



## **AUGMENTATIVE APPLICATIONS OF STEINERNEMA SCAPTERISCI (NEMATODA: STEINERNEMATIDAE) FOR MOLE CRICKET (ORTHOPTERA: GRYLLOTALPIDAE) CONTROL ON GOLF COURSES**

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# AUGMENTATIVE APPLICATIONS OF *STEINERNEMA SCAPTERISCI* (NEMATODA: STEINERNEMATIDAE) FOR MOLE CRICKET (ORTHOPTERA: GRYLLOTALPIDAE) CONTROL ON GOLF COURSES

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## ABSTRACT

The insect parasitic nematode, *Steinernema scapterisci* Nguyen and Smart, is a non-chemical alternative to pest mole cricket control in the southern United States. These ambush nematodes can become established after one application and spread into untreated areas through host movement in the soil. However, the nematode's persistence from previous inoculative applications in 1988 and 1989 and the effectiveness of subsequent augmentative applications on intensively managed golf courses were unknown. In 2001, two linear pitfall traps were placed in the roughs of 10 holes on each of two golf courses (20 traps per course) near areas of adult mole cricket activity, and half of the plots with traps were treated with *S. scapterisci*. Ten to 15% of mole crickets trapped before the augmentative nematode applications were infected by *S. scapterisci*. After this application, the percentage of infected mole crickets was higher than the baseline for 8 mo at one golf course and 17 mo at the other. The percentage of mole crickets infected on treated plots equaled or exceeded pretreatment levels about 4-8 wk post-application. The percentage of infected mole crickets in untreated areas at both sites equaled the percent infection in treated areas after about 5 mo. Mole cricket trap catches and percent of infection declined in the second year, but continued to fluctuate with mole cricket population density, age, and environmental conditions. Augmentative applications of *S. scapterisci* for pest mole cricket control can enhance mole cricket mortality on golf courses.

**Key Words:** insect parasitic nematodes, turfgrass, integrated pest management, augmentative biological control, predatory arthropods

## RESUMEN

El nematodo entomoparasítico, *Steinernema scapterisci* Nguyen and Smart, es una de las alternativas no químicas para el control de grillo topos en el sur de los Estados Unidos. Estos nematodos cazadores pueden establecerse después de una aplicación y moverse a áreas sin tratamiento con ayuda del huésped en el suelo. Sin embargo, la persistencia del nematodo debido a aplicaciones inoculativas entre 1988 y 1989, y la efectividad de aplicaciones aumentativas en campos de golf que han sido intensamente manejados, es desconocida. En 2001, dos trampas de caída se colocaron en las áreas periféricas de 10 hoyos en cada uno de dos campos de golf (20 trampas en cada campo) cerca de los focos de grillo topos, la mitad de los lotes con trampas fueron tratados con *S. scapterisci*. Después de las aplicaciones, el porcentaje de grillo topos infectados por el nematodo fue mayor que la línea base después de 8 meses en un campo y 17 meses en el otro. El porcentaje de grillo topos infectados igualaron o sobrepasaron los niveles del pretratamiento entre 4 y 8 semanas después de la aplicación. El porcentaje de grillo topos infectados en áreas sin tratamiento igualaron el porcentaje de infección de los lugares con tratamiento 5 meses después del inicio del experimento. El número de grillos capturados y el porcentaje de infección se redujeron en el segundo año, pero continuaron fluctuando con la población, edad y condiciones ambientales en las que se encontraban los grillo topos. Aplicaciones aumentativas de *S. scapterisci* para el control de grillo topos pueden incrementar los niveles de mortalidad en campos de golf.

Translation provided by the authors.

Pest mole crickets (*Scapteriscus* spp.) have been the targets of a classical biological control program during the past 25 years (Frank & Parkman 1999). Three species, including the tawny (*S. vicinus* Scudder), southern (*S. borellii* Gigliot), and shortwinged (*S. abbreviatus* Scudder)

mole crickets, were inadvertently brought without their natural enemies to the southern U.S. from South America via ship ballast in the early 1900s (Walker 1985). They became established and damaging in pastures and managed turfgrass areas, such as golf courses, athletic fields,

and home lawns. Their root-feeding and tunneling in the soil kills patches of turfgrass, which significantly reduces turfgrass quality and aesthetics. The importation, rearing, subsequent release, and dispersal of three natural enemies (*Larria bicolor* F., *Ormia depleta* (Wiedemann), *Steinernema scapterisci* Nguyen and Smart) has helped suppress mole cricket populations, but insecticides are still frequently used to provide control.

