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Activity of entomogenous nematodes (
(Rhabditida: Steinernematidae and Heterorhabditidae)
in multi-species structures

SUMMARY

Laboratory studies were conducted on the movement activity of invasive larvae of entomogenous nematodes Steinernema feltiae and Heterorhabditis bacteriophora. The experiments were carried out using the larvae of both nematode species simultaneously (in competitive conditions) with the lack and presence of the host insects: Galleria mellonella, Tenebrio molitor and Tribolium confusum. The results point out that the presence of a host and a competitive species leads to enhanced activity of invasive larvae of entomogenous nematodes. A more competitive species turned out to be S. feltiae, whose invasive larvae reach and infect the host faster.

STRESZCZENIE

Key words: entomogenous nematodes, *Rhabditida*, activity, competition, migration, distribution.

INTRODUCTION

Entomogenous nematodes belonging to the families of *Steinernematidae* and *Heterorhabditidae* are obligatory pathogens of insects. In the development of nematodes one observes a free-living stage, called the invasive larva (6, 26). Invasive larvae of entomogenous nematodes live in the water cover of soil particles (15); they are capable of active movement (9, 10, 11, 20, 32) as well as seeking and attacking the host (15). The factors that affect the occurrence and activity of invasive larvae in the environment include humidity (21, 33), temperature (13, 25), soil structure (13, 22), oxygen availability, environmental pH (23), presence of antagonistic organisms such as predators, pathogenic organisms or competitors, and the presence of potential hosts.

Entomogenous nematodes created two strategies of host finding, namely an ambush ("sitting and waiting for the host") and a cruise (active search for the host) (4). Both *Steinernema feltiae* and *Heterorhabditis bacteriophora* are active in picking up the host (24). As shown by Kreft’s experiment (19, 20) conducted in the conditions of simultaneous occurrence of three insect species and one species of entomogenous nematodes simultaneously, *Steinernema feltiae* and *Heterorhabditis bacteriophora* have the ability to localize the host in the environment choosing the more attractive insect.

Entomogenous nematodes are fairly common in the natural environment. They are also used in biological control of pests. Therefore, it seems highly significant to explain the interaction between the species of those organisms. The purpose of the study was to demonstrate the way in which the presence of a competitive species affects the activity of invasive larvae of *S. feltiae* and *H. bacteriophora*, the directions of migrations and the choice of a host.

MATERIALS AND METHODS

The experiment made use of invasive larvae of entomogenous nematodes of *Steinernema feltiae* Filipjev 1934 (*Nematoda: Steinernematidae*) (strain PLS81, isolated from forest soil in Bialowieża, 1981) and *Heterorhabditis bacteriophora* Poinar 1976 (*Nematoda: Heterorhabditidae*) (strain PLHb81, isolated from soil under grass, weed and trees near the Bysytzca river in Lublin, 1981), from a permanent laboratory culture. Since the moment of isolation the nematodes remained in continuous cultivation in the laboratory of Zoology and Ecology Department.

Before the experiment, the invasive larvae of nematodes were kept from one to three weeks at the temperature of 6–7°C, in water solution 0.001% of formaldehyde, the culture being aired at one-week’s intervals. Before the experiment was started the viability of nematode invasive larvae was checked under a microscope.

In the experiment the laboratory culture larvae of the final stage of development were used. They belonged to the following insect species: *Galleria mellonella* L. (*Lepidoptera: Pyralidae*), *Tenebrio molitor* L. (*Coleoptera: Tenebrionidae*) and *Tribolium confusum* Duv. (*Coleoptera: Tenebrionidae*).

The insects were selected according to weight criteria. The larval biomass of *T. confusum* ranged from 2.7 to 3.1 mg, *T. molitor* from 170 to 190 mg, while *Galleria mellonella* from 180 to 200 mg.
The experiment was carried out in glass crystallizers, 23 cm in diameter and 7 cm in height, filled with a 4.5 cm-deep layer of sterile earth, light silty medium loam sandy (36). The earth was roasted twice at 24-hours’ intervals, at the temperature of 200°C, for 12 hours; next, it was moistened with distilled water.

The experiment was performed in six repetitions. In order to establish the directions of migration and distribution of nematodes in the soil environment, where there is no potential host, 50 invasive larvae of each nematode species were introduced in the central place of the crystallizer. After 24, 48 and 72 hours the directions in which nematode larvae moved in the soil were determined according to the modified ambush method of Bedding and Akhurst (2). The earth in the crystallizers was divided into 7 fields, 6 of which were in the circumference of the crystallizer, and one in the centre (introduction field of nematodes). Soil samples from all the fields were taken and then placed on Petri dishes. Next, 5 larvae of *G. mellonella* were placed on each. After 5 days *G. mellonella* caterpillars were transferred onto Petri dishes, which were covered with tissue paper and dripped with 0.001% water solution of formaldehyde. Live and dead insects were placed in separate dishes. The infected insect larvae were selected in order to establish the number of the first generation of nematodes.

