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Source: Florida Entomologist, 87(3) : 300-305

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2004)087[0300:SAIOSS]2.0.CO;2

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SURVIVAL AND INFECTIVITY OF *STEINERNEMA SCAPTERISCI* (NEMATODA: STEINERNEMATIDAE) AFTER CONTACT WITH SOIL DRENCH SOLUTIONS

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ABSTRACT

After a nematode application, mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.) are frequently assayed to confirm nematode establishment and infectivity. However, the standard soap flush was suspected of providing false negatives under field conditions. Thus, we examined the effect of several potential flushing solutions on the survival and infectivity of *Steinernema scapterisci* Nguyen and Smart (Nematoda: Steinernematidae) as well as flushing ability under field conditions. Seventy percent of *S. scapterisci* died in lemon dish detergent solution, confirming that assays for nematode infection of soap-flushed mole crickets are likely to be inaccurate. When sampling for mole crickets in areas where *S. scapterisci* has been applied, a potential alternative to the standard soap drench is a dilute permethrin drench.

Key Words: Sampling, soap flush, soil drench, entomopathogenic nematode, biological control, integrated pest management.

RESUMEN

Después de una aplicación de nemátodos, los grillos topo (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.) son evaluados frecuentemente para confirmar el establecimento y la capacidad de infectar de los nemátodos. Sin embargo, la lavada con jabón que es el proceso usado usualmente, es sospechada de proveer datos negativos falsos bajo condiciones de campo. Por ello, nosotros examinamos el efecto de diferentes soluciones para la lavada sobre la sobrevivencia y la capacidad de infectar de los *Steinernema scapterisci* Nguyen y Smart (Nematoda: Steinernematidae) así como la habilidad para lavar bajo condiciones de campo. Setenta por ciento de los *S. scapterisci* mueren en una solución de detergente con limon para platos, confirmando que los ensayos para determinar la infección de nemátodos de grillo topos lavados con jabón son probablemente imprecisos. Cuando recolectan los grillos topo en áreas donde *S. scapterisci* ha sido aplicado, una alternativa a la lavada basica con jabón es una lavada con una solución diluida de permetrina.

Mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.) are subterranean pests of turfgrass in Florida and much of the southeastern United States (Walker & Nickle 1981; Walker 1985). Mole cricket damage and cost of control in Florida in 1986 was estimated at $45 million with an additional $33 million in Alabama, Georgia, and South Carolina combined (Frank & Parkman 1999). Estimates of annual expenditure in 1996 were over $18 million for insecticides in Florida turf, and over $12 million in control costs (Hudson et al. 1997). Mole crickets damage turf by their tunneling in the soil, which exposes and dries out roots and by direct root feeding. As a result, the turfgrass thins and bare patches appear. The tunneling and mounds that mole crickets make also disrupt the playing surface on golf courses, especially the roll of the golf ball on greens. Superintendents and golf course members have little tolerance for damage (Frank & Parkman 1999). Insecticides are usually targeted against the most destructive, nymphal stage. A more sustainable, environmentally friendly management approach for mole cricket control is needed.

Several biological control agents have been investigated for control of *Scapteriscus* spp. mole crickets in Florida (Hudson et al. 1988). One of these biological control agents is an entomopathogenic nematode, *Steinernema scapterisci* Nguyen and Smart. *Steinernema scapterisci* was originally collected in Uruguay in pitfall-trapped *Scapteriscus* mole crickets in the 1980s (Nguyen & Smart 1990). The nematode was cultured and released in several Florida counties in 1985, where it established a population, and was spread from the release site by infected *Scapteriscus* mole crickets (Hudson et al. 1988; Parkman & Frank 1992). The nematode kills the adult and late instar nymphs of *Scapteriscus borellii* Giglio-Tos and *S. vicinus* Scudder, and to a lesser extent *S. abbreviatus* Scudder. Fewer small to medium-sized nymphs of *S. borellii* and *S. vicinus* become infected (Nguyen 1988).