The insect parasitic nematode *Steinernema scapterisci* was discovered in Uruguay in the early 1980s, cultured in the U.S., and released in Florida in 1985 (Parkman et al. 1993b). This nematode infects older mole cricket nymphs and adults and recycles within the soil environment. The nematode, once inside the mole cricket host, releases *Xenorhabdus innexi* bacterium into the hemolymph (Lengyel et al. 2005). The bacterium reproduces and kills the mole cricket through septicemia, producing a nutrient rich bacterial soup that the nematodes consume. The infective juvenile nematodes then exit the body and infect other mole crickets in the soil (Nguyen & Smart 1991). *Steinernema scapterisci* (Nematac S®, Becker Underwood, Ames, IA) can be used in inoculative releases for mole cricket control (Parkman et al. 1994). Other insect parasitic nematodes (e.g., *Steinernema carpocapsae* Weiser; *S. feltiae* (Filipjev); *S. riobrave* Cabanillas, Poinar and Raulston; and *Heterorhabditis bacteriophora* Poinar) used against pest mole crickets have a broader host range and may infect non-target organisms.

Various methods to optimize the survival and establishment of insect parasitic nematodes have been tested. For example, commercial formulations may be sprayed onto turfgrass (Parkman et al. 1993a,b, 1994), chiseled, injected, or buried into the ground (Parkman et al. 1993b; Adjei et al. 2003), or target pests may be trapped into containers, treated with nematodes, and then released (Parkman & Frank 1992). To enhance the establishment of *S. scapterisci*, adult mole crickets can be attracted to a treated area with synthetic, electronic male mole cricket songs (Parkman & Frank 1992). Application timing is limited to when large nymphs and adults are present and actively tunneling through the soil, primarily late August to late October and March to May in the southern U.S. Usually only one application of *S. scapterisci* is necessary to successfully establish and recover *S. scapterisci* populations on a site (Parkman et al. 1993b), but annual applications are recommended (Lombardo et al. 1999).

Because nematode applications may be more expensive and labor-intensive than insecticide applications (Lombardo et al. 1999), spot treatments of nematodes may be more economical. Treating smaller areas of mole cricket damage should reduce costs, and it takes advantage of mole cricket behavior. Adult mole crickets infected with *S. scapterisci* can fly several kilometers before dying

(Walker 1985), thus spreading nematodes to uninfected sites. *Steinernema scapterisci* can also live in moist soil and survive without a host for at least 10 wk (Nguyen & Smart 1990), which increases its value in areas of low mole cricket density. The goals of this study were to assess the baseline level of mole cricket infection from a previous inoculative application and determine whether subsequent augmentative applications could increase mole cricket infection rates over time.

## MATERIALS AND METHODS

### Study Sites

The establishment and spread of *S. scapterisci* was monitored on two golf courses in Alachua Co., FL: Ironwood Golf Course and Gainesville Golf and Country Club. Ironwood Golf Course (IGC) was an 18-hole city-owned public golf course built in 1964. The roughs were bermudagrass (*Cynodon dactylon* Pers. × *C. transvaalensis* Burt-Davy) var. Tifway, mowed at 4.7 cm. Gainesville Golf and Country Club (GGCC) was an 18-hole private course located 16.6 km from Ironwood Golf Course. Gainesville Golf and Country Club was built in 1962 and originally planted with bermudagrass var. Ormond and the roughs were mowed at 3.2 cm. Ironwood Golf Course and GGCC had been previously treated with *S. scapterisci* in the late-1980s and did not have any subsequent treatments. Soil texture was sandy loam on both golf courses. Pesticides were not applied to these plots during the study.

