Nictating Behavior and Infectivity of Entomogenous Nematodes, *Steinernema* spp., to the Larvae of Common Cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae), on the Soil Surface

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Behavior and infectivity of *Steinernema feltiae* (DD-136), *S. bibionis*, and *S. glaseri* to the last instar larvae of common cutworm, *Spodoptera litura*, on the soil surface were investigated under laboratory conditions. Upward movement of *S. feltiae* was enhanced by placing an insect on the soil surface, while the movement of other steinernematids was less enhanced. On the soil surface, *S. feltiae* actively nictated taking a waving and straight postures; *S. bibionis* less frequently nictated for a shorter time without taking a straight form; *S. glaseri* usually crawled on the surface and rarely nictated except when it traveled to neighboring soil particles. In the presence of a *S. litura* larva, *S. bibionis* and *S. glaseri* were more attracted by the feces than by the insect itself, though *S. feltiae* was strongly attracted by the insect. Feeding and defecating activities of the insect significantly declined with the inoculation with *S. feltiae* but not with *S. bibionis* or *S. glaseri.*

*Steinernema feltiae* (DD-136, Mexican), *S. bibionis*, and *S. glaseri* were infective to the larvae of common cutworm, *Spodoptera litura*, although their infectivity differed considerably among nematode species (Kondo and Ishibashi, 1984, 1986a). For application of these nematodes to soil insect pest control, their behavior in soil and infectivity on a target pest should be considered in detail. As is widely known, infectivity of steinernematids is affected by biotic and abiotic soil conditions. Actually, survival, nictating activity, and infectivity of *S. feltiae* (DD-136) on *S. litura* larvae are considerably affected by soil moisture (Kondo and Ishibashi, 1985).

The present experiment was conducted to investigate behavior and infectivity of three species of steinernematid nematodes to *S. litura* larvae on soil under laboratory conditions.

**MATERIALS AND METHODS**

*Nematodes.* *Steinernema feltiae* (DD-136), *S. bibionis*, and *S. glaseri* were propagated at 25°C on a chicken liver medium (Kondo et al., 1985) and used within a day after harvest from 30–40 day old culture.

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Insect. The last (6th) instar larvae of common cutworm, Spodoptera litura, were used within a day after the final molt.

Inoculation method. About 1,000 infective juveniles (hereafter called J_{III}S) of steinernematids suspended in 0.4 ml of 0.1% formalin solution (hereafter called formalin solution) were inoculated onto a filter paper (5.5 cm in diam., Toyo #) in a glass petri dish (6 cm in inner diam.). Immediately after the inoculation, 10 g oven-sterilized soil (the same one used in a previous study, Kondo and Ishibashi, 1985) was added. Soil depth thus prepared was 3.3 mm on average. Then, formalin solution was added to adjust the soil moisture to 35% on a weight basis, the most favorable soil moisture for both movement and infection of S. feltiae (DD-136) (Kondo and Ishibashi, 1985). A preliminary experiment showed that S. bibionis and S. glaseri also actively moved around in this water content. To maintain a constant moisture, the petri dishes were encased in a square plastic box, tightly sealed, and incubated at a temperature of 25 ± 2°C. To attract and encourage invasion of nematodes to the insect, a cutworm was placed on the soil surface. For control an insect was placed on a non-inoculated soil.

Mobility and behavior. Upward movement of nematodes in soil was investigated in the presence or absence of a S. litura larva without an artificial insect diet placed on the soil. Twenty-four hours after the inoculation with ca. 1,000 J_{III}S, the number of nematodes on a petri dish lid, soil surface, and insect body, if present, were examined. Nematodes on the lid were washed off with 10 ml of formalin solution and counted. Those on the insect body surface were recovered by rinsing the insect with formalin solution for five minutes with an occasional shaking. These surface-washed insects were then dissected along a ventral line and placed on the surface of 1/2 diluted physiologically balanced
Behavior and Infectivity of *Steinernema* spp.

Fig. 3. Behavior of infective juveniles of *Steinernema* spp. on the soil surface. A and B: nictating juveniles of *S. feltiae* (DD-136) taking straight (A) and waving posture (B); C and D: nictating juveniles of *S. bibionis* taking waving posture, E and F: juveniles of *S. glaseri* crawling on the soil particles without nictating. Length of bars indicates 200 μm for all figures.

saline solution (NH₄H₂PO₄ 0.5 g, K₂HPO₄ 0.5 g, MgSO₄·7H₂O 0.2 g, NaCl 5.0 g, distilled water 2,000 ml) and nematodes swimming out were counted. Immediately after the removal of the insect, if present, behavior and number of nematodes on the soil surface were examined under a dissecting microscope at 15 to 30 magnification.

