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Effect of soil texture and moisture on the activity of entomopathogenic nematodes against female *Boophilus annulatus* ticks

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Abstract. Soil texture, chemistry and moisture have a profound effect upon the activity and persistence of entomopathogenic nematodes (EPNs). Whereas nematodes' natural habitat is within the soil, ticks and other arthropod pests prefer to stay on the soil surface and under stones or leaf litter; they spend much of their life cycle in the humid environment of the soil upper layer, therefore consideration of the effect of the soil environment on nematode activity is a pre-requisite for the sucessful use of EPNs against arthropod pests. In the present study we investigated the effects of soil type, and humidity on various nematode strains and on their effectiveness against ticks. Many infective juveniles (IJs) of *Steinernema carpocapsae* and *S. riobrave* were found in the uppermost soil layer whereas the heterorhabditid strains were almost absent from the upper 6 cm of the soil profile. The IJs of *S. feltiae*, and the *S. carpocapsae* strain S-20, exhibited an intermediate behavior. It was found that the activity of IJs of *S. carpocapsae* in the soil upper layer (1 cm depth) was strongly affected by soil type: the greatest number of IJs were recorded from sandy loam soil; less were found in the lighter soils – 'Marine sand' and 'Calcareous sandstone' – and only very few were recovered from heavy soils. Strikingly, even when the soil moisture was low and the number of nematodes found in the upper layer correspondingly low, tick mortality remained high. The results demonstrate: (a) the possible use of the nematodes as an anti-tick agent; (b) the importance of knowing the exact interaction of nematodes with the immediate environment of the pest, in order to optimize the pest-control activity of the nematode.

Key words: biocontrol, *Boophilus annulatus*, entomopathogenic nematodes, *Heterorhabditidae steinernematidae*, Ixodidae, ticks

Introduction

The third-stage infective juveniles (IJs) of entomopathogenic nematodes (EPNs) locate insect hosts, enter their natural body openings

and release symbiotic bacteria carried in their intestines (Dowds and Peters, 2002). Only the infective stage of these nematodes can survive outside insects, which die as a result of the infection. The nematodes feed upon the rapidly multiplying bacteria and debris, and subsequently mature, mate, and produce two or more generations within the insect cadaver before emerging into the environment as IJs in search of a new host. This behavior has been exploited in the control of many insect pests (Georgis and Manweiler, 1994; Gaugler and Han, 2002; Grewal, 2002).

Numerous studies have demonstrated wide variations in the virulence of EPNs, as shown by the rate and degree of mortality of their hosts (Poinar, 1979; Caroli et al., 1996). However, most studies were conducted in the laboratory; they demonstrated differences in infectivity under optimal or extreme conditions, in which the target pests were exposed to the nematode on an artificial substrate such as filter paper or agar (Glazer and Lewis, 1998).

Ticks cause enormous losses to people and to their domestic animals, mainly because they serve as vectors of many vertebrate diseases. The ticks spend the major part of their lives hidden in humid niches on the surface or in the upper layer of the ground. *Boophilus annulatus* (Ixodida, Ixodidae, Rhipicephalinae) ticks are one of the most important cattle ticks in wide areas of the world, and their control is, as yet, based almost entirely on chemical acaricides.

Studies during the last decade have shown that EPNs are pathogenic to ticks (Samish and Glazer, 1992, 2001; Mauleon et al., 1993; Zhioua et al., 1995; Hill, 1998; Samish et al., 2000b). Sixteen ixodid tick species from six genera and three argasid species from two genera were tested, and only one of these species seemed not to be susceptible to these nematodes (Samish and Glazer, 2001). Similarly to insects, wide differences in susceptibility to nematodes were recorded among engorged females of the various tick species in Petri-dish assays (Samish and Glazer, 2001). Fully engorged argasid and ixodid female ticks were generally very sensitive to EPN infection. Furthermore, the 42 nematode strains from various steinernematid and heterorhabditid species that were tested in the laboratory for their anti-tick activity varied in their virulence (Samish and Glazer, 2001). Heterorhabditids were generally more virulent to ticks than steinernematids (Hassanain et al., 1997; Kaaya et al., 1999; Glazer et al., 2001), and strains that were highly virulent to one tick species were mostly found to be highly virulent to other tick species also (Hassanain et al., 1997; Kaaya et al., 1999; Samish and Glazer, 2001).