Several techniques have been used to sample mole crickets including counts of dead nymphs and adults after insecticide applications (Short & Koehler 1979), estimation of surface burrowing (Walker et al. 1982; Cobb & Mack 1989), pitfall

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trapping (Lawrence 1982; Adjei et al. 2003), removal with a tractor mounted soil corer (Williams & Shaw 1982), sound trapping (Walker 1985) and soil drenching (Short & Koehler 1979; Walker 1979; Hudson 1989). However, results from each of these techniques are often inconsistent (Short & Koehler 1979; Lawrence 1982, Hudson 1988). Comparisons of different methods have indicated that soil drenching with soap solutions is the most practical and consistent at obtaining direct counts of mole crickets (Short & Koehler 1979; Hudson 1988).

Soil drenching with a solution of 15 ml of lemon dishwashing detergent in 3.8 L of water is inexpensive and commonly used by turfgrass managers to sample soil pests. Soil drenches with soap solutions irritate mole crickets and force them out of the soil. Soap flushes are often used for monitoring mole crickets to determine the size, age, and species present, the relative population density over time, and for control timing. However, it was suspected that soap flushes, when used to monitor mole crickets potentially infected with *S. scapterisci*, might be lethal to the nematodes because nematodes are rarely found in soap-flushed mole crickets (our observations, and K. B. Nguyen & G. C. Smart, Entomology and Nematology Dept., University of Florida, pers. comm.). Solutions such as pyrethroids, ammonia, vinegar, Lysol®, and other soap detergents have previously been tested as potential soil drench solutions (Short & Koehler 1979).

This study was conducted to determine if a standard soap detergent solution affects *S. scapterisci* survival and infectivity in pest mole crickets. Potential alternatives to the standard soap drench solution were also evaluated.

Materials and Methods

Nematodes and Mole Crickets

Steinernema scapterisci (Nematac S®, Becker Underwood, Ames, IA) were stored at 7°C in a cold room until used (<3 mo). Nematode viability was tested before each application by dissolving a pinch (~10 mg) of Nematac S® into water and observing nematode shape and mobility under a light microscope. Healthy nematodes were opaque in color and S-shaped with oscillating movements. Dead or unhealthy nematodes were translucent, straight, and lacked movement. The product was used if viability was >50% and discarded if <50% viable.

Scapteriscus vicinus were collected from pitfall traps or sound traps in Alachua Co., FL, and returned to the laboratory. Each mole cricket was placed in a 120-ml plastic vial (Thorton Plastics Salt Lake City, UT) with sterilized sand and held for ≥14 d to ensure health. Surviving mole crickets were used in this study. Mole crickets were maintained at 23°C with a photoperiod of 12:12 (L:D) and fed commercial cricket chow (Purina®, St. Louis, MO).

Bioassay

Nematode viability and infectivity were assessed after exposure to various drenching materials. *Steinernema scapterisci* were extracted from Nematac S® using a modified Baermann technique (K. B. Nguyen, pers. comm.). *Steinernema scapterisci* were kept at a density of 10,000 infective juveniles in solutions of water (control), lemon dishwashing detergent (Joy®), insecticidal soap (Safer Soap®, Woodstream Corporation, Litiz, PA), and permethrin (Spectracide Bug Stop®, Spectrum Brands, St. Louis, MO) for test 1. The mixtures were kept at room temperature (24°C) in a 125-ml Erlenmeyer flask with 125-ml per flask on a shaker at 65 rpm. There were five replicates for each treatment. Concentrations (Table 1) were selected based on recommendations for flush extraction of mole crickets in the field (Short & Koehler 1979) and label rates for mole cricket control. After 24h, 10-µl samples were taken from each treatment and placed on a microscope slide. The number of living and dead nematodes were counted with a dissecting microscope (10×); three 10-µl counts were taken and averaged to determine percent mortality for each replicate. Immobile nematodes were touched with a probe to determine survival.

Table 1. Mean nematode mortality and percent of mole crickets infected with *Steinernema scapterisci* after exposure for 24 h to various drenching solutions.