**Attraction by feces.** Two experiments were conducted to study the attraction of nematodes by the feces and body of *S. litura* larvae.

**Experiment 1.** A *S. litura* larva was exposed on the soil inoculated with ca. 1,000 J₁₅₈ of either *S. feltiae*, *S. bibionis*, or *S. glaseri* with an artificial diet for the insect. After 24 hr exposure, nematodes on the discharged feces and body of the larva were counted. For recovering nematodes on the feces mingled with soil particles, a simple assembly (see Fig. 1) was used; feces on the soil were transferred to an assembly containing about 10 ml of formalin solution and incubated at 25°C for 24 hr. Nematodes swimming out into a centrifugal tube were counted.

**Experiment 2.** The second experiment was conducted without placing an artificial diet on the soil. To lessen the defecation during 24 hr exposure, the newly molted last instar larva was allowed to starve and defecate for 24 hr. This starved larva was placed on the nematode-inoculated soil with eight fresh feces discharged by two-day-old healthy last instar larvae and eight willow wood pieces similar in shape and size to the feces. Twenty-four hours after the onset of exposure, nematodes on the wood pieces, feces, and the insect body were recovered and counted.

**Infectivity.** The last instar larva was exposed on the soil inoculated with ca. 1,000 J₁₅₈ of each species of steinernematids. The insect diet was placed on a piece of aluminum foil to keep it from the soil. At 24 hr intervals, the number of feces discharged and weight of diet consumed by an insect were measured for four days after the onset of exposure. The insects were allowed to feed on a diet until death or adult emergence.

**RESULTS**

**Mobility and nictating behavior**

Although all species of the three steinernematids tested was found to move upward, their mobility and behavior on the soil surface differed considerably. In the absence of a *S. litura* larva, numbers of nematodes on a petri dish lid and soil surface were significantly larger with *S. feltiae* than with *S. bibionis* or *S. glaseri* (Fig. 2). On the soil surface,
### Table 1. Attraction of Steinernema spp. by feces of Spodoptera litura larva under the presence of insect (Exp. 1)

<table>
<thead>
<tr>
<th>Nematodes inoculated</th>
<th>Number of nematodes recovered from</th>
<th>Rate of attraction by larval feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces (A)</td>
<td>Insect (B)</td>
</tr>
<tr>
<td>S. feltiae</td>
<td>3.4 a</td>
<td>266.9 a</td>
</tr>
<tr>
<td>S. bibionis</td>
<td>7.6 a</td>
<td>1.8 b</td>
</tr>
<tr>
<td>S. glaseri</td>
<td>16.8 a</td>
<td>1.0 b</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter do not significantly differ at 5\% level by DUNCAN's multiple range test. Each value is the mean of ten replicates.

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The Jlls of *S. feltiae* actively nictated taking a waving (Fig. 3B) or straight posture (Fig. 3A). The Jlls of *S. bibionis* nictated without taking a straight form (Figs. 3C–D); nictating time was usually less than 10 sec for *S. bibionis* and several minutes or longer for *S. feltiae*. The Jlls of *S. glaseri* were usually crawling on the soil surface and rarely nictated except when they traveled to neighboring soil particles (Figs. 3E–F).

An addition of a *S. litura* larva on the soil surface greatly altered mobility and behavior of *S. feltiae*; most Jlls were attracted by the host instead of moving to the petri dish lid or continuing nictate on the soil particles (Fig. 2). However, the addition of an insect had no significant effect on the mobility or behavior of *S. bibionis* and *S. glaseri*,
Behavior and Infectivity of Steinernema spp.

Table 2. Attraction of Steinernema spp. by wood piece or feces of S. litura larva under the presence of insect (Exp. 2)

<table>
<thead>
<tr>
<th>Nematodes inoculated</th>
<th>Number of nematodes recovered from</th>
<th>Rate of attraction by larval feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wood piece (A)</td>
<td>Feces (B)</td>
</tr>
<tr>
<td>S. feltiae</td>
<td>2.0 a</td>
<td>12.3 a</td>
</tr>
<tr>
<td>S. bibionis</td>
<td>0.8 a</td>
<td>26.9 a</td>
</tr>
<tr>
<td>S. glaseri</td>
<td>0.9 a</td>
<td>18.8 a</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter do not significantly differ at 5% level by Duncan's multiple range test. Each value is the mean of 15 replicates.

although the number of nematodes on each of the petri dish lid, soil surface, and insect body appeared slightly increased.