In contrast to laboratory studies, it was found that under field conditions steinernematid strains, particularly the Mexican and DT strains of *Steinernema carpocapsae*, were more effective against ticks than the heterorhabditid strains (Samish et al., 1999), which suggests that the simple Petri dish bioassays may not be reliable as predictors of the actual efficacy of EPNs in the field.

Entomopathogenic nematodes' behavior and efficacy are highly affected by environmental factors (Kaya, 1990; Lewis, 2002). The soil is the natural habitat of EPNs, and it has been shown that in the soil environment some nematode species (e.g., most *S. carpocapsae* strains) search for their host near to the soil surface (Moyle and Kaya, 1981; Kaya and Gaugler, 1993) adopting an 'ambush' searching approach, whereas others (e.g., most *Heterorhabditis bacteriophora* strains) have adopted a 'cruiser' searching approach, deeper in the soil (Choo et al., 1989; Kaya and Gaugler, 1993; Lewis, 2002). Such behavior cannot be examined in Petri dish infectivity bioassays but, nevertheless, soil texture, chemistry and moisture have profound effects upon the activity and persistence of EPNs (Kaya, 1990), therefore consideration of the effects of soil factors on nematode activity is a pre-requisite for the sucessful use of EPNs against target pests.

In the present study we investigated the effects of soil type and characteristics on nematode behavior and effectiveness against ticks. For this purpose we tested nematode strains from both Steinernema and Heterorhabditid genera that had shown the highest virulence against ticks in previous laboratory tests. Engorged females of one host tick species, *B. annulatus*, served as the target organisms.

Materials and methods

Ticks

Boophilus annulatus ticks were collected in 1994 from cattle in Israel and subsequently were fed every 2 months on Friesian calves. The off-host stages were incubated in the dark at 26 °C, 80% RH. The engorged female ticks were tested for nematode susceptibility within 24 h after repletion.

Nematodes

The following nematode species were tested: *Heterorhabditis bacteriophora* HP88, *Heterorhabditis* sp. IS-5 and IS-12, *Steinernema*

carpocapsae Mexican, S-20 and DT, *S. feltiae* SF, and *S. riobrave* TX. The origins of the nematode strains were presented previously (Glazer et al., 2001). The nematodes were reared at 25 °C in the last instar of the wax moth *Galleria mellonella* (L.), according to Kaya and Stock (1997). After 7–14 days of storage at 8 °C, they were left to acclimatize for 24 h at 21–23 °C.

Bioassay system

The experimental arena in the present study consisted of 10-l cylindrical plastic buckets (400 cm^2 soil surface and 21 cm deep), two-thirds filled with soil. Unless indicated otherwise sandy loam soil (Table 1) was used. The soil was oven dried (70 °C for 24 h) and then moistened to 13% (w/v) water content (i.e., 46% of field capacity in sandy loam). Stones and a layer of eucalyptus leaves were placed on the soil to create a habitat similar to natural conditions in the field. Infective juveniles (200 IJs cm^{-2}) were applied to the soil surface in 40 ml of water per bucket. One to two hours after the nematode application, 20 engorged *B. annulatus* females were placed on the soil surface of each bucket. The buckets were then covered with plastic bags to reduce evaporation, and were maintained in a climate-controlled greenhouse at 26 ± 2 °C and 80 ± 3% RH, under natural illumination. Tick mortality (indicated by absence of movement of legs when they were stretched artificially) was recorded. All treatments consisted of four replicates (buckets) and each experiment was repeated three times.