### Treatment Application

Nematodes (1 billion/378.5 L of water) were applied in an aqueous suspension with a boom sprayer calibrated at 0.5 L/m<sup>2</sup> at Ironwood Golf Course on 31 October 2001 at ~1600 h and at Gainesville Golf and Country Club on 5 November 2001 at ~0700 h. The weather at application at GGCC was cloudy, 20°C air temperature, 64-72% relative humidity, 4.3 km/h, and 21°C soil temperature (10.2 cm depth). The weather during application at IGC was partly cloudy, 28°C air temperature, 48-55% relative humidity, 0-2.5 km/h, and 20°C soil temperature (10.2 cm depth). All treated plots were irrigated with 0.6 cm of water before and 0.6 cm after application.

### Pitfall Trap Sampling

Two areas of mole cricket damage were located in the roughs adjacent to ten fairways on each golf course in September 2001. One area (20.1 × 20.1 m or 0.04 ha) was randomly assigned the nematode treatment and the other was the paired untreated control. A linear pitfall trap (modified from Lawrence 1982) was placed near the two areas per

hole, at least 80 m apart in September and early October 2001 (20 areas per golf course), before nematodes were applied. A 19-L plastic bucket was buried in the center with the top flush with the soil surface, and a 3.8-L bucket containing 3-5 cm deep sand was placed inside. Both buckets had water drainage holes. Four PVC pipes (3 m long, 7.6 cm diameter) were installed at right angles to the center of the bucket, with a 2.5-cm slit lengthwise along the top that was also flush with the soil surface. Each distal pipe end was capped. Before sampling, traps were cleaned, fresh sifted sand was added to the 3.8-L bucket, and all surface-active arthropods that had fallen into the 3.8-L bucket were collected 24 h later (Parkman et al. 1993a,b).

Pretreatment samples were collected from all 20 traps on 11, 18, and 25 October 2001. Samples were then collected weekly for 6 wk post-application, and once or twice a month thereafter for 1 year on Gainesville Golf and Country Club and for 2 years on Ironwood Golf Course. Traps were removed from GGCC after 1 year at the request of the golf course superintendent. Adult and juvenile mole crickets with pronotal lengths >4 mm (Hudson & Nguyen 1989a) were tested for nematode infection in the laboratory. Mole crickets were placed individually in 20-mL plastic scintillation vials (Fisher Scientific) with 1-2 drops of deionized water at an air temperature of 23°C

and 12:12 L:D. Mole crickets were examined 7 and 10 d after death under a dissecting microscope (10×) for the presence of nematodes. *Steinernema scapterisci* were identified by Dr. Khuong Nguyen, Entomology and Nematology Department, University of Florida. Potential natural enemies caught in the traps also were identified.

#### Statistical Analysis

Comparisons of percent infection between sites and years were subjected to analysis of variance and Tukey's studentized range test or Student's *t*-test (SAS Institute 2001). All comparisons were made at 0.05 significance level. Non-transformed means plus or minus one standard error of the monthly mean are presented.

#### RESULTS AND DISCUSSION

Spot treatments of *S. scapterisci* successfully increased the percentage of mole crickets infected in bermudagrass roughs within 4-8 wk after application at both golf courses, compared to pretreatment levels. The increased percentage of infection lasted for 17 months at IGC and 8 months at GGCC (Figs. 1 and 2). Likely because of mole cricket movement over time, infection rates in untreated plots (>80 m from treated areas) equaled

