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# Vertical Dispersal of *Steinernema scapterisci*[1]

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*Abstract:* When infective juveniles of *Steinernema scapterisci* Nguyen & Smart were released on the soil surface in the field and in the laboratory, some of them moved downward through the soil at least 10 cm in 5 days and infected and killed mole crickets. When released 2 cm below the soil surface, most of the juveniles moved into the upper 2 cm layer of soil, but some moved downward 10 cm. When placed at the center of a 16-cm soil column, infective juveniles moved in both directions with three times more moving downward than upward. Infective juveniles were more efficient in killing mole crickets in the field than in the laboratory.

*Key words:* entomogenous nematode, *Steinernema scapterisci,* Steinernematidae, vertical dispersal.

Dispersal of insect-parasitic nematodes is advantageous for both host seeking and survival. Vertical dispersal of most steinernematids and heterorhabditids depends on depth of placement. When juveniles are placed on or just under the soil surface, most remain near the surface, but when placed deeper, greater vertical dispersal occurs in both directions (1,3,7).

*Steinernema scapterisci* Nguyen & Smart (5) is pathogenic to mole crickets of the genus *Scapteriscus* (6). Mole crickets are soil dwellers that spend most of their lives in the top 20–25 cm of soil but forage just under or on the soil surface (2). Vertical dispersal of *S. scapterisci* should be considered when deciding how best to apply the nematode in the field for maximum mole cricket control. We conducted the experiments reported herein to determine the vertical dispersal of this species.

## Materials and Methods

*Steinernema scapterisci* were reared in mole crickets, *Scapteriscus* spp., or house crickets, *Acheta domesticus* L. The soil, consisting of 97.90% sand, 1.85% clay, and 0.25% silt, was autoclaved at 1 kg/cm² for 1 hour. In experiments in which perforated petri dishes and storage boxes were used, a needle heated in a flame was used to perforate the containers. The containers were soaked in water for a week after perforation, then scrubbed with a brush and rinsed thoroughly before use.

### *Vertical dispersal when placed on the soil surface*

*Laboratory experiment:* The purpose of this experiment was to determine if infective juveniles placed on the soil surface in a large container would move downward 6 cm. A 6-cm layer of soil was packed into a plastic storage box (31 × 23 × 10 cm) which had been perforated numerous times near the center in a 15-cm-d area. Ten thousand infective juveniles (56/cm²) in 4 ml water were distributed on the soil surface directly over the perforated area of the box. The top of a glass petri dish (15 cm d) was inverted, soil and five mole crickets were added, and the dish was placed underneath the perforated area of the box (Fig. 1A). The experiment, replicated four times, was checked daily for 5 days and any dead mole crickets were removed, washed in water, and placed individually in vials. Two days later the mole crickets were dissected and inspected for nematodes. Dispersal was determined by whether the mole cricket cadavers contained any life stage of *S. scapterisci.*

*Field experiment:* The purpose of this experiment was to determine if infective juvenile nematodes placed on the soil surface in the field would move downward. A petri dish, perforated numerous times through the top and bottom, was half-filled with soil and four mole crickets and some alfalfa seeds were added. The alfalfa seed would germinate and the seedlings would serve

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as a food source for the mole crickets. The four treatments, each replicated four times, were dishes buried in the field at depths of 4, 6, 8, and 10 cm in a 1-$m^2$ area. The soil removed from the holes was replaced around the dishes and packed firmly. Just before dark, 200,000 (20/$cm^2$) infective juveniles of *S. scapterisci* were sprayed (with a hand-operated household sprayer) on 1 $m^2$ of soil surface over the buried dishes. A total of eight controls, two at each treatment depth, were buried 2 m from the treatments. No nematodes were sprayed on the soil surface of the controls. After 5 days, the petri dishes were removed from the soil, and the dead mole crickets were dissected and examined for nematodes. Dispersal was determined by whether the mole cricket cadavers contained any life stage of *S. scapterisci.*