Attraction by feces

The Jttts of S. bibionis and S. glaseri were more attracted by the feces than by the body of S. litura (Tables 1 and 2), regardless of the addition of artificial diet to the nematode-inoculated soil.

In experiment 1, the insects inoculated with S. feltiae discharged fewer and smaller feces than did those with S. bibionis or S. glaseri. Insects inoculated with S. bibionis or S. glaseri discharged almost the same number of feces and of a similar size as did the non-inoculated control (Fig. 4). Under these conditions, the total number of nematodes recovered from the feces in four days was not significantly different among nematode species investigated. However, numbers of S. bibionis and S. glaseri recovered from the insect body were significantly fewer than those of S. feltiae which were more effectively attracted by the insect body than by the feces.

In the presence of eight fresh feces from two-day-old healthy S. litura larvae, attraction of steinernematid nematodes to the feces and body of the insect fluctuated (Table 2). The three species of steinernematids were recovered from the feces in a larger number in Exp. 2 than in Exp. 1. However, the number of S. feltiae recovered from the insect body decreased, while this was not the case with S. bibionis and S. glaseri. As indicated in the rate of attraction, S. feltiae was more effectively attracted by the insect body than S. bibionis or S. glaseri in Exp. 1. All three steinernematids used were more attracted by the feces than by the wood pieces of similar shape and size.

Infectivity

S. feltiae invaded the insect in a larger number than did S. bibionis or S. glaseri (Table 3). Consequently, the infectivity was the highest by S. feltiae, followed in order by S. bibionis and S. glaseri (Table 4). In fact, all S. litura larvae died within two days after the inoculation with ca. 1,000 Jttts of S. feltiae (Fig. 5A). The infectivity of S. bibionis was still higher (70%) than that of S. glaseri; about 1/3 of the insects died during their feeding period, and the other 2/3 died after development to spinning larvae, prepupae or pupae (Figs. 5B–E). Usually, the abdomen of a healthy pupa curved ventrally (Fig. 5F), however, that of an infected one was loosely stretched (Fig. 5E). Although S. glaseri moved more actively than S. bibionis (Fig. 1), its infectivity was not as high as the other two species (Table 4); no insect was killed during its larval stage.
Table 3. Attraction and invasion of *Steinernema* spp. to the *S. litura* larva

<table>
<thead>
<tr>
<th>Nematodes inoculated</th>
<th>Number of nematodes recovered per insect**</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Body surface</td>
</tr>
<tr>
<td><em>S. feliae</em></td>
<td>113.9 a</td>
<td>110.3 a</td>
</tr>
<tr>
<td><em>S. bibionis</em></td>
<td>1.2 b</td>
<td>0.5 b</td>
</tr>
<tr>
<td><em>S. glaseri</em></td>
<td>1.3 b</td>
<td>0.0 b</td>
</tr>
</tbody>
</table>

* Nematodes were recovered 24 hr after exposing the insect on the soil inoculated with ca. 1,000 infective juveniles per 10 g soil in a petri dish (6 cm in inner diam.).
* Means within columns followed by the same letter do not differ significantly at 5% level by Duncan’s multiple range test. Each value is the mean of ten replicates.

Table 4. Comparison of the infectivity of *Steinernema* spp. on the *S. litura* larva which was exposed on the soil inoculated with ca. 1,000 infective juveniles per petri dish

<table>
<thead>
<tr>
<th>Nematodes inoculated</th>
<th>Number of insect</th>
<th>Mortality of insect (%)</th>
<th>Developmental stages of dead insect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Used</td>
<td>Developed to adult</td>
<td>Feeding larvae</td>
</tr>
<tr>
<td><em>S. feliae</em></td>
<td>20</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td><em>S. bibionis</em></td>
<td>20</td>
<td>6</td>
<td>70.0</td>
</tr>
<tr>
<td><em>S. glaseri</em></td>
<td>20</td>
<td>19</td>
<td>5.0</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>20</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Fig. 5. Cadavers of nematode-infected *S. litura*. A: feeding last instar larva killed 24 hr after the inoculation, B: spinning larva, C: prepupa, D: prepupa with partially developed pupal cuticle, E: pupa with loosely stretched abdomen, F: non-inoculated healthy pupa with ventrally curved abdomen. Length of bars indicates 5 mm for all figures.