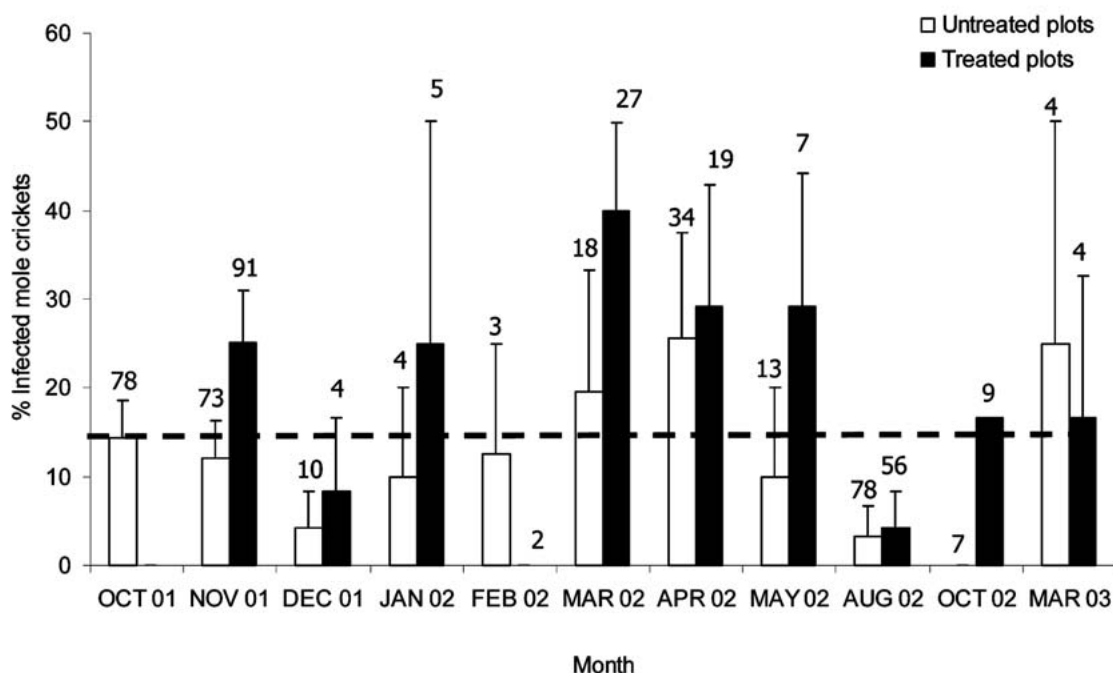


Fig. 1. Mean monthly ( $\pm$  SEM) percent infection of mole crickets collected in pitfall traps at Ironwood Golf Course from areas treated with *Steinernema scapterisci*. Only months with infection levels are presented. Untreated areas received no *S. scapterisci* and were >80 m from treated areas. Dashed line represents baseline pretreatment infection level. Data presented are for *Scapteriscus vicinus* and *Scapteriscus borellii* combined. Total numbers of mole crickets collected are presented above SEM bars.