*Vertical dispersal when placed 2 cm below the soil surface*

*Six-centimeter test:* The purpose of this experiment was to determine if infective juveniles placed 2 cm below the soil surface would move downward 6 cm. The experimental unit was constructed of pipe rings (2 cm high) cut from polyvinyl chloride (PVC) pipe (8 cm d) and a plastic petri dish (100 × 15 mm). Three of these rings were taped together and then to the perforated lid of the petri dish (Fig. 1B). The unit was placed on the bottom of the petri dish which contained two pieces of filter paper (Whatman No. 2) moistened with 2 ml water. Water was added to provide moisture for the mole crickets and any nematodes that might migrate into the dishes. The rings were filled with soil (14% moisture content), which was packed firmly by hand, and two treatments, each replicated five times, were applied.

The two treatments were 1) 5,000 juveniles in 2 ml water distributed drop by drop over the soil surface and two mole crickets in each petri dish (crickets) and 2) 5,000 juveniles in 2 ml water distributed drop by drop over the soil surface and no mole crickets in the petri dish (no crickets). No food was supplied to the mole crickets.

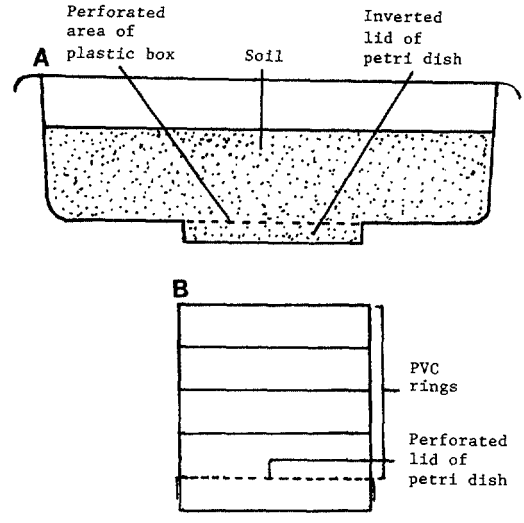


FIG. 1. Experimental units used in some of the tests. A) Plastic box with the bottom perforated in a 15-cm-d circle and packed with a 6-cm layer of soil. The box was placed with the perforated area over the inverted lid of a glass petri dish containing soil and five mole crickets. The unit was used to determine vertical dispersal of *Steinernema scapterisci* through 6 cm soil. B) Two-centimeter high rings of PVC pipe used in vertical dispersal tests. The rings were taped together and filled with soil. The lowermost ring was taped to a perforated top of a plastic petri dish. This unit was placed on the petri dish bottom to which mole crickets were added. The number of rings used depended upon the test performed.

Finally, an additional 2-cm-high ring was taped to the top of each unit and filled with soil to simulate placement of the nematodes 2 cm below the soil surface. The units were stored in the dark at 23 ± 2 C. The dishes were checked daily for 5 days, and any dead mole crickets were removed, washed by shaking them in a container of water, and placed individually in plastic vials; 2 days later they were dissected and examined for nematodes. The experiment was terminated after 5 days. At the end of the experiment the surviving mole crickets were placed individually, without food, in vials until they died; they were dissected and examined for nematodes 2 days later.

At the end of the experiment, the soil in each ring was removed and processed by a modified Baermann funnel for 24 hours to extract the nematodes. The numbers of nematodes collected at each depth of each nematode density were analyzed

TABLE 1. Mole cricket mortality at various soil depths from a surface application of 200,000/m² infective-stage juvenile *Steinernema scapterisci.*

| Depth† (cm) | Number dead/total crickets | |
|---|---|---|
| | Treatment | Control |
| 4 | 14/16 | 1/8 |
| 6 | 15/16 | 0/8 |
| 8 | 14/16 | 1/8 |
| 10 | 14/16 | 1/8 |

† Distance from the soil surface at which petri dishes containing mole crickets were buried.

by a *t*-test. The experiment was repeated once using 20,000 nematodes in the soil above.

