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Pathogenicity of *Steinernema scapterisci* to Selected Invertebrates[1]

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Abstract: *Steinernema scapterisci* was more pathogenic to insects tested in the order Orthoptera than to those in the orders Lepidoptera or Hymenoptera; it was not pathogenic to earthworms. The nematode also infected and killed the mole crickets *Scapteriscus acletus* and *S. vicinus* when released four successive times at 10-day intervals in containers of soil infested with the nematode.

Key words: biological control, entomopathogenic nematode, mole cricket, *Scapteriscus, Steinernema scapterisci.*

Three species of mole crickets, *Scapteriscus abbreviatus* (short-winged mole cricket), *S. acletus* (southern mole cricket), and *S. vicinus* (tawny mole cricket), were introduced accidentally into Florida from South America around the turn of the century (9). Currently, *S. vicinus* is present in the coastal plain regions of South Carolina through Alabama; *S. acletus* is present in the coastal plain regions of southern North Carolina through eastern Texas (8) and in Arizona (5); and *S. abbreviatus* is restricted to Puerto Rico (9) and to three coastal regions in Florida and one in Georgia.

The three species have become the most important insect pests of turf and pasture grasses in Florida (7). They also damage seedlings of agronomic and vegetable crops and ornamentals. Losses, including costs to control mole crickets in Florida, are about $45 million annually (4). Therefore, a mole cricket research project was established at the University of Florida in 1978 with emphasis on biological control. A search for natural enemies has extended to South America, which appears to be the native homeland for these mole crickets (8). Mole crickets infected with a steinernematid nematode, recently described as *Steinernema scapterisci* (6), were found in Brazil and Uruguay. In 1985, we brought several isolates of the nematode from Uruguay to the quarantine laboratory in Gainesville, Florida. We chose an isolate that, in initial pathogenicity tests in petri dishes, caused the highest mortality (38%) of *S. vicinus* and *S. acletus.* The virulence of the isolate was increased by serial passage (i.e., repeatedly exposing infective-stage juveniles collected from infected mole crickets to other mole crickets) through the two mole cricket species. These nematodes were used in all experiments.

The purpose of the research reported here was to determine the pathogenicity of *S. scapterisci* to a spectrum of insects and an earthworm and to determine whether uninfected mole crickets would become infected and die when released in containers of soil infested with *S. scapterisci.*

Materials and Methods

Pathogenicity tests: Test organisms were adults of Orthoptera: house cricket (*Acheta domesticus*), American cockroach (*Periplaneta americana*), field cricket (*Gryllus* sp.), and the mole crickets (*S. abbreviatus, S. acletus, and S. vicinus*); last-instar larvae of Lepidoptera: fall armyworm (*Spodoptera frugiperda*), granulate cutworm (*Feltia subterranea*), greater wax moth larva (*Galleria mellonella*), and velvet bean caterpillar (*Anticarsia gemmatalis*); adults of Hymenoptera: honeybee (*Apis mellifera*); and adults of Annelida: earthworm, *Allolobophora caliginosa.* The organisms (except the honeybee and earthworm) were tested in petri dishes (100 × 15 mm) containing two pieces of filter paper (Whatman #2, 90 mm d). Approximately 8,000 infective-stage ju-

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veniles of *S. scapterisci* in 2 ml water were added to each dish. Nematodes were used within 2 weeks after they were produced. Not all organisms tested nor the quantities desired were available at the same time. Therefore, the tests were not conducted simultaneously, and the number of test organisms per treatment and the number of replicates varied as follows: treatments with *Gryllus* sp. and *Feltia subterranea,* 10 specimens per replicate and 5 replicates; *S. acletus, S. vicinus,* and *S. frugiperda,* 5 specimens per replicate and 5 replicates; *G. mellonella* and *A. domesticus,* 10 specimens per replicate and 10 replicates; *A. gemmatalis,* 6 specimens per replicate and 5 replicates; and *S. abbreviatus,* 4 specimens per replicate and 3 replicates. Dishes were stored in the dark at 25 C. Numbers of dead organisms were counted after 3 days, except the earthworms which were examined after 5 and 10 days. Controls contained the test organism but no nematodes.

