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Source: Florida Entomologist, 85(4) : 537-544

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2002)085[0537:FEOTCP]2.0.CO;2

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# FIELD EFFICACY OF TWO COMMERCIAL PREPARATIONS OF ENTOMOPATHOGENIC NEMATODES AGAINST LARVAE OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) IN ALFISOL TYPE SOIL

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Abstract

Spring and fall field trials were conducted to determine the efficacy of two species of entomopathogenic nematodes for the control of larvae of *Diaprepes abbreviatus* in a citrus grove with alfisol type soil (sandy clay loam). Both *Steinernema riobrave* (Bio Vector 355) as a water-dispersible granule and *Heterorhabditis indica* (Grubstake™ 100) as a paste on sponge at rates from 22-108 IJ's/cm$^2$ failed to reduce larval populations in the tree rhizosphere at 25 d post-treatment. Larval parasitism by entomopathogenic nematodes in baited screen cages was sporadic over time, with the only significant treatment effect occurring at the highest rate (108 IJ's/cm$^2$) of *S. riobrave* in the fall at 7 d post-treatment. Possible constraints to nematode efficacy are discussed.

Key Words: biological control, citrus root weevils, *Diaprepes,* entomopathogenic nematodes, *Pachnaeus*

Resumen

Se llevaron a cabo pruebas de campo en la primavera y el otoño para determinar la eficacia de dos especies de nemátodos entomopatógenos para el control de larvas de *Diaprepes abbreviatus* en huertos de cítricos con suelo de tipo alfisol (marga arcilla arenosa). Las dos especies, *Steinernema riobrave* (Bio Vector 355) en la forma granular dispersable en agua, y *Heterorhabditis indica* (Grubstake™ 100) en la forma de una pasta puesta encima de una esponja al porcentaje de 22-108 IJ's/cm$^2$, no redujeron la población de larvas en la rizosfera a 25 d después del tratamiento. El parasitismo de larvas por nemátodos entomopatógenos en jaulas de tela metálica con cebo fué esporádico durante el tiempo del estudio, el único efecto significativo del tratamiento sucedió en la concentración más alta (108 IJ's/cm$^2$) de *S. riobrave* en el otoño a los 7 d después del tratamiento. Se discuten las posibles restricciones a la eficacia de nemátodos.

Several species of polyphagous root weevils, particularly *Diaprepes abbreviatus* L. and *Pachnaeus* spp., are important field and nursery pests of citrus, ornamentals, and some agronomic crops in Florida (McCoy 1999). Larval feeding injury to roots by *D. abbreviatus* can have a devastating effect on citrus trees since all stages feed on the roots for most of the year. Root injury appears to be cumulative, and most importantly, feeding sites can serve as infection courts for root rot diseases (Graham et al. 1996), thereby exacerbating economic loss. Tree decline can be particularly bad in poorly drained groves when water stress affects root health. There is no estimate of the total economic loss to the growers from larval root injury to citrus, but the end result can frequently be tree death. Current integrated pest management (IPM) strategies for weevil control include sound horticultural practices, fungal disease control, adult weevil monitoring, and a mix of suppressive tactics to control larvae and adults (McCoy & Duncan 2000).

Native and introduced entomopathogenic nematodes are infectious to all larval stages and possibly adults (Adair 1994, Beavers et al. 1983, Schroeder 1990). Naturally occurring species within the genera *Heterorhabditis* and *Steinernema* have been found in citrus groves throughout Florida infecting as much as 38-68% of caged *D. abbreviatus* larvae in the summer in deep sandy soils found on the central ridge and the sandy clay loam soils of the coastal and interior flatwoods (Beavers et al. 1983, McCoy et al. 2000). The density and distribution of endemic nematode populations, regardless of species, vary from grove to grove, within a grove and within a season. Inundative releases of mass-produced entomopathogenic nematodes (EPN) for larval control have been pursued by private industry for about 15 yrs (Duncan et al. 1999, Schroeder 1987). During this time, four nematode species have been sold in Florida to control *D. abbreviatus* in citrus: *Heterorhabditis bacteriophora* Poinar; *Heterorhabditis indica* Poinar, Karunaker and David; *Steinernema*

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*carpocapsae* (Weiser); and *Steinernema riobrave* (= riobravis) Cabanillas, Poinar and Raulston (Shapiro & McCoy 2000a). Currently, *H. indica* (Grubstake™ 100, Integrated BioControl Systems, Aurora, IN) and *S. riobrave* (Bio Vector 355, Certis Corporation, Columbia, MD) are sold commercially for use on Florida citrus.

