Establishment and Persistence of Steinernema scapterisci (Rhabditida: Steinernematidae) in Field Populations of Scapteriscus spp. Mole Crickets (Orthoptera: Gryllotalpidae)¹

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ABSTRACT The first successful inoculative releases of an entomopathogenic nematode, Steinernema scapterisci Nguyen and Smart, for the control of exotic pests, *Scapteriscus* spp. mole crickets, were made at three pasture sites in Alachua County, Florida in 1985. Based on the evaluation of field-collected crickets, the nematode was established at all sites and persisted for over 5 years. Mean yearly percentage of infected crickets ranged from 0 to 21.4% for individual release sites. Mean adult infection level for all years combined, 10.9%, was significantly greater than that for nymphs (2.5%) and infection levels for Scapteriscus borellii Giglio-Tos, 12.7%, was significantly greater than that for Scapteriscus vicinus Scudder (4.5%) for all years combined. Although 24 h trap catch results indicate mole cricket populations were significantly reduced, the nematode's effect on pest abundance could not be adequately assessed because of the variation in trap catch results and inadequate knowledge about the relationship between trap catch and the mole cricket field populations being sampled. Despite inadequacies in estimating pest abundance, the results indicate S. scapterisci has potential as a biological control agent for pest mole crickets in the genus Scapteriscus.

KEY WORDS Steinernema scapterisci, Scapteriscus spp., entomopathogenic nematode, mole crickets, biological control.

Mole crickets of the genus *Scapteriscus* are the most damaging insect pests of turf and pasture grasses in the southeastern United States. Native to South America, they probably arrived in ballast at southeastern seaports around 1900 (Walker and Nickle 1981) and have spread throughout lowland areas from Texas to North Carolina. One recent estimate of annual losses due to mole crickets for Florida's turfgrass industry was \$44 million (Hudson and Short 1988).

In the early 1980s an effort to identify natural mortality agents of *Scapteriscus* mole crickets in South America was initiated by the Institute of Food and Agricultural Sciences at the University of Florida. By 1984 steinernematid nematodes were determined to cause mortality in field-collected

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mole crickets in South America (Lima Costa et al. 1984). An isolate from Uruguay, brought to the University of Florida in January 1985, proved to be fairly specific for *Scapteriscus* spp. mole crickets and caused almost 100% mortality of individuals exposed to the nematode in the laboratory (Nguyen and Smart 1991). The structure and behavior of this isolate were different from those of all other species of *Steinernema* and the nematode was described as a new species, *S. scapterisci* Nguyen and Smart (Nguyen and Smart 1990).

The first releases of S. scapterisci were made in the summer of 1985 (Hudson et al. 1988) and data were collected for more than five years to monitor the establishment and persistence of the nematode in the field. This paper presents the results of these releases.

Materials and Methods

Three bahiagrass (*Paspalum notatum* Fluegge) pastures in Alachua County, FL, were selected for the initial releases of *S. scapterisci*. The pastures were approximately 5 km north of LaCrosse, 4 km northeast of Grove Park, and 9 km north of Alachua. Two pitfall traps (modified from Lawrence 1982) were installed no closer than 100 m apart in each field. Pitfall traps consisted of a 19liter plastic bucket placed in the ground and four arms which consisted of 3 m sections of 7.6 cm diameter PCV pipe made into troughs by cutting lengthwise slits 2.5 cm wide. The arms were placed in the ground so that the slits were at ground level. One end of each arm was inserted through the side wall of the bucket and the other was plugged so that insects falling into the arm slits eventually fell into the bucket. A soil-containing pail within the trap bucket expedited the process of emptying the traps.