The honeybee test was conducted in cylindrical screen wire cages (8.5 cm d × 20 cm high) containing 20 adult honeybees of different ages. About 8,000 infective-stage juveniles in a water-saturated cotton ball were placed in a petri dish which served as the bottom of each cage. The saturated cotton was a source of water for the bees and assured that they came in contact with the nematodes when they landed on the cotton. The controls were prepared in the same way but without nematodes. All cages were placed in the dark at 25 C. The treatment was replicated five times. Dead honeybees were collected daily for 3 days.

Dead organisms were placed individually in vials (25 ml). Two days later, the cadavers were dissected; if they contained *S. scapterisci* which had developed beyond the third stage, they were considered to have been killed by the nematode.

Pathogenicity tests against earthworms were conducted in cups (237 ml) containing organic soil (composted oak leaves). Approximately 8,000 infective juveniles and 10 earthworms were added to each cup. The treatment was replicated five times. The controls contained earthworms but no nematodes.

Additionally, to determine whether the nematode can develop in severely damaged earthworms, five earthworms were cut in half and placed in cups ⅓ filled with organic soil. Eight thousand infective juveniles in 2 ml water were added to each cup, and the cups were shaken well to mix the nematodes with the soil. The treatment was replicated five times. After 5 days, the earthworm segments were collected, dissected in water, and examined for nematodes. Also, two earthworms were cut in half and placed in a petri dish (60 × 15 mm) containing organic soil and 8,000 infective juveniles. The treatment was replicated five times. The earthworm segments were dissected after 5 days and examined for nematodes.

Serial infection of mole crickets in soil: Scapteriscus vicinus was used in one experiment and *S. acletus* in another. In both experiments, containers (11.4-liter capacity) were filled with soil (97.9% sand, 1.8% clay, 0.3% silt) to within 5 cm of the top. There were four treatments and 14 replicates per treatment for *S. vicinus* and four treatments and 10 replicates per treatment for *S. acletus.* The four treatments were 1) one live nematode-infected mole cricket per container, 2) two live nematode-infected mole crickets per container, 3) 12,000 infective-stage juveniles sprayed on the soil surface per container, and 4) control with mole crickets but no nematodes. After the treatments were applied, 10 uninfected mole crickets were released in each container and lids were applied to prevent the mole crickets from escaping. After 10 days the live and dead mole crickets were counted, and the live mole crickets were removed from the containers. The dead crickets were dissected; and if the nematode was detected, they were assumed to have been killed by it and were returned immediately to the original containers. On the same day, 10 other uninfected mole crickets were released into each container. The process was repeated three times for a total of four

TABLE 1. Mortality of organisms exposed to *Steinernema scapterisci*.

	Treated			Control	
	Tested (n)	Died (n)	Mortality† (%)	Tested (n)	Died (n)
Orthoptera					
Acheta domesticus	100	100	100	100	4
Gryllus spp.	50	11	22	50	0
Scapteriscus abbreviatus	12	10	75	12	4
Scapteriscus acletus	25	25	100	25	3
Scapteriscus vicinus	25	25	100	25	2
Periplaneta americana	50	2	4	50	0
Lepidoptera					
Anticarsia gemmatalis	30	1	3	30	0
Feltia subterranea	50	5	10	50	0
Galleria mellonella	100	9	9	100	0
Spodoptera frugiperda	25	2	8	25	0
Hymenoptera					
Apis mellifera	100	16	10	100	7
Annelida					
Allolobophora caliginosa	50	0	0	50	3

† Percentage of mortality corrected by Abbott's formula (1) which is $(X - Y)/X \times 100$, where X = % insects alive in the controls; Y = % insects alive in the nematode treatment.

releases per container at 10-day intervals. The containers were held at 25 C ± 4 C.

RESULTS

Pathogenicity tests: Steinernema scapterisci exhibited a higher degree of pathogenicity to insects in the order Orthoptera (except for the cockroach) than to those in the orders Lepidoptera and Hymenoptera; it was not pathogenic to earthworms (Table 1).

In dead or injured earthworms, one adult male and four adult female nematodes were dissected from the earthworm segments in the first experiment and none in the second experiment. Thus, although a few infective juveniles developed to the adult stage in dead earthworms, they did not reproduce. In both experiments, however, large numbers of diplogasterids and rhabditids developed on the bacteria associated with the earthworm segments.