*Ten-centimeter test:* The purpose of this experiment was to determine if infective juveniles placed 2 cm below the soil surface would move downward 10 cm. The experimental units were the same as used for the 6-cm test except that five 2-cm-high PVC rings were used. Treatments were 1) 10,000 infective juveniles in 2 ml water distributed on the soil surface and three mole crickets in the petri dish below and 2) no infective juveniles applied on the soil surface and three mole crickets in the petri dish below. The treatment with juveniles was replicated five times and the treatment without juveniles two times. The units were stored in the dark at 23 ± 2 C and examined daily. Any dead mole crickets were treated as for the 6-cm test. The experiment was terminated after 5 days. Downward movement was determined by whether mole cricket cadavers, when dissected, contained *S. scapterisci.*

*Vertical dispersal when placed in the center of a soil column*

The purpose of this experiment was to determine whether juveniles placed in the center of a vertical column of soil 16 cm long would move both up and down from the application site. Four 2-cm-high PVC rings were taped together and filled with soil. Ten thousand infective juveniles in 2 ml water were distributed on the soil surface. Another set of four rings was placed on top of this set, taped in place, and filled with soil. The treatment was replicated four times, and the experimental units were stored in the dark at 23 ± 2 C. After 5 days, the soil in each ring was removed and processed by a modified Baermann funnel to extract the nematodes. Differences in nematode recovery at the various depths were examined by ANOVA followed by Duncan's multiple-range test.

## RESULTS AND DISCUSSION

*Vertical dispersal when placed on the soil surface*

*Laboratory experiment:* After 5 days sufficient numbers of the juveniles had moved downward through the 6-cm soil layer to infect and kill some of the mole crickets in petri dishes below. Five of the twenty mole crickets were dead and 3 of those 5 contained developing *S. scapterisci* (developed beyond the infective stage).

*Field experiment:* Sufficient numbers of juveniles moved downward to infect and kill most of the mole crickets buried at each depth. At 4, 8, and 10 cm depths 14 of the 16 mole crickets died and all contained first generation adult *S. scapterisci.* At the 6 cm depth 15 of the 16 mole crickets died and 14 of them contained first generation adults (Table 1). None of three mole crickets that died in the controls contained *S. scapterisci.*

Because it takes 3 days at 24 C for the nematode to develop from third-stage infectives to first-generation adults (4), the third-stage infectives must have migrated the 10 cm and infected the mole crickets in 2 days (average soil temperature was 24.2 C during the experiment). This rate of movement may not occur naturally in the field, however, because to bury the petri dishes containing mole crickets involved removing the soil. When the soil was replaced, it was not possible to compact it as much as was the undisturbed soil. Therefore, movement of juveniles under our field conditions, as well as under laboratory conditions, may be greater than would occur in undisturbed soil.

Nonetheless, our data show that the juveniles moved downward through the soil and infected and killed mole crickets more

TABLE 2. Distribution of infective-stage juvenile *Steinernema scapterisci* in 2-cm layers of soil when 5,000 and 20,000 were applied 2 cm below the soil surface.

| | Percentage (total number) of nematodes recovered | | | |
|---|---|---|---|---|
| | 5,000 | | 20,000 | |
| Depth (cm)† | Crickets | No crickets | Crickets | No crickets |
| +0–2 | 92.6 (3,150) | 90.3 (2,070) | 81.6 (8,089) | 65.9 (7,890) |
| −0–2 | 4.6 (159) | 6.9 (160) | 13.6 (1,356)* | 23.8 (2,856) |
| −2–4 | 1.3 (47) | 1.8 (42) | 4.2 (420)* | 8.9 (1,068) |
| −4–6 | 1.2 (44) | 0.8 (20) | 0.4 (47)* | 1.3 (156) |

Average of five replicates.
* Significantly ($P \leq 0.05$) different from the corresponding treatment without crickets, according to a *t*-test.
† Distance nematodes traveled as measured in 2-cm increments; + = upward movement; − = downward movement. Broken line = release level.

efficiently in the field than in the laboratory. In the laboratory experiment, juveniles at a density of 56/cm² killed 15% of the mole crickets placed 6 cm below the release site, whereas in the field experiment with mole crickets placed at the same depth and a nematode density of 20/cm² (~ 3 times less), 88% of the mole crickets were killed.

