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EVALUATION OF THE NEMATODE *STEINERNEMA CARPOCAPSAE* TO CONTROL FIRE ANTS IN NURSERY STOCK

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Recent developments in low-cost, large scale production of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* (Bedding 1984) have facilitated large-scale field testing against various soil-infesting insects (Kaya 1985, Poinar 1986), with good potential for control in some applications. Early research by Poole (1976) and Quattlebaum (1980) indicated that the potential included fire ants. Citrus growers in Florida using nematodes (BioVector®; Biosys, Inc.) for control of soil insects have reported fewer problems with fire ants (personal communications to W. R. Martin). Jouvenaz et al. (1990), however, did not achieve significant control of red imported fire ants, *Solenopsis invicta* Buren, in field trials of nematodes (Biosys, Inc.) conducted in Florida.

Steinernematid nematodes may be moderately effective against fire ants in irrigated soil. Morris et al. (1990) reported 47% control with *Steinernema carpocapsae* versus 39% control with the Amdro standard at sites containing irrigated turf. This level of control is far below normal for Amdro (Williams et al. 1987; Jouvenaz et al. 1990), indicating that it may have been broadcast on wet soil (personal communication, D. F. Williams, USDA, ARS). Unmarked mounds found outside the blocks after treatment were not included in the assessment for activity; thus, control by nematodes may have been overestimated due to mound relocation. Considerable relocation of fire ant nests occurred in the tests conducted by Jouvenaz et al. (1990). Had not mound movement been taken into consideration (Poole and Quattlebaum scored all mounds uninhabited after treatment as dead), the apparent control would have been over 77% (of 44 treated mounds, 34 were inactive). We subsequently confirmed the aversion of fire ants to nematodes by controlling the movement of small laboratory colonies between containers of sandy soil with single applications of BioSafe® Lawn and Garden Insect Control (*Steinernema carpocapsae*; Biosys, Inc., Palo Alto, CA).

The readiness with which fire ants relocated their nests in the field and vacated soil in containers to avoid nematodes prompted us to evaluate the potential of these parasites to eliminate fire ants from nursery stock. The early spread of fire ants in the southeastern United States was greatly facilitated by the shipment of infested nursery stock (Lofgren 1986). Recently these pests have been transported to Arizona and California; if they become established in the West, their range will increase substantially.

Currently, federal certification of nursery stock for shipment through quarantine is based solely on incorporation of chlorpyrifos. Granular chlorpyrifos is not as effective as previously believed, drenching poses problems of worker exposure, and both treatments are expensive (personal communication, H. Collins, USDA, APHIS). The improvement of methods for control of fire ants is a critical need of the nursery industry (Regelbrugge 1991).

In the present study, three sequential tests were conducted using BioSafe against fire ants nesting in nursery pots containing *Pittosporum* sp. shrubs. A colony of *S. invicta* consisting of five queens, 3,000-5,000 workers and about 1.5 g brood was introduced into each of 50 1 gal pots containing shrubs 20-35 cm in height. The ants were

maintained on honey agar, boiled eggs, and Lepidoptera larvae, and were allowed 48 h for acclimation before treatment with nematodes. The fire ant-infested potted shrubs were held individually in plastic trays, the inner walls of which were coated with Fluon® (ICI America) to prevent migration of the colonies. The trays were arranged in a grid on the cement floor of a shed and treated in a randomized complete block design. Daily soil temperatures in the pots ranged from 16-32°C at a depth of three inches. Soil moisture was monitored and the plants were watered as deemed necessary. Suspensions of nematodes were prepared according to label directions and kept agitated; viability was confirmed microscopically immediately before use.

In the first test, 50 ml of water containing 30,000 nematodes (about 1,000 per square inch of soil surface) were poured into each of 20 pots, 50 ml of water containing 300,000 nematodes was poured into each of 20 other pots, and the remaining 10 pots received 50 ml of water only. At the time of treatment, an additional pot of watered potting soil (but without a plant) was placed in each tray to provide the ants with untreated soil in which to relocate their nests. Without "escape pots", ants cluster around the bases of evacuated containers (personal observation), and evaluation is difficult due to the open drainage holes. Also, this represents a more natural situation, since ants are free to relocate in untreated soil in nurseries. Three days after treatment, 10 pots treated with each dose of nematodes (total of 20) were examined for the presence of living ants by spreading the soil on a 1 × 1.5 m white plastic cloth. The remaining 20 treated pots and the 10 control pots were examined similarly 14 days after treatment. The number of worker ants was estimated and categorized as none, fewer than 100, 100-1,000, or over 1,000.