The distribution of the various nematode strains in the soil profile was determined 3 days after their application by using a plastic cylinder (2.5 cm in diameter) to take vertical cores from the soil. Three soil samples were taken from each bucket. The column of soil in the cylinder was slowly pushed out and cut into slices 1 cm thick, i.e., about 5 cm^3 in volume. Slices from depths of 0–1, 1–2, 2–3 and 5–6 cm were placed on a 60-mesh screen mounted above a 1-cm layer of water in 9-cm-diameter Petri dishes. After 24 h of incubation in room conditions the number of nematodes in the water was determined.

In order to determine the effect of soil texure on nematode distribution and efficacy, the above described buckets were filled with different soils which are listed in Table 1. Soils were chosen according to the most common soils on which animals are grazing in Israel and the marine sand added for comparison. The soils were dug, dried (70 °C for 24 h), gently mixed and transferred to the buckets. Under this treatment it is expected that the natural soil aggregates were not

Table 1. Types of soils studied, their sources and compositions

Soil type	Location	Texture	Code name	Sand (%)	Silt (%)	Clay* (%)	Calcium carbonate (%)	pH	Field capacity (%)
Sandy loam (C-Horizon Rhodoxeralf)	Bet Dagan	Sandy loam	SL	88	0	11	1.4	8.4	28
Marine sand	Beach	Sand	MS	90	0	9	5.5	8.3	28
Calcareous sandstone	Nes Ziona	Sandy loam	CSS	83	5	11	26.8	8.3	28
Haploxeroll soil (Mollichorizon)	Shalabim	Clay	HS	23	27	49	15.9	7.6	72
Haplargid soil	Bet Nir	Clay	HSO	19	27	53	18.2	7.8	68
Calcareous xerochrep	Tel Goded	Sandy clay loam	CX	45	23	31	27.7	6.8	70

*The dominant clay (>85%) in all tested soils is Montmorillonite.

disturbed. Nematode distribution and efficacy against engorged *B. annulatus* females was determined as described above.

Statistical analysis

Data on the virulence were analyzed with the General Linear Model procedure and Tukey's multiple range test (Anonymous, 1996). Mortality data expressed as percentages were arcsine square root transformed, and analyzed by means of a contingency table and the χ^2 test. All comparisons were made at a significance level of $p = 0.05$.

Results

The distribution of nematodes with depth in the various sandy loam soils varied considerably among the various nematode strains (Figure 1a). For all strains very few nematodes were found as deep as 6 cm. Among the steinernematids, most IJs were found at 1–2 cm. The majority (>80%) of the IJs of the *S. carpocapsae* Mexican and DT strains and of the *S. riobrave* TX strain were found in the upper soil layer, whereas appreciable numbers of *S. feltiae* and *S. carpocapsae* S-20 IJs were also found in the 1–2 and 2–3 cm layers (Figure 1a). Of the three heterorhabditid strains tested, small numbers (<3) of the HP88 strain of *H. bacteriophora* and the IS-12 strain of *Heterorhabditis* sp. were recovered from each depth. IJs of the IS-5 strain of *Heterorhabditis* sp. were found at similar concentrations to those of steinernematids. More IJs of this strain than of all the other strains tested were found at the depth of 6 cm (Figure 1a).

The highest (>85%) mortality of engorged females of *B. annulatus* was achieved with the heterorhabditid strains IS-12 and IS-5, and somewhat lower but still high mortality was observd with the *H. bacteriophora* HP88 and *S. riobrave* TX strains (Figure 1b). Under the same conditions the mortality caused by all the other tested steinernematid strains was about 50% or lower (Figure 1b).

