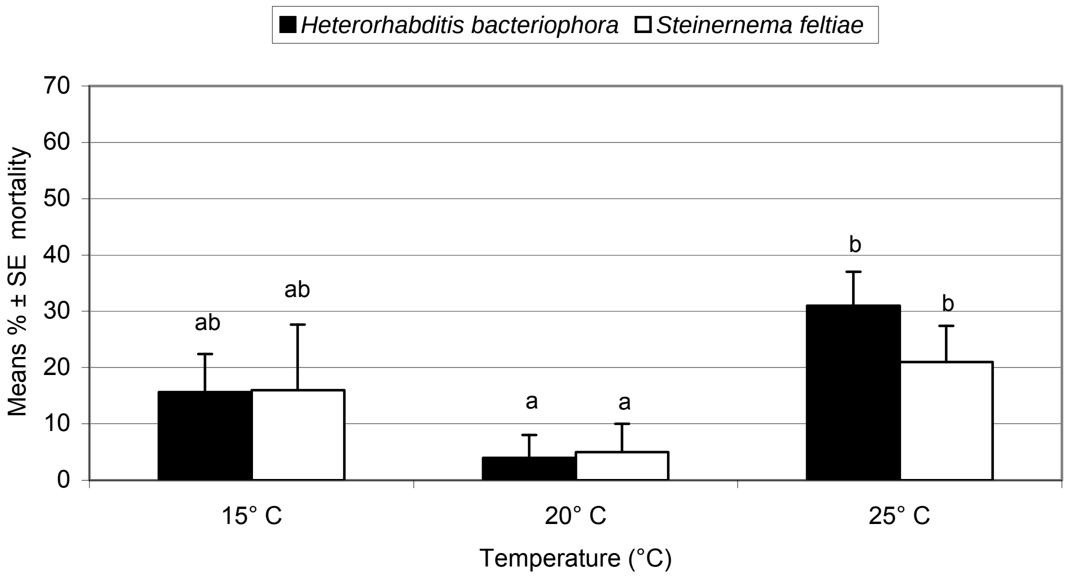

Figure 2: Mean adult mortality (±SE) of *Hercinothrips femoralis* treated with two different species of entomopathogenic nematodes (200 IJs/individual) depending on rearing temperature. Data shown are corrected for control mortality and analyzed by multifactor ANOVA. Mean values followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple range test. The letters correspond to the grouping of means for temperature, while for EPN species no signifficant diferences were established.

4 DISCUSSION

Though the first record of *H. femoralis* in Slovenia on some of the ornamental plants was expected (Trdan and Vierbergen, 2001), it was somewhat surprising when it was found on young maize plants grown in the laboratory (Trdan, 2002). Some sources (*Hercinothrips*..., 2002) quoted maize as one of the potential hosts of banded greenhouse thrips. The thrips most likely entered into the laboratory with some ornamental plants, and remained in the same laboratory since 2002 or even earlier.

The fact that indoor work and living places are important habitats of banded greenhouse thrips has implications for the control methods that can be used. Special attention should be paid to guard against secondary damage to human comfort or health. With the choice of some of the environmentally acceptable control methods, we may be able to curcumvent some of the problems associated with chemical pesticides. Among these methods we include EPNs, whose efficacy against Thysanoptera species has been particularly studied with western flowers thrips in the greenhouses. Higher efficacy of EPNs against this pest has been shown with a soil application of the suspension (Buitenhuis and Shipp, 2005), with earlier and multiple applications at lower concentration (Belay *et al*., 2005), at higher soil moisture (Ebssa *et al*., 2004a) and temperatures of 25°C (Ebssa *et al*., 2004b).

Foliar applications of EPNs on plants attacked by thrips represents one of the more recent potential methods of their use. Although previous results of such research do not lend strong support to foliar application of EPNs (compared to soil application), particularly due to their sensitivity to desiccation and UV radiation (Shapiro-Ilan *et al*., 2006), this method could nevertheless be suitable for the control of thrips on (tropical) plants, which often require water applications to leaves and which preferentially grow in places not exposed to direct sunlight. In fact, many hosts of *H. femoralis* like such habitats.

In our research, the larvae of banded greenhouse thrips were more sensitive to nematode infection than were adults. This confirms the known fact that EPNs are most efficient against the larvae and other preimaginal stages of insects, as they can enter their bodies more easily (LeBeck *et al*. 1993). For this reason, a weaker activity associated with foliar applications *S. feltiae* and *H. bacteriophora* on adults of *H. femoralis* is no surprise. Furthermore,, in recent related research on adults of western flower thrips, similar conclusions were reached (Buitenhuis and Shipp, 2005). A poorer efficacy of the nematodes in our research might also be attributed to lower concentration of suspension (200 infective juveniles/individual), which was applied to bean leaves only once. It is often necessary to apply such a suspension repeatedly in order to achieve satisfactory efficacy of these biological agents (Belay *et al*., 2005), and in this particular detail we see a chance to notably improve the efficacy against *H. femoralis* on ornamental plants via repeated EPN foliar applications.

Temperature had no influence on nematode efficacy against larvae, but this was not the pattern for adults where the mortality was significantly higher at 25°C. This is in accordance with nematode efficacy in controlling western flower thrips (Ebssa *et al*., 2004b). We attribute the weak activity of the nematodes against the banded greenhouse adults at 20°C to the highest vitality of thrips at this temperature, most likely promoting its natural resistance to infection with EPNs.

H. femoralis also survives on above-ground parts of host plants during pre-pupal and pupal stages, especially on older leaves (Oetting *et al*., 1993). Foliar application of the nematodes is therefore a legitimate method of pest control. We note that western flower thrips and many other damaging Thysanoptera species (Tommasini and Maini, 1995) pupate in the soil. Furthermore, thrips in pre-pupal and pupal stages are especially susceptible to EPN infection (Ebssa *et al*., 2001) and are almost immobile.

Thus these first results concerning the control *H. femoralis* with foliar applications of EPNs showed that these biological agents could be, along with optimal abiotic and biotic factors, an effective enough alternative to the current prevailing (conventional) methods of pest control. Therefore, further research from our group will be oriented to the optimization of foliar application methods of EPNs to control all above-ground developmental stages of the pest.

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