Can pre-colonisation of the soil substrate increase the efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae)?

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Abstract
The effects of soil pre-colonisation with entomopathogenic nematodes *Steinernema feltiae*, *S. carpocapsae* and *S. arenarium* on the mortality of *Tenebrio molitor* larvae were tested under laboratory conditions. The soil was inhabited with the nematodes for 7 days prior to the addition of the larvae. Nematode concentrations of 50 and 500 infective juveniles per box were used. *S. carpocapsae* reacted to pre-colonisation negatively and the efficacy decreased at the concentrations tested. *S. arenarium* showed generally low efficacy against *Tenebrio molitor* larvae and pre-colonisation showed only a slight positive effect on the efficacy of this species. *S. feltiae* increased its efficacy at the concentration of 500 infective juveniles per box compared with other tested nematodes.

Key words: efficacy; *Steinernema feltiae*; *S. carpocapsae*; *S. arenarium*; pre-colonisation; *Tenebrio molitor*

INTRODUCTION
Entomopathogenic nematodes (EPNs) have been used successfully in agricultural systems. As well as parasitoids or predators, EPNs have chemoreceptors, are motile, highly virulent, and kill their hosts quickly. They can be cultured easily *in vivo* or *in vitro* (Ehlers 2001). EPNs have been used against many different pests in the soil, in cryptic habitats and on foliage (Begley 1990). Soil is the natural reservoir of EPNs (Akhurst 1986, Gaugler 1988) offering excellent conditions for nematode survival and activity. The opportunities for the use of EPN are promising, as more than 90% of insect pests spend part of their life cycle in the soil (Ehlers 2001).

Free-living infective juveniles (IJJs) or dauer juveniles (Fodor et al. 1990) in the soil, penetrate insect hosts through natural body openings such as the mouth and spiracles. Once inside the host, species-specific, symbiotic bacteria are released...
from the IJ gut into the insect hemocoel, and through the synergistic actions of both nematode and bacterial virulence factors, the host is killed within 24–48 h post-infection (Boemare 2002). The host carcass, subsequently, becomes a nutritionally rich environment for EPN mating and progeny development, which includes the egg and four juvenile stages, J1–J4 (Adams and Nguyen 2002). Both steinernematids and heterorhabditids can undergo one or more rounds of development within a host cadaver at the optimal incubation temperature of 23 °C (Wang and Bedding 1996). When food becomes limited, a facultative developmental choice is made at the J2 stage to form the specialized IJ. This is the only stage in the life cycle when it can travel and survive outside the host (Campbell et al. 1995) and it has been shown to be resistant to heat, desiccation, and other environmental stresses (Kung et al. 1990).

EPNs have generally been used for short-term inundative or augmentative biological control against a broad spectrum of the agriculturally important pest orders: Diptera, Coleoptera, Lepidoptera and Orthoptera (Begley 1990, Klein 1990, Ogura 1993, Parkman et al. 1993, Cabanillas and Raulston 1994, Georgis and Manweiler 1994, Williams and Walters 2000, Jagdale et al. 2004). All the abovementioned authors studied the effect of the direct application of EPNs into the environment for curative purposes. Nevertheless, some of the biological pesticides, i.e. mycoparasitic and entomopathogenic fungi, are used in preventive application programmes in ornamental or nursery plants to provide better control against phytopathogenic fungi or pests. Fungi applied early colonise the soil substrate and are able to survive on the alternate substrate represented by organic matter in the soil or to grow saprophytically in the rhizosphere (Wang et al. 2005). The ability to reproduce in non host conditions gives a guarantee of permanent protection and increasing inoculum density in the soil for some time after application; consequently the positive effect of the higher density of fungal spores in the soil increases the efficacy of the applied fungi.