The population and activity of IJs of *S. carpocapsae* DT in the upper soil layer (1 cm depth) were strongly affected by the soil type. The largest number of IJs was recorded in pots containing sandy loam soil (Figure 2); 50–70% fewer nematodes were found in the lighter – marine sand and calcareous sandstone – soils; and very few nematodes were recoved from the heavy soils, Haploxeroll and Haplargid (Figure 2, Table 1). Almost 100% mortality of engorged *B. annulatus*

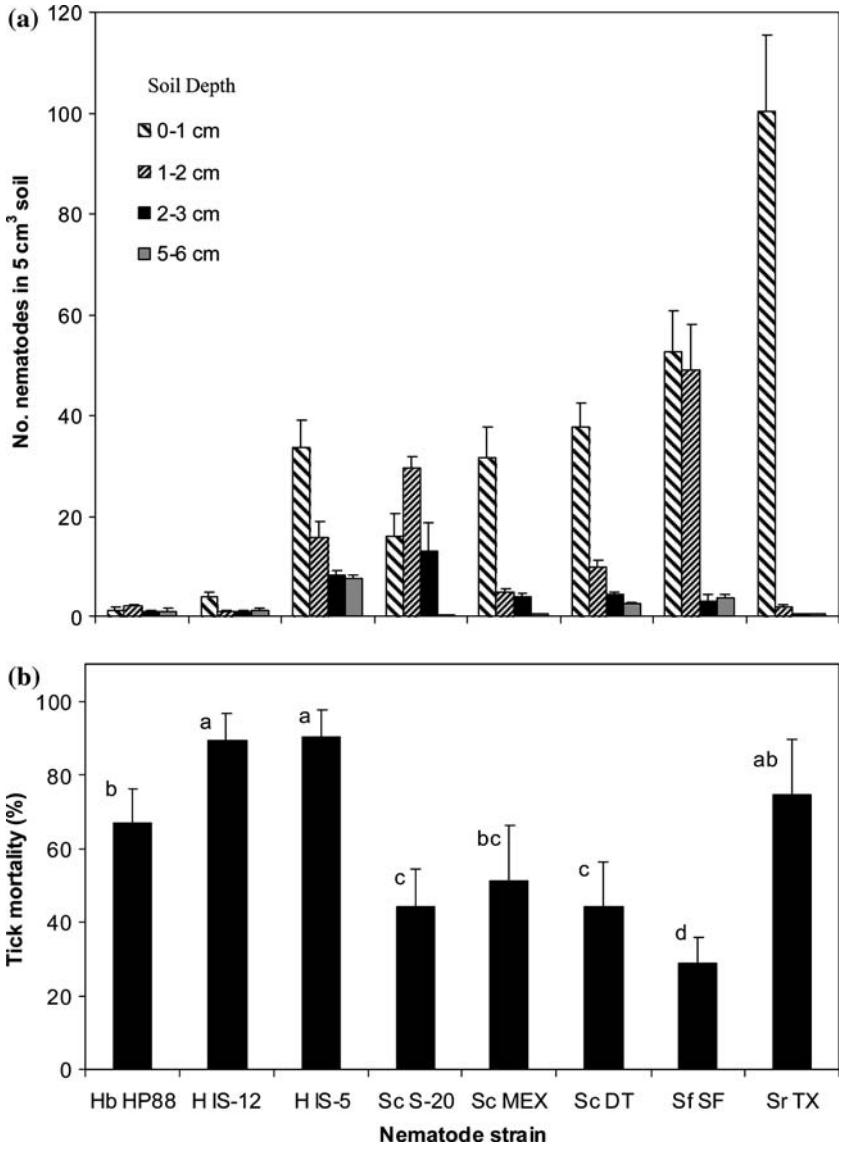

Figure 1. Distribution of the various nematode strains at various depths in sandy loam soil 3 days post application (mean ± S.E.M) (a) and their effects on mortality of engorged *Boophilus annulatus* female ticks (b). Nematode abriviations: Hb = *Heterorhabditis bacteriophora*; H = *Heterorhabditis* sp.; Sc = *Steinernema carpocapsae*; Sf = *S. feltiae*; Sr = *S. riobrave*. Columns marked with the same letter are not significantly different at $p = 0.05$.