In each repetition 3 larvae of each, *G. mellonella*, *T. molitor* and *T. confusum* were used. The dose of nematodes was 100 invasive larvae per one insect: 50 invasive larvae of *S. feltiae* and 50 invasive larvae of *H. bacteriophora*. The experiment was conducted in three time variants differing in the period of the contact between nematodes and insects, which was 24, 48 and 72 hours.

The insects were placed in copper net cages with the dimensions of 1 × 1 × 3 cm, previously filled with earth. The larvae of each species were placed separately. Next, the cages with insects were uniformly placed in the soil with the circumference of the crystallizers, at the depth of 2 cm (Fig. 1).

![Fig. 1. Distribution of insect larvae in crystallizers](image)

Explanations: 1 — a field, where *T. confusum* (Tc) larvae were placed; 2 — an empty field; 3 — a field, where *G. mellonella* (Gm) larvae were placed; 4 — an empty field; 5 — a field, where *T. molitor* (T m.) larvae were placed; 6 — an empty field; 7 — a field in the centre of a crystallizer, where invasive larvae of nematodes, *H. bacteriophora* (Hb) and *S. feltiae* (Sf) were introduced.

Crystallizers were placed in a climatic chamber at the temperature of 23°C and relative humidity of the air 99.8% RH.

After 24 hours, both *S. feltiae* and *H. bacteriophora* were introduced in the central part of the crystallizers. Afterwards, insect larvae were removed from them, correspondingly after 24, 48 and
72 hours. The dead insect larvae were dissected in order to establish the number of generation I of the nematode population. Dissection of the insect larvae infected by *S. feltiae* was performed four days after the contact with insects, while in the case of insect larvae infected by *H. bacteriophora* it was after six days. Dispersion of the other invasive larvae of nematodes in the earth was determined according to the ambush method Bedding and Akhurst (2).

The statistical analysis of the results was performed with the use of Pearson’s $\chi^2$ test, with the hierarchical-logarithmic-linear methods. Calculations were made using the program SPSS/PC+ 4.0 at the Computer Centre of the Catholic University of Lublin.

RESULTS

Migration and distribution of invasive larvae, *S. feltiae* and *H. bacteriophora* in the soil with no host in the environment were accidental in nature. In the particular time variants nematodes gathered in other fields, and the differences in the distribution of invasive larvae are statistically significant (for *S. feltiae*: $\chi^2 = 131.18757$, DF = 12, level of significance = 0.000, for *H. bacteriophora*: $\chi^2 = 201.73975$, DF = 12, level of significance = 0.000) (Figs. 2, 3).

The migration rate of invasive larvae *S. feltiae* increased with the time they stayed in the soil. In all the time variants the majority of the recovered nematodes were found in the region of introduction of invasive larvae. In the 24-hours' variant, in the vicinity of the place where nematodes were introduced to the environment, the studies found out the presence of 89.9% of the recovered invasive larvae *S. feltiae*, in the 48-hours' variant — 86.8%, and in the 72-hours' variant — 61.2%.

Fig. 2. Percentage of *Steinernema feltiae* invasive larvae in particular fields in no-host conditions after simultaneous introduction of two nematode species, after 24, 48 and 72 hours of nematodes staying in the earth
The activity of invasive larvae *H. bacteriophora* in the absence of a host in the environment is considerably greater than of *S. feltiae*. The greatest activity of *H. bacteriophora* was found out in the 24-hours’ variant, while in the other time variants this activity decreased (Fig. 3).

Migration of invasive larvae *S. feltiae* and *H. bacteriophora* clearly changed after introducing insects into the soil and with the increase of the time of contact. In the 24-hours’ variant in the vicinity of the place where nematodes were introduced to the soil the studies found out 23.4% of the recovered *S. feltiae* and 3.5% *H. bacteriophora*, in the 48-hours’ variant — 5.1% *S. feltiae* and 2.2% *H. bacteriophora*, while in the 72-hours’ variant 1.9% *S. feltiae* with no *H. bacteriophora* (Tables 1, 2). Differences in the number of active nematodes in particular time variants are statistically significant (for *S. feltiae*: $\chi^2 = 163.896$, DF=2, level of significance = 0.000, for *H. bacteriophora*: $\chi^2 = 27.145$, DF=2, level of significance = 0.000).

Significant differences in the distribution of invasive larvae *S. feltiae* are visible depending on the presence or absence of a host in a given region ($\chi^2 = 5643.593$; DF=6; level of significance = 0.000) and on the period of nematodes’ contact with the host ($\chi^2 = 383.458$; DF=12; level of significance = 0.000) — Table 1.