The ability to multiply outside the host has not been adopted by entomopathogenic nematodes because of their biological aspects. Anyway, there exist many other methods to intensify the efficacy of the application of EPNs. The factors most frequently affecting efficacy are a) environmental conditions, b) application techniques and c) the proper selection of the strain or species of the EPN. Also combinations of nematodes with chemicals and pathogens have been used to increase nematode efficacy in laboratory and field applications. Nematodes have been combined with pesticides (Head et al. 2000, Koppenhöfer et al. 2003), adjuvants (Baur et al. 1997), bacteria (Koppenhöfer and Kaya 1997, Koppenhöfer et al. 2000), viruses (Agra Gothama et al. 1996) and fungi (Choo et al. 1996). These methods involve a mechanism of stressing the host so that is more susceptible to the nematode infection. Physical and chemical stressors could be used to compromise insect defences in order to enhance nematode infection. Stresses induced by physical extremes such as temperature have been responsible for increased parasitism in a variety of plants and animals (Shibata 2000, Daane and Williams 2003, Pickett et al. 2003). There have been studies published about the negative/positive effects of abiotic factors on the development and infectivity of EPNs (Jaworska and Gorczyca 2002, Bornstein-Forst et al. 2005, Jagdale and Grewal 2007).

As mentioned above, the efficacy of applying EPNs depends very much on the strains or species used and the environmental conditions. Factors of major importance are the age and lipid reserves of the IJs. These characteristics directly influence the ability of nematodes to survive for a period of time without a host and their ability to find and infect a host (Womersley 1993). Many studies have tested the persistence and efficacy of EPNs under various conditions. The data generally indicates a survival of weeks rather than months (Fan and Hominick 1991, Womersley 1993).

The short-term exposure of EPNs to non-host conditions could lead to an increase in efficacy. Nematodes could inhabit the environment in advance and adapt to the new conditions after application and then provide better results in protection against the pest.

Based on this hypothesis we examined the ability of EPNs to increase their efficacy by a simple pre-colonisation effect. Our objective was to determine the effect of pre-colonisation on the efficacy of three steinernematids (S. feltiae, S. carpocapsae and S. arenarium) under laboratory conditions.

MATERIALS AND METHODS

Insect host: Tenebrio molitor larvae were used as hosts for the bioassays. The larvae were held at 15 °C and fed on grain grit.
Nematodes: Three nematode species were tested: *Steinernema carpocapsae* (Weiser) and *S. feltiae* (Ust.) and *S. arenarium* (Slov), were obtained from the Institute of Entomology (Academy of Sciences of the Czech Republic, České Budějovice), and were reared in wax moth larvae following the procedures of Kaya and Stock (1997). Larvae of the greater wax moth *Galleria mellonella* were held at 28 °C and reared according to the description of Woodering and Kaya (1988). Third stage juveniles were harvested from water surrounding White traps (White 1927) within 10 days of emerging from their hosts. A stock suspension of the IJs in distilled water was stored at 9 °C for 2 weeks before use.

The experiments were conducted in plastic boxes (90×70×50 mm) filled with 40 ml of a soil substrate (sand : potting substrate, 2 : 3). The soil substrate was sterilized (121 °C for 2 h) before the use to reduce soil microbes and until completely dry.

Experiment: The soil substrate was moistened with sterile water to a final moisture content of 12.5% (w/w). Ten plastic boxes received 50 or 500 IJs of *S. carpocapsae*, *S. feltiae* or *S. arenarium*. The nematodes were left for 24 h at room temperature prior to the application. Sterile water was used as a control variant. Each box was well shaken to distribute the nematodes regularly. Each of five boxes received 10 larvae of *T. molitor*. Five boxes were left without the host. The boxes were covered with lids and kept in darkness at 25±1 °C. Larvae mortality was assessed after 7 days. At the same time each of the boxes previously incubated without a host received 10 larvae of *T. molitor* and were kept in incubation. The boxes were checked on days 7 and 14 after host introduction.

Larvae mortality was statistically assessed using program Statistica 8.0. Data were transformed using an arcsine transformation before use of one-way ANOVA. Tukey’s test at P ≤ 0.05 was used to assess significant differences among the experimental groups.