TABLE 2. Mole crickets *Scapteriscus vicinus* and *S. acletus* killed by *Steinernema scapterisci* in each of four releases.†

Source of inoculum	Average mortality 1	2	3	4
	S. vicinus			
1 infected cricket	3.7 b	5.3 b	3.2 ab	4.0 a
2 infected crickets	4.5 b	5.6 b	2.7 ab	4.1 a
12,000 juveniles‡	4.1 b	7.5 c	3.9 b	3.9 a
Control	1.1 a	1.7 a	1.7 a	3.3 a
	S. acletus			
1 infected cricket	1.8 ab	3.0 b	1.8 a	4.6 a
2 infected crickets	1.0 a	2.7 b	1.1 a	3.4 a
12,000 juveniles‡	2.5 b	5.1 c	1.8 a	2.6 a
Control	0.7 a	0.6 a	0.7 a	3.5 a

For each mole cricket species, numbers within a column followed by the same letter are not significantly different ($P = 0.05$) based on Duncan's multiple-range test.

† Ten uninfected mole crickets released into containers of soil at 10-day intervals.

‡ Infective-stage juveniles sprayed over the soil surface.

In the first experiment, mortality of *S. vicinus* in all nematode treatments was significantly different from the controls for the first two releases (Table 2). In the first release, there was no difference in mortality when nematode-infected mole crickets or nematodes sprayed on the soil surface were used as inoculum, but in the second release mortality was significantly greater when nematodes were sprayed on the soil surface. In the third release, only the treatment with nematodes sprayed on the soil surface was significantly different from the control, and in the fourth release, there were no significant differences among treatments.

Mortality of *S. acletus* by the nematode was less than that of *S. vicinus*. In the first release, only the treatment with nematodes sprayed on the soil surface was significantly different from the control. In the second release, all three treatments were significantly different from the control, with mortality greatest with nematodes sprayed on the soil surface. There were no significant differences among treatments in the third and fourth releases. In the fourth release of both mole cricket species, the percentage of mortality in the controls increased to much higher levels than at the other releases.

Discussion

Infectivity of Lepidoptera by *S. scapterisci* was much lower than expected, especially since the lepidopteran *Galleria mellonella* is used commonly as a host for in vivo culture of other *Steinernema* spp. This indicates that the biology of *S. scapterisci* differs from that of other species of *Steinernema*.

The live, intact earthworm, *Allolobophora caliginosa*, was not affected by *S. scapterisci*, and the nematode did not reproduce in damaged ones. In contrast, Capinera et al. (3) reported that *S. carpocapsae* reproduced in the damaged earthworm, *Aporrectodea* sp., although it did not affect undamaged ones. The low susceptibility of the lepidopterans and of *Apis mellifera* and the nonsusceptibility of *A. caliginosa* when exposed to rather high concentrations of *S. scapterisci* suggest that the nematode can be used to control mole crickets with little or no harm to the nontarget organisms tested.

In the experiment with serial infection of mole crickets, the reason for greater mortality at the second release of both *S. vicinus* and *S. acletus* when nematodes were sprayed on the soil surface than when infected mole crickets were used as inoculum is almost certainly the more even dispersal of nematodes. In the controls, mortality was higher in the fourth release than in previous releases. Whereas the cause of increased mortality in these tests is not known, experience in keeping mole crickets in containers of soil has shown that after prolonged storage many of them were killed by a fungus, *Sorosporella* sp. The fungus causes the mole cricket's body to turn brick-red (2), a symptom observed in these experiments. Also, we have observed that a mite, *Rhizoglyphus* sp., often is present externally on mole crickets. This mite, when placed in a petri dish containing infective juveniles of *S. scapterisci*, consumed large numbers of them. Possibly, the mites were present in the experimental containers and decreased the number of nematodes resulting in less infection and mortality of mole crickets. Thus, the fungus and the mite may have been contributing factors in mortality of mole crickets. Whatever the cause, the data show that lack of significant differences between treatments and controls in the fourth release was due to higher mortality in the controls and not to lower mortality in the treatments.

Spraying infective juveniles in an aqueous suspension onto the soil surface is effective when soil moisture and relative humidity are adequate for nematode survival and penetration into the soil or for host finding. Nematode-infected mole crickets also provide a good source of nematode inoculum because up to 80,000 (av. 50,000) infective-stage juveniles may be produced in each cadaver. This assures a continuing source of inoculum once the mole crickets have been infected.

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