Feeding and defecating activities of *S. litura* larvae also differed depending on the nematode species inoculated. Larvae inoculated with *S. feliae* consumed a small amount of diet and defecation ceased two days after inoculation (Fig. 4). The amount of diet consumed and the number of feces discharged in four days by the non-inoculated control were nearly the same as those by the insects inoculated with *S. bibionis* or *S. glaseri*. 

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DISCUSSION

Exposure of a test insect to a nematode-inoculated moist filter paper has been adopted as a method of evaluating the infectivity of entomogenous nematodes. However, the nematode infectivity obtained by this method appeared generally high, because the nematodes readily came to the top of the thin filter paper and there frequently encountered insects by chance. Actually, the infectivity of the three species of steinernematids used in the present soil-inoculation experiment was lower than that obtained in a previous experiment in which the nematodes were inoculated on a moist filter paper (Kondo and Ishibashi, 1986a). Accordingly, even such a shallow soil depth as 3.3 mm might have obstructed the infection of insect larvae by nematodes with a lower mobility and/or of less attraction to the host insect.

To kill soil insect pests, entomogenous nematodes have to move through soil particles to invade it. Nematode mobility is known to be considerably affected by such physical factors as soil type (Georgis and Poinar, 1983) or moisture (Silverman et al., 1982; Kondo and Ishibashi, 1985). In the present experiment conducted under a suitable soil moisture and temperature for the nematode infection, the behavior and infection of the three steinernematid species on the larvae of the common cutworm, Spodoptera litura, differed considerably among nematode species.

The infective juveniles (J$_{118}$) of steinernematids tend to move upward from the inoculation site in soil (Moyle and Kaya, 1981; Georgis and Poinar, 1983). In the present experiment, Steinernema feltiae (DD-136), S. bibionis, and S. glaseri moved upward, though their mobility was different: the greatest mobility by S. feltiae, followed by S. glaseri and S. bibionis.

The behavior of these steinernematids on the soil surface was also different among species: S. feltiae actively nictated alternately taking waving and a straight posture; S. bibionis nictated for a shorter time without taking a straight form; S. glaseri usually crawled on the soil particles and rarely nictated except when it traveled to neighboring particles. Although the nictating behavior is considered to be associated with host finding (Poinar, 1979) and is actually positively related to the infectivity of DD-136 on S. litura larvae (Kondo and Ishibashi, 1985), direct evidence for the infection has not been obtained. To clarify the role of nictating behavior on the infection, further investigations require a neurophysiological and ethological approach.

Positive attraction by an insect host is important for effective nematode infection. Steinernematid nematodes are known to be attracted by such excretory products of insects as uric acid, xanthine, allantoin, ammonia, and arginine in feces (Schmidt and All, 1979). The attraction of feces appears to be purposive because the nematodes are expected to reach an insect host by ascending the gradient of stimuli emanated from the insect feces. However, once in the vicinity of the insect, the nematodes have to be attracted not by the feces but by the insect itself. Therefore, too strong an attraction by the feces may lessen the chance of infection of an insect host. In the present test of attraction of steinernematids by the feces and body of S. litura larvae, S. feltiae was more highly attracted by the body than by feces the insect had discharged, although its attraction to the insect was slightly disturbed by the presence of fresh feces. Contrarily, S. bibionis and S. glaseri were mostly attracted by the insect feces. In addition to the strong invasive ability of S. feltiae (Kondo and Ishibashi, 1986), its high mobility in soil and positive attraction to the body of S. litura are evident by its higher infectivity than
S. bibionis and S. glaseri.

In conclusion, of the three species of steinernematids used, S. feltiae (DD-136) was the most infectious to S. litura larva on soil over S. bibionis and S. glaseri. For the field application of these entomogenous nematodes, the effects of biotic and abiotic factors of soil on the nematode infectivity should be further taken into consideration. Infection effectiveness of the most infectious nematode, S. feltiae (DD-136) will be described in detail in our following paper (Kondo and Ishibashi, 1986 b).

REFERENCES