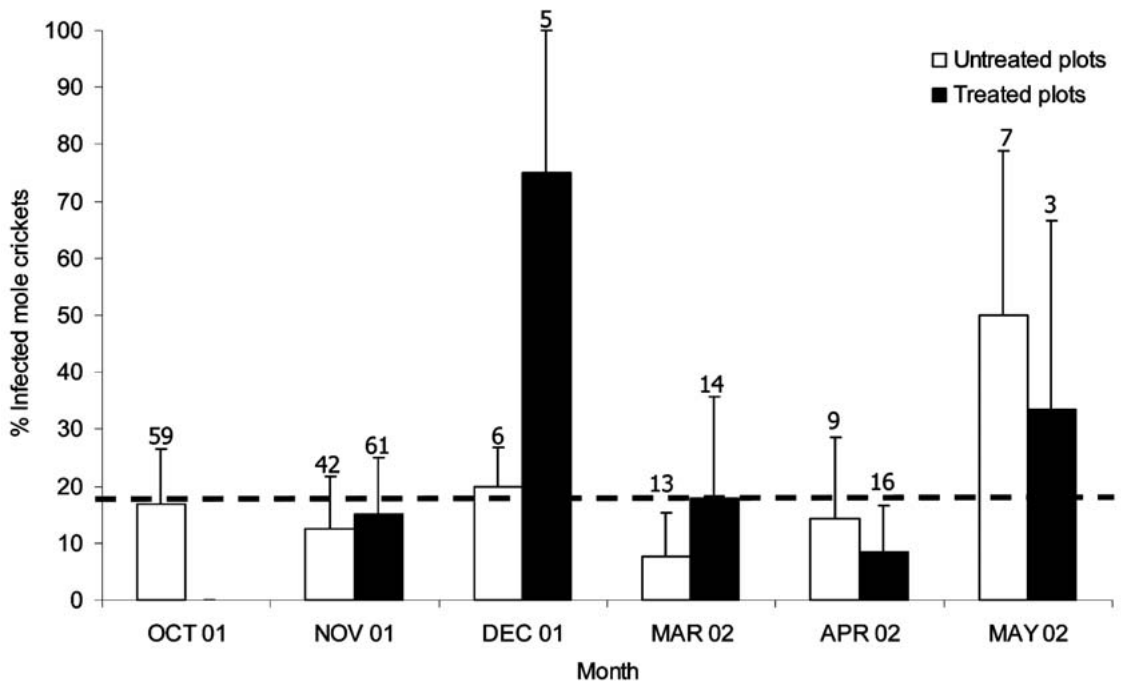


Fig. 2. Mean monthly ( $\pm$  SEM) percent infection of mole crickets collected in pitfall traps at Gainesville Golf and Country Club from areas treated with *Steinernema scapterisci*. Only months with infection levels  $<0$  are presented. Untreated areas received no *S. scapterisci* and were  $>80$  m from treated areas. Dashed line represents baseline infection level. Data presented are for *Scapteriscus vicinus* and *Scapteriscus borellii* combined. Total numbers of mole crickets collected are presented above SEM bars.

the percent infection in treated plots after ~5 months. Turfgrass density ratings (0-9 scale), which indicate the relative amount of damaged turfgrass, did not differ among nematode-treated and control plots in this test at each sampling time (Barbara 2005), and are thus not reliable indicators of nematode establishment.

Nematode infection levels seemed to fluctuate primarily with mole cricket population density, age, and environmental conditions over time (15-40% of mole crickets infected at IGC, 15-75% at GGCC). However, the mean cumulative percentages ( $\pm$  SE) of infection for mole cricket trap collections from 2001 to 2003 from the sites GGCC ( $22.1 \pm 10.5\%$ ) and IGC ( $15.8 \pm 4.6\%$ ) did not differ ( $t = 2.00$ ;  $df = 2, 56$ ;  $P > 0.05$ ). Fewer mole crickets were collected in year 2 than in year 1 at IGC ( $t = 2.47$ ;  $df = 1, 37$ ;  $P < 0.01$ ). Although *S. scapterisci* persisted throughout the study period, the percent infection also decreased in year 2 at IGC compared to year 1 ( $t = 6.63$ ;  $df = 1, 37$ ;  $P < 0.01$ ). Mole crickets that were large enough to be vulnerable to *S. scapterisci* infection were present from late August to April, but cooler winter temperatures likely reduced mole cricket activity and possibly nematode infectivity (Molyneux 1985) from December to February. Similar to Parkman et al. (1994), we found more mole cricket adults infected

with *S. scapterisci* than nymphs. But, contrary to Parkman et al. (1994), more *S. vicinus* were collected in traps and infected than *S. borellii* (Table 1). Other factors that may have influenced mole cricket numbers, nematode infectivity, and/or sur-

TABLE 1. MEAN PERCENTAGE ( $\pm$  SEM) OF MOLE CRICKETS INFECTED BY *STEINERNEMA SCAPTERISCI* ON TWO GOLF COURSES IN GAINESVILLE, FL.

|                    | Mean % of infected mole crickets<br>(total # crickets collected) |                      |
|--------------------|--|----------------------|
|                    | GGCC <sup>1</sup>  | IGC <sup>2</sup>     |
| <i>S. borellii</i> |  |                      |
| Nymphs             | 0 (1)  | 0 (15)               |
| Adults             | 0 (1)  | 3.3 $\pm$ 2.6 (20)   |
| Total              | 0 (2)*   | 1.6 $\pm$ 1.3 (35)*  |
| <i>S. vicinus</i>  |  |                      |
| Nymphs             | 7.7 $\pm$ 4.0 (70)   | 9.7 $\pm$ 3.4 (208)* |
| Adults             | 13.9 $\pm$ 4.5 (163)   | 33.2 $\pm$ 7.0 (313) |
| Total              | 10.8 $\pm$ 3.0 (233)   | 11.9 $\pm$ 2.5 (521) |

Pairs of means within columns followed by asterisks are different,  $t$ -test ( $P > 0.05$ ).

<sup>1</sup>GGCC:  $F = 8.57$ ;  $df = 2, 111$ ;  $P = 0.0003$ .