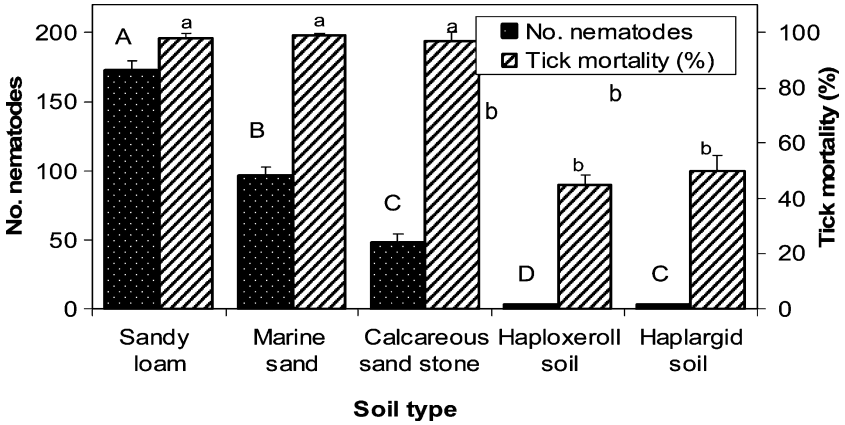

Figure 2. Effects of different soil types (Table 1) on the appearance (mean ± S.E.M) of nematodes (*Steinernema carpocapsae* DT) in the upper 1 cm of soil, in buckets covered with plastic bags. Data are expressed as the numbers of nematodes recovered from 5 cm^3 of soil, 3 days post application. Columns marked with the same letter are not significantly different at $p = 0.05$. The levels of mortality (mean ± S.E.M) of engorged *Boophilus annulatus* females 7 days after exposure are expressed in precentages.

females was recorded in pots containing sandy soil, marine sand and calcareous sandstone; whereas only 40–45% mortality was found in pots containing Haploxeroll or Haplargid soils (Figure 2).

In the upper layer of sandy loam soil nematode numbers were drastically reduced (by 70–80%) at a moisture content of 40–80%, compared with the numbers in soil moistened to full field capacity (Figure 3). Despite the reductions in nematode numbers, soil moisture of 40% and above was sufficient to ensure high efficacy (>85% mortality) of the IJs among engorged female ticks (Figure 3). Hardly any nematodes were recovered from the upper layer of very dry soil.

Discussion

Most of the tested entomopathogenic nematode strains displayed typical dispersal profiles in the soil according to their search behavior characteristics. The species *S. carpocapsae* and *S. riobrave*, which were categorized as 'ambushers' (Lewis, 2002), were found, as expected, in relatively high numbers on the soil surface (Figure 1a). Heterorhabditid strains that were characterized as 'cruisers' in their foraging behavior (Lewis, 2002) almost disappeared from the soil profile, even as deep as 6 cm. The IJs of *S. feltiae*, which are known to exhibit intermediate