Treatment	Rate	Mean % nematode mortality (± SEM)[1]	% Mole crickets infected with *S. scapterisci*[2]
Water	n/a	6.2 ± 3.95	16.7
Lemon Joy	15 ml/ 3.79 L	70.6 ± 4.52*	8.3
Insecticidal Soap	15 ml/ 3.79 L	90.0 ± 7.82*	0
Permethrin	18 ml/ 3.79 L	35.6 ± 1.97*	16.7

*Statistically significant values using Dunnett's method comparing treatments to water.
[1]n = 20, $F = 54.68$, $df = 19, 3$, $P = < 0.0001$.
[2]n = 12, $R^2 = 0.2971$, $df = 11, 3$, $\chi^2 = 4.843$ (likelihood), $P = 0.18$.

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A second test was initiated to further test potential drench materials. Treatments for test 2 included water (control), azadirachtin (Safer® Brand BioNeem, Woodstream Corporation, Litiz, PA), citrus oil (Green Sense®, Garland, TX), garlic extract (Garlic Barrier®, Garlic Research Labs, Inc., Glendale, CA), lemon juice (Realemon®, Rye Brook, NY), permethrin (Spectracide Bug Stop®, Spectrum Brands, St. Louis, MO) and cyfluthrin (Bayer Advanced Lawn and Garden®, Bayer Environmental Sciences, Montvale, NJ). Concentrations (Table 2) were selected based on label and half label rates for mole cricket control. Methods from test 1 were repeated.

Nematode infectivity was assessed by filtering nematodes from above solutions and adding 50 living infective juveniles to 120-ml plastic cups (Fisher Scientific, Pittsburgh, PA) containing 20 g sterilized sand, 4% deionized water, and one *S. vicinus* adult. Dead mole crickets were dissected and the presence or absence of nematodes was recorded.

The above solutions were tested for their effectiveness at flushing mole crickets at the University of Florida G. C. Horn Turfgrass Research Unit in Gainesville, FL, on 20 and 28 May 2003. Each treatment from tests 1 and 2 (3.8 L of each solution) was applied to areas of bermudagrass (*Cynodon dactylon* [L.] Persoon) that had mole cricket damage (75 cm^2). The numbers of adult and first instar mole crickets emerging from the soil within 3 min were counted. Five replicates for each solution were completed. Any turfgrass phytotoxicity was noted at 1 h and 1 wk posttreatment.

The effect of nematode infected crickets exposed to soap solutions was also tested. *Scapteriscus abbreviatus* adults were obtained from a lab colony at the University of Florida Entomology and Nematology Department, Gainesville, FL and were inoculated with about 10,000 nematodes by applying predetermined amount (approximately 150 µl) of concentrated nematode solution onto a piece of filter paper (Fisher #P8, 5.5 cm) inside a petri dish with one *S. abbreviatus* adult. The mole cricket was allowed to incubate in the petri dish for 1, 5, 8, 12, or 24 h (five mole crickets per treatment). *Scapteriscus abbreviatus* was used because *S. vicinus* adults were unavailable at the time of the test. All infected mole crickets were then dipped into a 118-ml Solo soufflé cup (Gainesville Paper Co., Gainesville, FL) containing the soapy water or soapy water followed by a deionized water rinse for 5 sec. Untreated controls were healthy, uninfected mole crickets dipped in water. Mole crickets were placed into 20-dram plastic scintillation vials (Fisher Scientific, Pittsburgh, PA) and observed every 24 h for 10 days. On day 10, mole crickets were dissected and the presence of nematodes was noted.

Statistical Analysis

Nematode mortality and field test data were subjected to an analysis of variance (SAS Institute 2001). Treatments were compared to the control (water) by Dunnett's means comparison method ($\alpha = 0.05$). Nematode infectivity data were subjected to Chi-square analysis (SAS Institute 2001). Treatments were compared to the control (water) and the standard soap flush solution (15 ml lemon dish detergent/3.8 L water) by Dunnett's means comparison method ($\alpha = 0.05$). Nematode mortality data were transformed by arcsine-square root transformation before statistical analysis; nontransformed data are presented. Effects of nematode infected crickets exposed to soap solutions data were subjected to PROC GLM (SAS Institute 2001) procedure.

Results and Discussion

Insecticidal soap, lemon dishwashing soap, and permethrin at the label rate for mole cricket control caused significantly more nematode mortality than water (Table 1). Nematodes exposed to

Table 2. Mean nematode mortality and infectivity after exposure for 24 h to various drenching solutions.