<sup>2</sup>IGC:  $F = 14.05$ ;  $df = 2, 155$ ;  $P < 0.0001$ .

vival include pesticide use (Barbara & Buss 2005), exposure to ultraviolet light (Gaugler & Boush 1978), soil saturation after excessive rain or irrigation (Molyneux & Bedding 1984; Hudson & Nguyen 1989b), infection by or competition with other nematodes (i.e., *Heterorhabditis* spp., *Steinernema* spp.), infection by fungi (i.e., *Beauveria bassiana*), microbial consumption of the nematodes, parasitism by *Larria bicolor* F. or *Ormia depleta* (Wiedemann), or predation. Over 8,400 predaceous arthropods were collected in the pitfall traps with the mole crickets (Table 2), and the most abundant families included Carabidae (>16.6%), Formicidae (>19.9%), and Staphylinidae (>24.8%). However, earwigs were specifically observed attacking mole crickets in the traps. Fluctuations in predator and parasitoid abundance with mole cricket numbers were not examined.

*Steinernema scapterisci* may become established after one application to turfgrass, but this is the first known study to demonstrate that augmentative spot treatments can also increase the percent of mole crickets infected by a nematode. Given the background infection levels on both golf courses, it is possible that *S. scapterisci* popula-

tions can either persist on intensively managed golf courses for  $\geq 13$  years (initial applications had been made in 1988 and 1989) (Parkman et al. 1994), and/or infected mole crickets may reintroduce nematodes periodically during mating flights from other areas. Because infected or septic mole crickets might not be as mobile as healthy mole crickets, and thus not fall into traps and be detected, the reported percentage of mole cricket infection may be an underestimate. It is possible that *S. scapterisci* can synchronously cycle with mole cricket density over time, since it can reproduce in its host. These nematodes can also kill adult mole crickets before they lay all of their egg clutches (Barbara 2005), which reduces the potential population size of the next generation. Insect parasitic nematodes are viable, environmentally-friendly alternatives to insecticides for long-term mole cricket management.

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TABLE 2. RELATIVE ABUNDANCE OF PREDATORY ARTHROPODS IN TWO GOLF COURSE BERMUDAGRASS HABITATS, GAINESVILLE, FL 2001-2002.

| Taxon           | No. per site |       | % of total <sup>1</sup> |       |
|-----------------|--------------|-------|-------------------------|-------|
|                 | IGC          | GGCC  | IGC                     | GGCC  |
| Arachnida       | 403          | 265   | 9.5                     | 6.4   |
| Chilopoda       | 9            | 8     | 0.2                     | 0.2   |
| Insecta         |              |       |                         |       |
| Coleoptera      |              |       |                         |       |
| Carabidae       | 927          | 690   | 21.8                    | 16.6  |
| Coccinellidae   | 2            | 2     | 0.1                     | 0.1   |
| Histeridae      | 69           | 180   | 1.6                     | 4.3   |
| Lampyridae      | 1            | 0     | <0.1                    | 0     |
| Phengodidae     | 1            | 0     | <0.1                    | 0     |
| Staphylinidae   | 1,056        | 2,031 | 24.8                    | 48.9  |
| Dermaptera      |              |       |                         |       |
| Carcinophoridae | 4            | 14    | 0.1                     | 0.3   |
| Forficulidae    | 0            | 1     | 0                       | <0.1  |
| Labiduridae     | 98           | 114   | 2.3                     | 2.7   |
| Hemiptera       |              |       |                         |       |
| Anthracoridae   | 1            | 3     | <0.1                    | 0.1   |
| Geocoridae      | 2            | 0     | 0.1                     | 0     |
| Nabidae         | 29           | 4     | 0.7                     | 0.1   |
| Reduviidae      | 14           | 13    | 0.3                     | 0.3   |
| Hymenoptera     |              |       |                         |       |
| Formicidae      | 1,646        | 825   | 38.6                    | 19.9  |
| Total           | 4,262        | 4,150 | 100.0                   | 100.0 |

<sup>1</sup>Values represent percent of total collection at each site.

- nematode density on laboratory infection of *Scapteriscus vicinus* and *S. acletus* (Orthoptera: Gryllotalpidae) by *Neoplectana* sp. (Rhabditida: Steinernematidae). Environ. Entomol. 18: 719-722.
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