The second test was conducted using replanted shrubs, new ant colonies, a new and different lot of BioSafe®, and a different brand of potting soil (Hyponex Professional Mix®; Hyponex Corp., Atlanta, Ga. in lieu of Pennington Potting Mix®; Pennington Lawn & Garden Supplies, Orlando, Fla.). The same procedures used for the first test were employed except that 50 ml of water containing the nematodes were sprayed onto the soil surface with a Solo #485 backpack sprayer.

The third test consisted of 30 pots infested with new fire ant colonies, of which 20 were sprayed (same sprayer) with a single dose of 75,000 nematodes (about 2,500 nematodes per square inch of soil surface) in 50 ml water. The remaining 10 pots received water only. The batch of BioSafe®, method of application, and potting soil were as in the second test; however, escape pots were not provided. Instead, all 30 pots were arranged and treated in a random pattern on the ground, which was covered with short grass and shaded by two oak trees. Thus, the ants were free to relocate in field soil (fine sand). This test was evaluated four days after treatment.

We found that fire ants were not eliminated from 1 gal pots containing *Pittosporum* shrubs treated with either 30,000 or 300,000 nematodes (1,000 or 10,000 per square inch of soil surface). The numbers of cadavers in the refuse piles did not indicate elevated mortality in treated colonies.

In the first and second tests, queens and brood remained in all 80 treated pots and all 20 control pots; neither was found in the escape pots three or 14 days after treatment. Most of the worker ants also remained in the treated pots.

In the first test, fewer than 100 workers were found in each of the 20 escape pots examined three days after treatment. Fewer than 100 workers per pot were found in 18 of the 20 escape pots examined 14 days after treatment; the remaining two escape pots harbored 100-1000 workers each. Fewer than 100 workers were found in each of the 10 control escape pots, all of which were examined 14 days after treatment.

In the second test, fewer than 100 workers per pot were found in 18 of the 20 escape pots examined three days after treatment; the remaining two escape pots harbored 100-1000 workers each. Of the 20 escape pots examined 14 days after treatment, 10

harbored fewer than 100 workers and 10 harbored 100-1000 workers each. Of the 10 control pots, five harbored fewer than 100 workers and five harbored 100-1000 workers each.

In the third test, queens, brood and workers remained in 11 (55%) of the 20 treated colonies and five (50%) of the 10 control colonies four days after treatment. Brood was absent from nine (45%) and queens from seven (35%) of the treated pots. However, brood was absent also from five (50%) and queens from three (30%) of the 10 control pots. Four of the treated pots and two of the control pots (20% each) also were free of worker ants.

After completion of the above tests, a bioassay using wax moth, *Galleria melonella*, larvae was conducted to assess the distribution of nematodes in treated potting soil. Ten pots containing shrubs, but without ants, were sprayed with 300,000 nematodes each. Five pots were bioassayed after 48 h and the remaining five after 72 h. Soil samples were taken from the periphery of the upper part of the root ball (about one inch below the surface), the periphery of the lower part of the root ball, the interior of the root ball, and from below the root ball. Each soil sample was packed loosely in a separate petri dish, to which five *G. mellonella* larvae were added. The larvae were examined for nematode parasitization after one week incubation at 28°C.

The bioassay with larvae of *G. melonella* indicated that distribution of nematodes was not uniform. Of the 50 larvae (10 treated pots) exposed to soil from the periphery of the upper root ball, the periphery of the lower root ball, the interior of the root ball, or below the root ball, 48 (96%), 42 (84%), 34 (68%), and 20 (40%), respectively, became parasitized. Since one or very few nematodes are required to initiate parasitization of *G. melonella* larvae, it also appears that few active nematodes were present.

A practical treatment for eliminating fire ants from nursery stock must be essentially 100% effective in all types of potting soil, and also be quite persistent. Unfortunately, fire ant colonies living in nursery stock were unaffected by steinernematid nematode drenches. Also, the *G. melonella* bioassay indicated that premixing nematodes in potting soil would not be effective. Thus, it appears unlikely that these parasites can be manipulated to reliably eliminate fire ants from nursery stock.

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