ORIGINAL ARTICLE

Effect of soil temperature and moisture on the pathogenicity of two species of entomopathogenic nematodes (Rhabditida: Steinernematidae)

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Abstract

This study investigated the impact of soil temperature and soil moisture on the virulence of the entomopathogenic nematodes *Steinernema carpocapsae* and *S. feltiae*. The effects of temperatures of 10, 15 and 25 °C and humidity of 6% and 12.5% were tested against the larvae of *Tenebrio molitor*. The nematodes were tested in two concentrations of 50 nematodes and 500 nematodes per box. *S. carpocapsae* was generally significantly more efficient at the highest temperature (25 °C) than *S. feltiae*, especially at the lower concentration of 50 nematodes per box. *S. feltiae* recorded higher insect mortality at lower temperatures (15 °C and 10 °C). The virulence of both tested nematode species was low (0–26%) at 10 °C after 7 days, but in the case of *S. feltiae* increased to 66% after 14 days. The efficacy when tested with 6% moisture at 15 °C was low (4% for both nematode species) compared with 12.5% moisture, where after seven days it reached 54% for *S. carpocapsae* and 70% for *S. feltiae*, although generally, *S. feltiae* was more efficient under dry conditions than *S. carpocapsae*. Insect mortality increased significantly after remoistening of the soil, especially with *S. feltiae* (500 nematodes per box) where mortality reached 46% two weeks after remoistening.

Key words: *Steinernema carpocapsae*; *Steinernema feltiae*; efficacy; soil conditions; *Tenebrio molitor*

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INTRODUCTION

Entomopathogenic nematodes (EPNs) (Rhabditida: Heterorhabditidae, Steinernematidae) are lethal insect parasites used in biological control (Gaugler and Kaya 1990, Georgis 1992, Kaya and Gaugler 1993). The only free-living stage is an infective juvenile that is produced when host

nutrients are depleted, and which then leaves the cadavers to seek out new hosts.

Soil as the natural habitat for entomopathogenic nematodes is a difficult environment for the persistence of any organism, considering the complexity of its physical, chemical and biological components (Poinar 1990, Hominick et al. 1996). It is assumed that in the course of evolution, entomopathogenic nematodes, just like other terrestrial organisms, adopted unique survival mechanisms to resist environmental extremes. Entomopathogenic nematodes of the genera *Steinernema* are ideal biological agents to control soil insect pests because of their broad host range, their marked virulence, their ability to search for hosts and their high reproductive potential (Grewal et al. 2005). However, temperature and moisture are the most important factors limiting the success of EPNs. Both factors directly influence host searching (Byers and Poinar 1982), pathogenicity (Molyneux 1984, 1986) and survival (Kaya 1990, Kung and Gaugler 1990, 1991). Because low temperatures induce inactivity in infective juveniles, they seem to be the main barrier to the use of EPNs in temperate regions (Dolmans 1983, Rutherford et al. 1987). Such inactivity is characterised by decreased enzymatic activity and mobility, both reducing metabolic expenditures (Molyneux 1985, Fan and Hominick 1991).

Moisture conditions have also been recognized as one of the most important factors in the soil environment, as they affect the survival, virulence and persistence of nematodes (Klein 1990, Curran 1993), which need high relative humidity to survive and a film of free water for movement. They may become dormant at very low soil moistures. Some free-living stages of several animal and plant-parasitic nematodes can survive exposure to desiccation for long periods (Cooper and Van Gundy 1971, Wharton 1986), but, on exposed surfaces, steinernematids and heterorhabditids can survive no longer than several hours, depending on the species, temperature and relative humidity (Glazer 1992). In dry soil, entomopathogenic nematodes can persist for 2–3 weeks (Kaya 1990, Kung and Gaugler 1990). Some nematode species can be more tolerant to cold or the level of humidity because of their natural adaptation and seeking strategy. Some studies that have investigated the effects of desiccation on the survival ability of steinernematid nematodes, have concentrated on *S. carpocapsae* (Ishibashi et al. 1987, Womersley 1990, Glazer 1992). The general finding has been that under slow drying conditions various strains

of *S. carpocapsae* can survive for appreciable lengths of time.

The aim of our research was to investigate the impact of soil temperature and soil moisture on the virulence of two species of entomopathogenic nematodes: *S. carpocapsae* and *S. feltiae*.

MATERIALS AND METHODS

Insect host: *Tenebrio molitor* larvae were used as host for the bioassays. The larvae were held at 15 °C and fed on grain grit.