Nematodes were released at dusk on 13 June 1985 at Grove Park and on 14 June 1985 at LaCrosse by burying nematode-infected mole cricket cadavers 2-4 cm beneath the soil surface at one site (i.e., around one pitfall trap) in each field and by sprinkling an aqueous suspension of nematodes onto the soil surface at the other site in each field. The suspension was applied as a drench using handheld watering cans at approximately 200,000 infective stage juveniles per m². Cadavers which would produce, on average, 50,000 infective stage juvenile nematodes were buried four per m² (200,000 per m²). Fifty m² of pasture were treated around each pitfall trap. The sites in the Alachua pasture were treated on 15 October 1985 using the drench method only. Nematodes were applied as before, in a 50 m^2 area around each pitfall trap at the level of approximately 200,000 infective-stage juveniles per m². To increase the local population density of mole crickets at the sites and perhaps enhance the probability of successful nematode establishment, electronic callers (Walker 1982) broadcasting the mating call for the southern mole cricket, Scapteriscus borellii Giglio-Tos (= Scapteriscus acletus Rehn and Hebard) were operated at the drench release site on the night of 17 June 1985 at Lacrosse and on 18 June 1985 at Grove Park. Callers were operated at both the drench and cadaver release sites on 19 June at LaCrosse and on 20 June at Grove Park.

Pitfall traps were sampled by visiting the traps 2 d in succession. On the first day all contents of a trap were removed and fresh soil was placed in the trap. Mole crickets collected the second day were counted and those ≥ 1.3 cm in

length were returned to the laboratory where they were held individually in vials and examined daily for 7 d. Any dying within 7 d were held for an additional 14-18 d after death to determine whether they were infected with the nematode. Individuals of the tawny mole cricket, *S. vicinus* Scudder, a native species of mole cricket, *Neocurtilla hexadactyla* (Perty), and *S. borellii* were collected for evaluation.

Sampling at all three sites began 30 May 1985. During the pretreatment period (14 and 15 d for the Grove Park and LaCrosse sites, respectively, and 138 d for the Alachua site) trap catches were made five times at the Grove Park and LaCrosse sites and 26 times at the Alachua site. Thereafter, collections were made once a week until September of 1990.

A bahiagrass pasture approximately 1.6 km west of the Grove Park release site was used as a control field. Two pitfall traps were installed and serviced at this site in the same manner as the release sites. Collections at the control site began 30 May 1985.

Means of percent infection levels were derived from data collected throughout the study beginning at the time of release for each site. Means of 24 h pitfall trap catches were derived from data collected during the pretreatment period (30 May to 15 June) for the Grove Park and LaCrosse sites and for the pretreatment period (30 May to 15 October) for the Alachua and control sites. These data were compared with those obtained during the same time periods of each year for the respective sites. Infection and 24 h pitfall trap catch data were subjected to analysis of variance using the SAS general linear model (GLM) procedure (SAS Institute 1985). To stabilize the variance percent infection data were transformed by arcsine square root of proportion data and 24 h trap catch data were transformed by square root of count data + 0.5 before analysis.

Results and Discussion

Steinernema scapterisci was established at all release sites as determined by the collection of nematode-infected crickets in pitfall traps within one week after release. Methods of application appeared to establish the nematode equally well as there was no significant difference in infection levels for the drench and cadaver methods at the Grove Park (F = 2.91; df = 1, 46; P < 0.05) and LaCrosse (F = 0.85; df = 1, 70; P < 0.1) sites for collections made the first year after release. Establishment at all sites indicates electronic mole cricket callers, used to increase potential host population levels immediately after nematode release at two of the sites, were not necessary to establish the nematode.

Percent infection levels for *Scapteriscus* spp. collected from 24 h pitfall trap catches are presented in Table 1. Infection levels fluctuated yearly with greatest mean levels of infection at the Alachua and LaCrosse sites occurring two years after the nematode was released. No infected mole crickets were found in 24 h pitfall trap catches at the Grove Park site during 1987, 1989, and 1990; however, infected mole crickets were trapped in the pitfall traps at this site during these years in collections other than the 24 h trap catches. Infected individuals were collected from the control site in the spring of 1988 and in 1990 (Table 1) indicating that *S. scapterisci* may have been dispersed from the nearest release site, Grove Park, to the control site by infected mole crickets before they sickened and died. Numerous nematode-infected mole crickets have been caught in mole cricket sound traps (Parkman and Frank 1992) proving that infected individuals can disperse *S. scapterisci* through flight. Only flying mole crickets are caught in sound traps (Walker 1982).