A greater number of invasive larvae *S. feltiae* accumulated near the insects than in the regions with no potential hosts. This regularity was already observable in the 24-hours’ variant, but it grew after a longer contact. In those time variants more *S. feltiae* stayed in the vicinity of all the insect species than in the "empty fields" (Table 1). The number *S. feltiae* accumulating near the larvae of *T. molitor* and *T. confusum* grew in all the time variants, while in
Table 1. Recovered invasive larvae *Steinernema feltiae* in particular fields in the conditions of simultaneous presence of three hosts in the environment

<table>
<thead>
<tr>
<th>No. of field</th>
<th>Period of contact</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tribolium confusum</td>
<td>22</td>
<td>63</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>Empty</td>
<td>11</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Galleria mellonella</td>
<td>370</td>
<td>765</td>
<td>656</td>
</tr>
<tr>
<td>4</td>
<td>Empty</td>
<td>13</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Tenebrio molitor</td>
<td>130</td>
<td>348</td>
<td>436</td>
</tr>
<tr>
<td>6</td>
<td>Empty</td>
<td>24</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>Area of nematodes’ introduction</td>
<td>174</td>
<td>66</td>
<td>24</td>
</tr>
</tbody>
</table>

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | Area of nematodes’ introduction | 23.4% | 5.1% | 1.9% |

the vicinity of *G. mellonella* caterpillars it increased in the 24- and 48-hours’ variants, going down by 6.5% in the 72-hours’ variant. Differences in the number of nematodes *S. feltiae* recovered by means of an ambush method from the areas of particular insect species are statistically significant ($\chi^2 = 1439.873; \text{DF} = 2; \text{level of significance} = 0.000$).

During the experiment there was found no distinct accumulation of *H. bacteriophora* invasive larvae in the hosts’ vicinity, or any lowered activity of nematodes in the “empty fields” (Tables 2). In each time variant, the greatest number of *H. bacteriophora* there was found in the area of another host species, and these are statistically significant differences ($\chi^2 = 28.885; \text{DF} = 2; \text{level of significance} = 0.000$). In the 24-hours’ variant the greatest number of nematodes accumulated in the vicinity of *G. mellonella* (35.5% of the recovered nematodes), a smaller number near *T. confusum* (13.5%), and the smallest in the area of *T. molitor* (11.3%). In the 48-hours’ variant the highest number of *H. bacteriophora* was found near *T. molitor* (31.7%), a lower one in the area of *G. mellonella* and *T. confusum* (17.3% each). In the 72-hours’ variant, most nematodes (52.1%) accumulated near *T. confusum*, fewer in the neighbourhood of *T. molitor* (15.5%), and the fewest number in the area of *G. mellonella* (only 9.9% of the recovered nematodes) — Table 2.

The analysis of the migration of *H. bacteriophora* invasive larvae showed that in the presence of a competitor species they accumulate in the areas where a host less infected by the competitor was found or in the fields with no insects. Only
Table 2. Recovered invasive larvae *Heterorhabditis bacteriophora* in particular fields in the conditions of simultaneous presence of three hosts in the environment

<table>
<thead>
<tr>
<th>No. of field</th>
<th>Period of contact</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tribolium confusum</td>
<td>19%</td>
<td>24%</td>
<td>37%</td>
</tr>
<tr>
<td>2</td>
<td>Empty</td>
<td>29%</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>3</td>
<td>Galleria mellonella</td>
<td>50%</td>
<td>24%</td>
<td>7%</td>
</tr>
<tr>
<td>4</td>
<td>Empty</td>
<td>16%</td>
<td>22%</td>
<td>1%</td>
</tr>
<tr>
<td>5</td>
<td>Tenebrio molitor</td>
<td>11.3%</td>
<td>31.7%</td>
<td>15.5%</td>
</tr>
<tr>
<td>6</td>
<td>Empty</td>
<td>6%</td>
<td>13%</td>
<td>6%</td>
</tr>
<tr>
<td>7</td>
<td>Area of nematodes' introduction</td>
<td>5%</td>
<td>3%</td>
<td>0%</td>
</tr>
</tbody>
</table>

in the 24-hours' variant, *H. bacteriophora* accumulated most intensively in the neighbourhood of *G. mellonella* caterpillars (Table 2). The statistical analysis of the results showed that the differences which occurred in the distribution of *H. bacteriophora* invasive larvae in competitive conditions in particular time variants are statistically significant ($\chi^2 = 90.66369$; DF = 12; level of significance = 0.000).

Summing up the results it can be stated that in competitive conditions *S. feltiae* invasive larvae showed a much greater activity than *H. bacteriophora*. Together with a longer contact of nematodes with insects the number of *S. feltiae* accumulating in the neighbourhood of insects increased, while the number of *H. bacteriophora* increased after 48 hours, going down with time. *H. bacteriophora* nematodes showed a growing accumulation only in the area of the presence of *T. confusum* larvae also after 72 hours.