Treatment	Rate	Mean % nematode mortality (± SEM)[1]	% Mole crickets infected with *S. scapterisci*[2]
Water	n/a	12.9 ± 7.89	3.7
Citrus Oil	15 ml/ 3.79 L	32.1 ± 9.47	3.7
Cyfluthrin	8 ml/ 3.79 L	6.4 ± 6.44	3.7
Cyfluthrin	15 ml/ 3.79 L	4.1 ± 4.12	3.7
Garlic Extract	111 ml/ 3.79 L	0	3.7
Lemon Juice	15 ml/ 3.79 L	6.8 ± 6.76	0
BioNeem	60 ml/ 3.79 L	4.4 ± 4.38	0
Permethrin	9 ml/ 3.79 L	10.8 ± 6.73	3.7
Permethrin	18 ml/ 3.79 L	0	3.7

[1]n = 45, $F = 2.70$, $df = 44, 8$, $P = 0.193$.
[2]n = 27, $R^2 = 0.1349$, $df = 26, 8$, $\chi^2 = 4.170$ (likelihood), $P = 0.18$.

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all treatments showed similar infectivity in mole crickets (R^2 = 0.2971; *df* = 2,11; χ^2 = 4.843; *P* < 0.184) (Table 1). Nematode mortality was similar among all treatments in test 2 (Table 2). Nematodes surviving all treatments except azadirachtin and lemon juice, demonstrated a low percentage infectivity of mole crickets, no significant treatment differences were observed (R^2 = 0.1349; *df* = 2,26; χ^2 = 4.170; *P* < 0.842) (Table 2).

In the field, insecticidal soap and the higher rate of permethrin flushed significantly more mole crickets than water (Table 3). However, when all treatments were compared to the standard lemon dish detergent, insecticidal soap and permethrin brought a similar number of mole crickets to the surface (n = 55; *F* = 2.88; *df* = 10,54; *P* = 0.008). None of the mixtures tested produced any noticeable phytotoxicity to the turf.

Soil drenches with a mixture of lemon dish detergent and water are commonly used to monitor turfgrass insects such as mole crickets, chinch bugs (*Blissus* spp.), big-eyed bugs (*Geocoris* spp.), and several species of caterpillars (Short & Koehler 1979; Hudson 1989). Soil drenches are inexpensive and are not labor intensive when compared with other methods of monitoring mole cricket populations. These other methods include large pitfall traps (Lawrence 1982; Adjei et al. 2003), an emitter producing a synthetic song of male mole crickets (Parkman & Frank 1993), and a soil-coring device (Williams & Shaw 1982). Each method requires more than one person, are labor intensive or costly (Lawrence 1982; Williams & Shaw 1982).

Seventy percent of *S. scapterisci* died in the lemon dish detergent solution. Assays for nematode infection of soap-flushed mole crickets, the method currently used by many turfgrass managers, are likely to be inaccurate. Krishnayya & Grewal (2002) reported a toxic effect of a common soap surfactant (Ajax®) on *S. feltiae* Bovien nematodes. They found 24% mortality of nematodes when incubated at 4, 24, 72, and 120 h (Krishnayya & Grewal 2002). Kaya et al. (1995) reported an insecticidal soap (M-Pede®) adversely affected *S. carpocapse* (Weiser) and *Heterorhabditis bacteriophora* Poinar survival and infectivity. However, infectivity may not be affected if the nematodes are combined with an insecticidal soap and applied immediately (Kaya et al. 1995). Nematodes cannot be stored in an insecticidal soap solution because without aeration, nematode survival can be adversely affected (Kaya et al. 1995). The toxicity of metal ions present in soap may be responsible for the high mortality in soap solutions (Jaworska et al. 1994; Krishnayya & Grewal 2002).

Tests of exposure of nematode infected mole crickets to soap solutions show that soap flush solution does not greatly affect nematode infection at least 8 h post infection (Table 4). The soap flush solutions may potentially kill nematodes in certain areas of the body (i.e., mouth) and further testing should be done to determine this. Immediately rinsing flushed mole crickets with clean water may potentially increase the accuracy of determining nematode infection. The unavailability of *S. vicinus* at the time of experimentation may have also led to inconsistent, low levels of infection. It is known that *S. scapterisci* does not infect *S. abbreviatus* as successfully as *S. vicinus* or *S. borellii* (Nguyen 1988).