RESULTS

Results show significant differences among each tested nematode species (Table 1) especially at the higher concentration (500 IJs per box) in both models (F=63.82; p<0.05; df=3, F=75.30; P<0.05; df=3). *S. arenarium* recorded the lowest efficacy against larvae of *T. molitor* compared with *S. feltiae* and *S. carpocapsae* even at the concentration of 500 IJs per box. The highest larvae mortality was recorded with *S. carpocapsae* in the model without pre-colonisation. *S. carpocapsae* reacted to pre-colonisation negatively and its efficacy decreased at both tested concentrations. The difference was statistically significant (F=8.06; P<0.05; df=1) at the concentration of 500 IJ per box. *S. feltiae* increased its efficacy compared with other tested nematodes; however this increase is not statistically significant (P>0.05). If compared with the model without pre-colonisation, a significant difference was detected

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Species</th>
<th>T. molitor mortality 7 day after application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>0±0 b</td>
</tr>
<tr>
<td>50 IJ</td>
<td><em>S. feltiae</em></td>
<td>0.38±0.19 aA</td>
</tr>
<tr>
<td></td>
<td><em>S. carpocapsae</em></td>
<td>0.24±0.23 abA</td>
</tr>
<tr>
<td></td>
<td><em>S. arenarium</em></td>
<td>0.16±0.09 abA</td>
</tr>
<tr>
<td>500 IJ</td>
<td>control</td>
<td>0±0 d</td>
</tr>
<tr>
<td></td>
<td><em>S. feltiae</em></td>
<td>0.94±0.09 aA</td>
</tr>
<tr>
<td></td>
<td><em>S. carpocapsae</em></td>
<td>0.60±0.14 bB</td>
</tr>
<tr>
<td></td>
<td><em>S. arenarium</em></td>
<td>0.38±0.08 ca</td>
</tr>
</tbody>
</table>

Table 1. Mortality (mean±standard deviation) of *Tenebrio molitor* larvae after the exposure to *S. feltiae*, *S. carpocapsae* or *S. arenarium* in the soil with or without pre-colonisation at 25 °C after 7 days

a, b, c = significant differences among nematode species in a column within the group are marked with small letters; A, B, C = significant differences among pre-colonised / non pre-colonised treatment in a row within the group are marked with capital letters (Tukey’s test at P ≤ 0.05)
The mortality of *T. molitor* larvae showed a similar trend in the efficacy of each nematode species after 14 days (Table 2). The statistical differences among all tested species were recorded at both tested concentrations. A decrease in the efficacy in the pre-colonised model was confirmed with *S. carpocapsae* \( F=21.67; \, P<0.05; \, df=1 \). *S. feltiae* as well as *S. arenarium* achieved better results in the pre-colonised model although the difference is not statistically significant.

### Table 2. Mortality (mean±standard deviation) of *Tenebrio molitor* larvae after the exposure to *S. feltiae*, *S. carpocapsae* or *S. arenarium* in the soil with or without pre-colonisation at 25 °C after 14 days

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Species</th>
<th>pre-colonisation</th>
<th>without pre-colonisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 IJ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>0.02±0.04 b</td>
<td>0.02±0.04 c</td>
</tr>
<tr>
<td></td>
<td><em>S. feltiae</em></td>
<td>0.42±0.16 aA</td>
<td>0.28±0.16 bA</td>
</tr>
<tr>
<td></td>
<td><em>S. carpocapsae</em></td>
<td>0.30±0.27 abA</td>
<td>0.60±0.17 aA</td>
</tr>
<tr>
<td></td>
<td><em>S. arenarium</em></td>
<td>0.18±0.08 abA</td>
<td>0.16±0.09 bcA</td>
</tr>
<tr>
<td>500 IJ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>0.02±0.04 c</td>
<td>0.02±0.04 c</td>
</tr>
<tr>
<td></td>
<td><em>S. feltiae</em></td>
<td>0.96±0.09 aA</td>
<td>0.82±0.18 aA</td>
</tr>
<tr>
<td></td>
<td><em>S. carpocapsae</em></td>
<td>0.60±0.14 bB</td>
<td>0.94±0.06 aA</td>
</tr>
<tr>
<td></td>
<td><em>S. arenarium</em></td>
<td>0.52±0.16 bA</td>
<td>0.38±0.08 bA</td>
</tr>
</tbody>
</table>

a, b, c = significant differences among nematode species in a column within the group are marked with small letters (Tukey’s test at \( P \leq 0.05 \)); A, B, C = significant differences among pre-colonised/ non-pre-colonised treatment in a row within the group are marked with capital letters (Tukey’s test at \( P \leq 0.05 \))