*Vertical dispersal when placed 2 cm below the soil surface*

*Six-centimeter test:* In both the 5,000 and 20,000 nematode density trials, sufficient numbers of juveniles moved downward 6 cm to infect and kill mole crickets. At the 5,000 density level, two of the mole crickets in petri dishes died within 5 days. The eight survivors, placed in vials after the 5-day test period, died within 3 days. The cadavers of the two mole crickets that died within the 5-day test period and three of the five that died later contained developing *S. scapterisci.*

At the 20,000 density, six crickets died during the 5-day test period and the other four died 3 days later. Cadavers of the six that died within the test period and one of the four that died later contained developing *S. scapterisci.* No mole crickets died in the controls within the test period and none of those that died later contained the nematode.

When the 2-cm-deep soil layers were examined, the majority (65.9–92.6%) of the juveniles that were recovered had moved into the upper 2-cm layer of soil (Table 2). That is similar to the findings for *Steinernema* (= *Neoaplectana*) *carpocapsae* Weiser, *Heterorhabditis bacteriophora* Poinar, and *H. heliothidis* (Khan, Brooks & Hirshmann) Poinar (1,3,7).

Most of the *S. scapterisci* that moved downward (4.6–23.8%) were in the −0–2 cm layer immediately below the release site, but in all cases, some of the nematodes (0.4–1.3%) moved to the bottom soil layer. At the 5,000 density, the number of juveniles that moved into each soil layer was not significantly different whether or not mole crickets were present. This indicates that a host is not necessary for dispersal to occur.

At the 20,000 density, the number of juveniles that moved into the upper 2-cm layer of soil was not significantly different whether or not mole crickets were present, but below the application site, significantly greater numbers of juveniles were present in each soil layer when mole crickets were absent than when they were present. We cannot account for the difference in nematode numbers when mole crickets were absent, but the number of juveniles that moved into the petri dishes and entered the mole crickets is not known. Thus, it is possible that when mole crickets were present in the petri dishes, many of the juveniles traveled through the soil layers and entered the dishes.

*Ten-centimeter test:* After 5 days sufficient numbers of the juveniles moved downward the 10 cm to infect and kill mole crickets. Eight of the fifteen mole crickets were dead

TABLE 3. Recovery of infective-stage juvenile *Steinernema scapterisci* from each 2-cm layer of soil 5 days after release at the center of a soil column.

| Depth (cm)† | Percentage (number) of nematodes recovered |
|---|---|
| +6–8 | 2.4 (108) c |
| +4–6 | 1.5 (69) c |
| +2–4 | 3.3 (151) c |
| +0–2 | 17.0 (781) b |
| −0–2 | 32.2 (1,475) a |
| −2–4 | 18.4 (843) b |
| −4–6 | 5.4 (246) c |
| −6–8 | 19.7 (901) b |

Numbers with the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple-range test.
† + = upward movement; − = downward movement. Broken line = release level.

after 5 days and all eight of them contained developing *S. scapterisci.*

*Vertical dispersal when placed in the center of a soil column*

When placed at the center of a 16-cm soil column, the juveniles moved in both directions with 75.8% recovered below the release site and 24.2% above it (Table 3); 49.2% were within ± 2 cm of the release site. Of the total number recovered, significantly more (19.7%) were in the bottom layer of soil (−6–8 cm) than in the layer immediately above it (5.4%). This indicates that the nematode can move farther than 8 cm in 5 days.

Our data are similar to those of Moyle and Kaya (3) who found that when they placed juveniles of *S. carpocapsae* 2.5 cm below the surface, an average of 55% were recovered within 1 cm of the release site. When they placed them 5 cm deep, an average of 42.9% were recovered at the 4–5-cm layer, 56.4% below that layer, and only 0.8% above it. When they placed them 15 cm below the surface, 23% were recovered below that layer and 77% above it. For the species studied, most of the juveniles placed on or just below the soil surface remain near the surface, with a few moving deeper, but when placed 5 cm deep or deeper in the soil, greater dispersal occurs.

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