Although permethrin solutions killed some nematodes in our experiments, *S. scapterisci* infectivity was not compromised and field flushes successfully extracted mole crickets from the soil. The field data concur with Short & Koehler (1979) who reported that pyrethrins were the most effective material, flushing a mean of 11.5 mole crickets/0.6 m^2. Hudson (1988) compared three sampling tech-

TABLE 3. MEAN NUMBER OF MOLE CRICKETS EMERGING FROM BERMUDAGRASS WITH VARIOUS DRENCHING SOLUTIONS IN MAY 2003.

Treatment	Rate	Mean number of mole crickets flushed (± SEM)
Water	n/a	0
Citrus oil	15 ml/ 3.79 L	2.6 ± 1.6
Cyfluthrin	8 ml/ 3.79 L	0.2 ± 0.2
Cyfluthrin	15 ml/ 3.79 L	4.0 ± 2.1
Garlic extract	111 ml/ 3.79 L	0.4 ± 0.2
Lemon juice	15 ml/ 3.79 L	0.6 ± 0.4
BioNeem	60 ml/ 3.79 L	3.2 ± 1.2
Permethrin	9 ml/ 3.79 L	2.6 ± 1.1
Permethrin	18 ml/ 3.79 L	5.8 ± 1.4*
Insecticidal soap	15 ml/ 3.79 L	5.4 ± 1.3*
Lemon Joy	15 ml/ 3.79 L	4.6 ± 2.1

*Means statistically significant values by Dunnett's method comparing treatments to water.
n = 54, *F* = 2.88, *df* = 59,10, *P* = 0.01.

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TABLE 4. PERCENT NEMATODE INFECTION FROM MOLE CRICKETS EXPOSED TO TREATMENT SOLUTIONS 1, 5, 8, 12, OR 24-H POST INFECTION.

Time Post Infection	1 H	5 H	8 H	12 H	24 H
Joy (15 ml/ 3.79 L)	0	40	60*	60*	100*
Joy (15 ml/ 3.79 L) + H_2O rinse	40	40	100*	80*	100*
Control[†]	0	0	0	0	0

n = 75, $F = 6.77$, $df = 14, 2$, $P < 0.0001$.
*Means within columns statistically significant values when compared to control.
[†]Control = uninfected, healthy mole crickets immersed in water.

niques, soil flushing with lemon dish detergent or synergized pyrethrins, and a tractor mounted soil corer. None of the methods were significantly different. Our results from the field test show drenching solutions of permethrin are useful in determining if mole crickets collected in the field are infected with *S. scapterisci* nematodes. A soil drench containing permethrin may be the best monitoring tool to flush mole crickets to determine the presence of *S. scapterisci*.

However, there are disadvantages to pyrethroids as soil drenches for mole crickets. Pyrethroid drenches at the half or full label rate may cause more mole cricket mortality than a soap solution. Subsurface mortality of mole crickets can be as high as 65% with pyrethroids or similar insecticides (Ulagaraj 1974; Walker 1979; Hudson 1988). Applicator exposure to insecticides is increased with a pyrethroid soil drench.

Soil drenches are effective, non labor-intensive methods to sample soil insect populations. Soap detergent solutions, although inexpensive, may not accurately indicate mole crickets infected with *S. scapterisci*. Permethrin solutions are less cost effective (Short & Koehler 1979), but are effective at flushing mole crickets potentially infected with nematodes.

ACKNOWLEDGMENTS

We are grateful to G. Diaz-Saavedra and T. Hinks (Becker Underwood) for providing nematodes and technical assistance, and to Y. Wang and B. Owens for assistance in field tests. We thank N. Leppla and J. H. Frank for advice and review of earlier versions of the manuscript. Funding was provided by the Florida State Legislature and the Department of Agriculture and Consumer Services. This is the Florida Agricultural Experiment Station Journal Series No. R-10104.

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