In all the time variants there was a significant difference between the number of invasive larvae *S. feltiae* recovered from the hosts’ areas and those from the “empty fields”. In the case of *H. bacteriophora* the number of nematodes recovered from the areas where insects occurred is frequently close or even lower than that from the “empty fields”.

**DISCUSSION**

Results of the studies show that introducing a host into the environment significantly affects the migration of entomogenous nematodes, which confirms...
earlier information (9, 10, 11, 14, 16, 27, 28, 32). When no insects are present in the environment the area of nematodes’ introduction was left by 17% of the recovered S. feltiae and 73% of the recovered H. bacteriophora on the average. On the other hand, when insects were introduced, the place where nematodes were introduced to the environment was left by 91.9% feltiae and 97.7% H. bacteriophora. When there are no insects in the environment, H. bacteriophora invasive larvae show a greater movement activity, while in the condition when a host is available S. feltiae move faster. Also Jaworska (14), who analyzed the migration of S. feltiae and H. bacteriophora in a horizontal direction, stated that the former reached the host earlier than H. bacteriophora.

After S. feltiae invasive larvae had been introduced to the earth, G. mellonella caterpillars lived 3.45 days on average, while after H. bacteriophora had been introduced they survived 4.90 days.

Recognition of a host by invasive larvae is important for further development of entomogenous nematodes. However, despite a lot of studies (1, 3, 7, 8, 12, 24, 29, 30, 31, 34, 35) the mechanism of recognizing and localizing the host by entomogenous nematodes has not been fully explained. Kreft’s research (19, 20) showed that invasive larvae H. bacteriophora and S. feltiae are able of recognizing and choosing an attractive host. In the presence of many insect species in the environment there are significant differences in the number of invasive larvae of nematodes clustering in the neighbourhood of particular insect species. In the conditions of no competitor in the environment both species of entomogenous nematodes accumulate most numerously in the vicinity of G. mellonella (on the average 60.1% S. feltiae and 65.9% H. bacteriophora), less numerously in the area of T. molitor (on the average 18.7% S. feltiae and 19.8% H. bacteriophora) and the least numerously in the neighbourhood of T. confusum (on the average 6.7% S. feltiae and 6.0% H. bacteriophora) (19, 20).

The studies presented here show that when nematodes S. feltiae and H. bacteriophora occur simultaneously the winning species is S. feltiae. In competitive conditions invasive larvae S. feltiae are faster to migrate towards the insects accumulating nearby, and they make the same choice of a host as when there are no nematodes of the competitive species H. bacteriophora (20). On the other hand, in the presence of S. feltiae, H. bacteriophora moves to less attractive areas with no host. It infects a small number of insects, mainly those of little attraction. The results of experiments suggest that S. feltiae is more sensitive to kaironomes excreted to the environment by the host than H. bacteriophora (Figs. 2, 3, Tables 1, 2).

In no-host conditions S. feltiae was much less active than H. bacteriophora. After introducing a host into the environment its activity increased rapidly and considerably exceeded the activity of H. bacteriophora. In the case of H. bacte-
riophora the increased activity in the presence of insects in the environment was smaller. *S. feltiae* invasive larvae, owing to their sensitivity to attractants, find the host in a faster and more efficient manner. Already in the shortest time variant, of 24 hours, more *S. feltiae* accumulated in the neighbourhood of each insect species than in no-host areas. On the other hand, *H. bacteriophora* occurred in greater numbers in the areas with no host than near *T. confusum* and *T. molitor*.

*S. feltiae* invasive larvae move faster than *H. bacteriophora* invasive larvae. In a shorter time more *S. feltiae* move to the distance of 12 cm from the introduction area near the insects than in the case of *H. bacteriophora*. The superiority of *S. feltiae* is then caused by a greater speed of movement and by more effective infection of the host than in the case of *H. bacteriophora*, and not — as suggested by Molyneux (25) — by the ability of Steinernematidae to survive in the environment for a longer period of time.

Choo et al. (5), Koppenhofer and Kaya (17, 18) investigated the activity of entomogenous nematodes in the conditions of the occurrence of invasive larvae of another species of entomogenous nematodes that differed in their strategy of seeking the host. The results suggest that the presence of invasive larvae of entomogenous nematodes with various strategies of finding the host does not significantly affect the change of their activity or the effectiveness of infection. Koppenhofer and Kaya (18) found out that in laboratory conditions nematodes of different strategies of picking up the host can coexist.

The results presented here can show that the use of two species of entomogenous species exposing the same strategy of picking up the host will increase the effectiveness of entomogenous nematodes in controlling the numbers of insects. A competitor’s presence increases the activity of invasive larvae, which is manifested in the speed of migration and host infection.

REFERENCES