### DISCUSSION

Due to the cost of the product and in some cases a lack of consistency in the environment, microbial control agents are usually used to achieve economic pest control in a curative rather than preventative manner. Exceptions in which microbial control agents have been implemented or considered in a prophylactic approach tend to be in protected or controlled environments where prolonged environmental consistency is expected, e.g., in storage facilities or greenhouses. For example, the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin can be applied as a prophylactic treatment to control *O. sulcatus* in potted plants and in the greenhouse (Bruck 2005, Shah et al. 2007). As with other microbial control agents in commercial settings entomopathogenic nematodes are applied almost exclusively in a curative rather than prophylactic manner (Grewal et al. 2005).

Research has indicated that curative applications of entomopathogenic nematode *Steinernema feltiae* can effectively reduce many soil inhabiting pests especially dipteran (Sciarids and Lycoriella) (Tomalak 1994, Fenton et al. 2002, Jagdale et al. 2004). *S. feltiae* is an efficacious and economical replacement for chemical insecticides in the floriculture industry in The Netherlands, England and Germany (Jagdale et al. 2004). In this study it was demonstrated that preventative treatments are also efficacious and that their efficacy can be improved on. An opposite reaction to the short-term absence of the host in the environment was recorded with *S. carpocapsae*, which reacted to the pre-colonisation effect negatively and its efficacy decreased at both tested concentrations. These results are in contrast to the findings of Toepfer et al. (2008) who confirmed successful preventative application of entomopathogenic nematodes during sowing of corn (*Zea mays* L.). The damage caused by the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) was reduced. Also Kim et al. (2004) reported that *S. carpocapsae*, applied for the control of a fungus gnat, *Bradysia agrestis* Sasakawa (Diptera: Sciaridae) at
the time of sowing, reduced the mortality of watermelon plants in a seedling propagation house. The lower efficacy of S. carpocapsae during the test could be caused by the phased infectivity of IJs (Hominick and Reid 1990). According to the switch model, individuals in a population are either infectious or non-infectious, and the proportion of non-infectious individuals in the population may change over time (Hominick and Reid 1990, Bohan and Hominick 1996). To demonstrate a non-infectious proportion, IJs must have unlimited opportunity to infect potential hosts. Phased infectivity of this species will not be detected when nematodes have unlimited access to hosts, as under such circumstances all individuals capable of infecting will eventually do so. Campbell et al. (1999) tested infectivity with “sufficient suitable hosts” and found no evidence for the existence of a non-infectious proportion in three species of Steinernema (S. glaseri, S. feltiae and S. carpocapsae).

S. arenarium showed generally low efficacy against the larvae of T. molitor and pre-colonisation made only a slight positive difference to the efficacy of this species though it was not statistically significant. The low virulence of IJs of S. arenarium could be affected by the physiological and morphological pre-dispositions of the host, particularly the size of the natural openings (anus and mouth). S. arenarium is double sized when compared with two other tested nematodes and this could play an important role in the invasion of the host body by IJs. Eidt and Thurston (1995) reported, that the width of both openings in wireworms may exclude IJs.

Based on regulatory trends, organophosphate insecticide usage continues to decline. Therefore, efficacious and economically sound alternative pest management strategies must be developed. Many studies have indicated that curative applications of entomopathogenic nematodes can effectively reduce pest populations (Cabanillas and Raulston 1994, Georgis and Manweiler 1994, Tomalak 1994, Williams and Walters 2000, Fenton et al. 2002, Jagdale et al., 2004). This study demonstrates that preventative treatments can be also efficacious especially with S. feltiae. Preventative approaches are important for pests that are undetected until significant damage has been done, i.e. soil borne insects. Additional research is needed because our findings show that each species reacts to a prophylactic application differently and also the selection of strains might increase or decrease the efficacy of the approach.

ACKNOWLEDGEMENTS

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