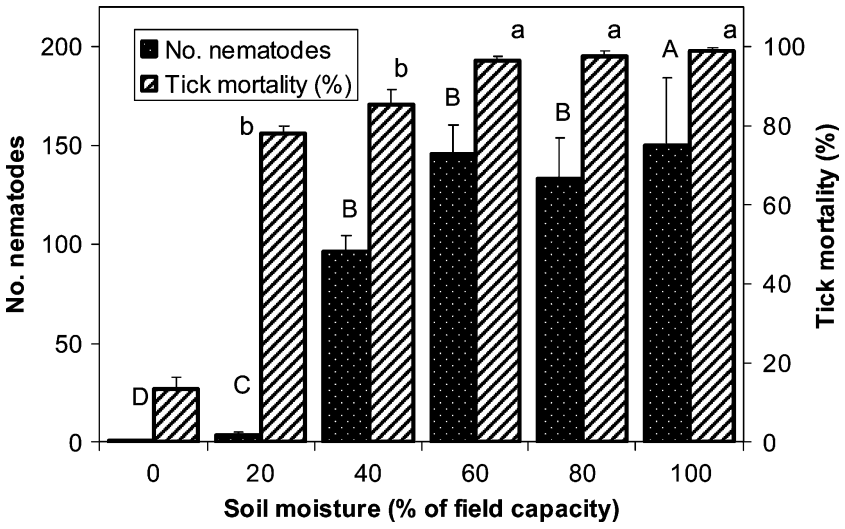

Figure 3. Effect of moisture level in sandy loam soil (expressed as percentage of field capacity) on the appearance (mean ± S.E.M) of nematodes (*Steinernema carpocapsae* DT) in the upper 1 cm of soil. Data are expressed as the number of nematodes recovered from 5 cm^3 of soil 3 days post application. Columns marked with the same letter are not significantly different at $p = 0.05$. The levels of mortality (mean ± S.E.M) of engorged *Boophilus annulatus* females 7 days after exposure are expressed as percentages.

behavior (Lewis, 2002), and the S-20 strain of *S. carpocapsae*, which is genetically selected for its attraction to CO_2 sources (Lewis, 2002), were found in larger numbers in deeper soil layers (Figure 1a).

Ticks typically live on the soil surface and prefer the humid environments of the upper soil layer, under stones or leaf litter; conditions that also favor entomopathogenic nematodes. Although one would expect the efficacy of nematode to be related to the number of IJs found on the surface, no such relationship was found in the present study. On the contrary, the IS-12 strain of the *Heterorhabditis* sp., which was recovered only in small numbers, had the highest killing efficacy, whereas most of the steinernematid strains tested persisted in relatively high abundance on the surface and exhibited a lower anti-tick efficacy (Figures 1 and 2). This can be explained either by assuming that once a host is placed on the surface the nematodes from the deeper soil layer emerge to infect it, or that, although nematodes were applied uniformly on the soil surface, some local population concentrations formed in protected micro-niches such as under stones or leaf litter, which also represent a favorable environment for the engorged female ticks. The differences in efficacy could be also attrib-

uted to differences in virulence between the various strains. It is noteworthy that the present efficacy data are consistent with the performance of the various strains in our laboratory assays (Samish et al., 2000a, b, c; Samish and Glazer, 2001), indicating that these laboratory tests may be effective for identification of nematode strains that are highly effective against particular tick species.

All nematodes are aquatic organisms and need to have a film of water enveloping their body in order to be able to move (Norton, 1978), therefore dry conditions impair nematode motility and survival. The present findings regarding the effects of soil texture and moisture strongly indicate that moisture affected the presence and activity of nematodes at the upper soil layer.

Soil texture

The small numbers of nematodes recovered from the upper layers of light soils can be attributed to the fact that water evaporation is more rapid in these soils than in sandy loam soil (Figure 2). The IJs either died because of the fast desiccation or migrated to deeper soil layers which retained sufficient humidity. The drastic decline in the nematode populations in the upper layer of the heavy soils (Haploxeroll and Haplargid soils) can probably be attributed to the inability of the IJs to readily penetrate into these soil types; they remained exposed to rapid desiccation. This decline also reduced nematode efficacy. Nevertheless, tick mortality was also high when soil moisture was low (Figure 2), and this can be explained by the above hypotheses, i.e., that nematodes are attracted to the host from deeper soil layers, or that protected niches contain higher concentrations of nematodes. Kung et al. (1990) also demonstrated that nematode movement is inhibited in soils with small particles, so that the sandy loam and marine sand soils in the present study appear to be media through which IJs move easily.

Soil moisture

The present results show that tick mortality remained high even when the number of nematodes found in the upper layer was much diminished as a result of reduced moisture content (Figure 3). These findings indicate that the smaller number of nematodes which remained on the surface was enough to infect and kill the ticks. It could also be that the presence of the target tick attracted the nematodes from deeper

layers (see review by Lewis, 2002). When soil mositure remains constant in different texture it is most likely that water film and availbility to nematode will vary among the different soils. The influence of water availability will be studied in a future work.

The results obtained here strongly imply that environmental conditions must be carefully evaluated before EPNs can be effectively applied against ticks on the soil surface. Nevertheless, some nematode strains were highly effective even under conditions that were less favorable for their activity and persistence in the soil. Our data support the assumption that EPNs can be used as an effective biological control agent against ticks.

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