Nematodes: The two nematodes tested – *Steinernema carpocapsae* (Weiser) and *S. feltiae* (Ust.) – were obtained from the Institute of Entomology (Academy of Sciences of the Czech Republic, České Budějovice) and were reared in wax moth larvae according to the procedures of Kaya and Stock (1997). Larvae of the greater wax moth *Galleria mellonella* were held at 28 °C and reared as described by Woodering and Kaya (1988). Third stage juveniles (IJs) were harvested from the 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 soil substrate (sand : potting substrate – 2 : 3). To reduce soil microbes the soil substrate was sterilized (121 °C for 2 hours) before use until completely dry.

Experiment 1. Testing of temperature

effects: The soil substrate was moistened with sterile water to a final moisture content of 12.5% (w/w). Each plastic box received 50 or 500 IJs of *S. carpocapsae* or *S. feltiae*. Sterile water was used as a control variant. Each box was well shaken to distribute the nematodes regularly. Ten larvae of *T. molitor* were placed onto the soil surface and the boxes were covered with lids. 50 individuals were tested in each nematode/concentration. Three different temperatures of 10, 15 and 25 °C were used in the test. Larvae mortality was assessed after 5, 7 and 14 days.

Experiment 2. Testing of humidity effects:

The soil substrate was moistened by sterile water to a final moisture content of 6% (w/w). The subsequent steps were the same as in experiment 1. All boxes were held at 15±1 °C. For this experiment, a rehydration process was used. An amount of sterile water was applied sufficient to

increase the moisture to 12.5% after 7 days of the test. Larvae mortality was assessed after 5, 7, 14 and 21 days. The dead larvae were removed from the soil, washed with sterile water and put onto the White traps to recognize reproduction.

Statistical analyses were performed with the Statistica 8.0 programme. The percentage of *Tenebrio molitor* mortality was arcsine transformed before statistical analysis. The One-way ANOVA was used for the analysis of temperature test. Tukey's test at $P \leq 0.05$ was used to assess significant differences among groups. Two-way ANOVA was used for analysis of moisture effects.

RESULTS

S. carpocapsae was generally significantly more efficient ($F=16.88$; $df=2, 12$; $P<0.05$) at higher temperature than *S. feltiae* especially with the lower nematode concentration (50 IJs). The results for the higher concentration of 500 IJs were not statistically significant between the nematode species (Table 1). *S. feltiae* showed higher insect mortality at lower temperatures but at the concentration of 50 IJs per box the

rate was lower (12%) than *S. carpocapsae* (38%). Both tested nematode species showed low efficacy (0–26%) on days 5 and 7 of the test at 10 °C, but the mortality rate with *S. feltiae* increased to 66% after 14 days.

The results of experiment 2 present the impact of moisture on the ability of nematodes to infect the insect host. With 6% moisture, insect mortality was very low at 0% for *S. carpocapsae* and 2% for *S. feltiae*, compared with rates of 20% and 12% respectively with 12.5% moisture after 7 days (Fig. 1). The results were more significant at the concentration of 500 IJs per box ($F=25.77$; $df=2, 24$; $P<0.05$) (Fig. 2). *S. feltiae* was generally more efficient under dry conditions than *S. carpocapsae*. Insect mortality increased significantly ($F=8.67$; $df=2, 24$; $P<0.05$) after remoistening of the soil especially in the case of *S. feltiae* (500 IJs per box) where 46% was reached two weeks after remoistening. This is approximately half the efficacy of the same concentration at 12.5% soil moisture after two weeks of the test. *S. carpocapsae* caused only 16% insect mortality on day 21 of the test. Nematode reproduction was not influenced by either of the abiotic factors studied.

Table 1. Mortality [mean±standard deviation (SD)] of *Tenebrio molitor* larvae after exposure to entomopathogenic nematodes of *S. feltiae* or *S. carpocapsae* in soil containing 12.5% moisture (w/w) at 25, 15 and 10 °C (significant differences among nematode species in a column within the group are marked with different letters)