The number of trap catches used to determine means of infection levels (Table 1) and 24 h trap catches (Fig. 1) were not similar from year to year for each site. Moderate to heavy rainfall often flooded traps at all sites, preventing collection of specimens and catch data. Also, trap catches containing only small nymphs (<1.3 cm in length) were not used to determine infection levels. Nymphs of this approximate size, those with a pronotal length <4 mm, were found less susceptible to infection by *S. scapterisci* than larger nymphs (Hudson and Nguyen 1989).

Scapteriscus spp. nymphs caught in pitfall traps exhibited lower infection levels than did adults (Table 2). Mean percent infection for the nymphs (\pm SE) of both species combined collected during the entire study, 2.5 \pm 0.8, was significantly less than that for adults combined, 10.9 \pm 1.3 (F = 24.81; df = 1, 594; P <0.001). This concurs with the findings of Hudson and Nguyen (1989) who reported that *S. vicinus* nymphs are less susceptible to infection and mortality by *S. scapterisci* than are adults.

Scapteriscus borellii and S. vicinus were equally susceptible to infection by S. scapterisci in the laboratory (Hudson et al. 1988). However, a significantly greater percentage of S. borellii (12.7 ± 1.7) than of S. vincinus (4.5 ± 0.9) captured during the study were infected with the nematode (F = 20.97; df = 1, 639; P < 0.001). Similar results were found for adult S. borellii and S. vicinus captured in sound traps (Parkman and Frank 1992) where a disparity in the flight behavior of the two species was believed to have resulted in greater levels of infection for S. borellii. The two species also differ in their feeding behavior. S. borellii is primarily predacious and, therefore, more active above and below the soil surface than S. vicinus which is mainly herbivorous (Matheny 1981). The predacious activity of S. borellii may increase the probability that individuals will contact infective-stage nematodes.

Mean number of mole crickets captured in 24 h during 1986 and subsequent years was significantly less than that for 1985 at all three release sites (Alachua: F = 74.17; df = 1, 82; P < 0.001; Grove Park: F = 17.37; df = 1, 14; P < 0.001; LaCrosse: F = 5.45; df = 1, 14; P < 0.05). Trap catch results for each *Scapteriscus* species at each site are presented in Fig. 1.

Although trap catch means at the release sites remained below those for 1986 for the duration of the study, these reductions in trap catches do not necessarily reflect similar reductions in field population numbers. The number of mole crickets collected in a pitfall trap may be affected as much by mole cricket activity (which, in turn, is affected by environmental conditions, e. g., temperature, relative humidity, soil moisture) as it is by population density in the vicinity of the trap. This often results in extreme variability in trap catch results as seen in the standard errors for mean infection levels and 24 h trap catch means. Therefore, the relationship between pitfall trap catch and the population being sampled is unclear. Some data (Hudson 1985) suggest that the relationship may be exponential: numbers caught increase or decrease at a rate

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	Alachua	Grove	Park	LaCrosse	Con	crol
1985	$7.2 \pm 2.6 (26)$	11.9±	3.5 (48)	7.1 ± 2.0 (72)	0.0	(3)
1986	$7.1 \pm 2.5 (58)$	$10.6\pm$	4.8 (36)	$1.9 \pm 1.0 (58)$	0.0	(4)
1987	$20.3 \pm 7.4 \ (23)$	0.0	(1)	21.4 ± 14.9 (7)	0.0	(2)
1988	$11.8 \pm 4.0 \ (36)$	10.0 ± 1	0.0 (5)	$9.5 \pm 5.6 (21)$	6.3 ±	6.0 (10)
1989	$5.0 \pm 3.7 \ (30)$	0.0	(12)	$4.2 \pm 4.2 (24)$	0.0	(15)
1990	$10.1 \pm 4.8 (13)$	0.0	(17)	$3.3 \pm 3.3 (10)$	60.0 ± 2^{4}	1.5 (5)
* (n) denotes number	of trap catches used to determine the	mean.				

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Fig.1. Mean 24 h pitfall trap catch (+ SE) of Scapteriscus spp. mole crickets collected from pastures treated with S. scapterisci and a control pasture, Alachua Co., FL. No standard error indicates mean of 0.0.

greater than than of the population being sampled. At best, the trap catches may provide data on long-term trends in population densities. These trap catch results suggest mole cricket populations were reduced after the nematode releases and were maintained at levels below those which occurred before the releases.