Temperature	Concentration *	Species **	Mortality (mean±SD) %					
			Day 5		Day 7		Day 14	
25 °C	50 IJ	control	0	a	0	a	12±8.4	a
		Sf	10±7.1	a	14±11.4	a	28±16.4	a
		Sc	48±11.0	b	50±12.6	b	60±17.3	b
	500 IJ	control	0	a	0	a	12±8.4	a
		Sf	74±9.0	b	78±13.0	b	82±17.9	b
		Sc	80±10.0	b	86±11.4	b	94±5.5	b
15 °C	50 IJ	control	0	a	0	a	2±4.5	a
		Sf	2±4.5	a	12±13.0	ab	12±13.0	a
		Sc	20±10.0	b	20±10.0	b	38±16.4	b
	500 IJ	control	0	a	0	a	2±4.5	a
		Sf	46±24.1	b	70±14.1	b	80±12.3	b
		Sc	52±17.9	b	54±20.7	b	62±22.1	b
10 °C	50 IJ	control	0	a	0	a	4±4.4	a
		Sf	0	a	4±5.6	a	10±12.3	a
		Sc	0	a	0	a	6±5.48	a
	500 IJ	control	0	a	0	a	4±4.4	a
		Sf	0	a	26±25.1	b	66±20.7	c
		Sc	0	a	0	a	26±5.5	b

* IJ: infective juveniles

** Sf: *Steinernema feltiae*, Sc: *Steinernema carpocapsae*; SD: standard deviation

A) 12.5% soil moisture

B) 6% soil moisture

Fig. 1. *T. molitor* mortality (%) in soil inoculated with 50 infective juveniles per box of *S. feltiae* or *S. carpocapsae* at 12.5% (A) or 6% (B) soil moisture at 15 °C and rehydrated to the level of 12.5% after 7 days of the test (remoistening is marked in the graph)

A) 12.5% soil moisture

B) 6% soil moisture

Fig. 2. *T. molitor* mortality (%) in soil inoculated with 500 infective juveniles per box of *S. feltiae* or *S. carpocapsae* at 12.5% (A) or 6% (B) soil moisture at 15 °C and rehydrated to the level of 12.5% after 7 days of the test (remoistening is marked in the graph)

DISCUSSION

In nature, native populations of EPN are exposed to fluctuations in temperature and soil moisture throughout their development, and studies have examined the effects of such conditions on EPNs vitality and pathogenicity (Grewal et al. 1994, Lewis 2002, Chen et al. 2003). The results of this present study widen our knowledge of the impact of the two most important factors and show how it might be possible to use these findings and implement them into the practical use of EPNs.

In this study, *S. feltiae* showed very good tolerance to cold and ability to infect *T. molitor* at the lower temperatures (15 and 10 °C). Grewal et al. (1994) concluded that *S. feltiae* has adapted to cooler environments and this is the reason why this species is extremely effective against the economically significant members of the family *Bibionidae* (Diptera). Commercial products based on *S. feltiae* are available in both glasshouse and mushroom markets (Grewal and Smith 1995). Chen et al. (2003) confirmed in their study that *S. feltiae* was the only species that destroyed *Delia radicum* larvae at 10 °C; *S. carpocapsae* and two other species were only effective between 15 °C and 20 °C. In this study, *S. carpocapsae* showed the best results at 25 °C and lower temperatures decreased the efficiency of this species. These results also correspond with the narrow reproductive thermal niche of this species (Grewal et al. 1994). The findings of the experiment which tested the effects of moisture were interesting. *S. feltiae* showed a higher efficiency than *S. carpocapsae* which is considered to be a species with better adaptation to dry conditions (Glazer 1992). In this study, the moisture level showed a deep impact on the pathogenicity of the tested nematode species. The ability to infect the host *T. molitor* in 6% soil moisture decreased significantly compared with 12.5% moisture. There were also significant differences between the tested species. *S. feltiae* surprisingly showed better results than *S. carpocapsae*. Campbell and Kaya (2000) have noted that *S. carpocapsae* is an ambusher that uses a 'sit-and-wait' strategy to find hosts. In such an ambush search infective juveniles exhibit a nictation behaviour displacing over 90% of their body in the air in search of highly mobile hosts on or near the soil surface. This behaviour exposes infective juveniles to a rapid desiccation environment. Therefore, it could be predicted that ambush foraging nematodes would survive desiccation and would be adapted to desiccation tolerance.

Low soil moisture induces a quiescent dehydration-survival state in *S. carpocapsae* in contrast to *S. feltiae* which avoids desiccation by moving deeply into the soil, and are therefore more active and subsequently more infectious (Lewis 2002). A noticeable effect of remoistening was recorded at the concentration of 500 IJs per box. This effect was most significant in *S. feltiae* where efficacy increased from 4 to 40%. This increasing *S. feltiae* infectivity could have been affected by prompting of the searching activity induced by the improvement in the moisture condition of the soil.

The findings of this study show that *S. feltiae* especially can be successfully used under colder conditions without major problems. On the other hand, both nematode species recorded problems with desiccation tolerance. Thus, during application and post-application, desiccation of soil should be avoided or soil moisture should at least be maintained at a level sufficient for minimal nematode activity.

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