In their preliminary report of these releases Hudson et al. (1988) found mortality of collected mole crickets due to pathogens was quite low indicating these mortality factors had a negligible effect upon mole cricket abundance. A complex of native generalist predators is believed responsible for the annual decline in the number of mole cricket nymphs (Hudson et al. 1988), however, the role of these beneficial species in the apparent long-term reduction of mole crickets at the release sites is unknown.

Mean number (\pm SE) of *Scapteriscus* spp. trapped at the control site did not differ statistically for 1985 (8.2 \pm 2.3) ad 1986 (9.8 \pm 2.5) but did decrease significantly for 1987 and successive years (F = 14.95; df = 5, 206; P < 0.001) (Fig. 1). Reductions in trap catches at the control site in 1987 and successive years may be due in part to a persistent fire ant, *Solenopsis invicta* (Buren), infestation at that site. Fire ants can alter trap catch results by foraging within pitfall traps and removing trapped crickets before the trap contents can be censused and collected. Also, nematode-infected crickets were collected at the control site in the summer of 1988 (Table 1) and since that time *S. scapterisc*i may have affected mole cricket abundance at this site.

LaCrosse was the only site where individuals of *N. hexadactyla* were collected. None of the 187 *N. hexadactyla* collected from pitfall traps was infected with *S. scapterisci*. Although fluctuating during the study, 24 h pitfall trap catches of *N. hexadactyla* did not decrease significantly from the time of nematode release. Mean number of *N. hexadactyla* (\pm SE) caught per pitfall trap in 24 h was 0.5 ± 0.2 , 1.7 ± 0.5 , 1.0 ± 0.4 , 0.3 ± 0.1 , 0.1 ± 0.04 , and 0.2 ± 0.1 for 1985, 1986, 1987, 1988, 1989, and 1990, respectively.

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	Nympl	hs	Adults	Nymł	phs	Adults
1985	7.3 ± 2.9	(61)	15.6 ± 3.1 (79)	2.2 ± 1.6	6 (63)	2.2 ± 1.3 (48)
1986	5.4 ± 3.0	(99)	$6.5 \pm 2.9 (62)$	0.0	(43)	$9.4 \pm 3.0 \ (63)$
1987	1	(0)	$33.3 \pm 11.8 (13)$	0.0	(1)	$9.2 \pm 5.6 \ (20)$
1988	0.0	(2)	$24.7 \pm 7.8 (27)$	0.0	(23)	$18.4 \pm 6.8 \ (28)$
1989	0.0	(3)	42.9 ± 20.2 (7)	0.0	(38)	0.0 (31)
1990	1	(0)	14.3 ± 9.2 (7)	0.0	(2)	$2.2 \pm 1.3 \ (36)$
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Only a few species of entomopathogenic nematodes have been employed as classical biological control agents (Ehler 1990) and inoculative releases of entomopathogenic nematodes have not been attempted (Kaya 1990). These releases of *S. scapterisci* may be considered inoculative because relatively small areas were treated, single applications established the nematode at each site, and subsequent nematode generations exerted some control on the pest populations. Therefore, they represent the first successful inoculative releases of an entomopathogenic nematode for the control of exotic pests.

Although the difficulty in estimating mole cricket abundance limited our ability to determine the nematode's effect on mole cricket numbers, the multiyear trap catch data suggest the pest populations were adversely affected. Also, based on informal observation, grass cover in all three release pastures has increased substantially since the beginning of the study indicating that damage inflicted by the pests may be reduced as well. The ability of the infective stage juveniles to survive in the soil and remain infective for several weeks (Nguyen and Smart 1989) without encountering a host allows the nematode to persist in an area when suitable hosts are not abundant and to infect mole crickets immigrating to that area from untreated areas. The ability to disperse enables the nematode to establish in untreated areas and cause mortality in populations beyond the area of application. The potential of *S. scapterisci* to provide longterm mole cricket control for pastures and other affected areas such as golf courses, athletic and recreational grounds, and lawns appears substantial.

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