The label rate for Bio Vector 355 is $4.9 \times 10^8$ viable IJ's/treated hectare at 250 trees/grove hectare, that is, 2,000,000 IJ's/tree (Knapp 2000). The label rate for Grubstake™ 100 is one-half the Bio-Vector rate. These field rates can be highly variable since the area of soil treated per hectare can change according to tree size and method of application. To circumvent this problem, private industry suggests that growers apply Grubstake™ 100 at 11 IJ's/cm$^2$ and Bio Vector 355 at twice that rate. To date, no published field research has shown that these rates are effective against *Diaprepes*. Field trials by Bullock & Miller (1994), Bullock et al. (1999), and Schroeder (1990), showed that rates of 2-5 million IJ's of *S. carpocapsae* or *S. riobrave* per tree applied within a ~0.3 m$^2$ area surrounding the base of the tree in the spring significantly reduced adult emergence of both *D. abbreviatus* and *Pachnaeus litus* (Germar). In addition, field trials in groves on the central ridge using *S. riobrave* and *H. bacteriophora* showed that rates of 120-250 IJ's/cm$^2$ reduced larval populations significantly within 4 weeks post-treatment (Downing et al. 1991, Duncan et al. 1996, Duncan & McCoy 1996). In three separate trials, McCoy et al. (2000) showed that nematode parasitism by either *S. riobrave* or *H. indica* at 22 IJ's/cm$^2$ or less was no different than parasitism in the untreated control in a flatwoods grove. In fact, 108-216 IJ's/cm$^2$ of *S. riobrave* were required to increase parasitism to 40-60%.

Although published data cited above clearly show that higher rates of entomopathogenic nematodes result in (i) higher parasitism, (ii) greater suppression of larvae in the soil, and (iii) reduced adult emergence from the soil, data also suggest that efficacy is influenced by other unknown factors relating to nematode, host and/or environment (Kaya & Gaugler 1993). For example, *S. carpocapsae* at rates greater than 100 IJ's/cm$^2$ gave no control in central ridge and coastal flatwoods groves (Adair 1994; Bullock et al. 1999; Duncan et al. 1996). Laboratory studies have shown that *S. riobrave* is more effective at warmer soil temperatures, and host age also affects susceptibility of *D. abbreviatus* larvae to the nematode (Shapiro & McCoy 2000b; Shapiro et al. 1999). In addition, soil type can affect virulence and persistence of *S. riobrave* and *H. bacteriophora* (Shapiro et al. 2000), whereas culture and formulation method have no effect on larval mortality for *S. riobrave* (Shapiro & McCoy 2000a).

The objectives of this study were to further test the efficacy of a commercial formulation of *S. riobrave* (Bio Vector 355) in a flatwoods-like grove with alfisol type (sandy clay loam) soil at different rates per soil surface area and, for the first time, test the field efficacy of high rates of *H. indica* (Grubstake™ 100) in the same soil. Tree destruction and baited traps were used to assess larval population survival and nematode parasitism, respectively.

## Materials and Methods

### Experimental Site

Two field trials were conducted near Poinciana, FL, (Osceola County) in a declining mature planting of Hamlin oranges grafted to Swingle citrumelo rootstock. The grove was planted on two row beds with a setting pattern of $6.1 \times 8.5$ m. The grove was equipped with under-tree micro-jet sprinkler irrigation. The alfisol soil type for the grove was classified as Floridana fine sand 68.8% sand, 11.8% silt, 19.4% clay. The surface layer was about 35.6 cm loam and the subsurface layers about 76.2 cm grey fine sand followed by clay. The soil was poorly drained with a low to moderate organic content and a pH of 4.8. The trials were conducted within 40 m of each other. Adult weevil injury to the leaf (McCoy 1999) was evident in all trees throughout the grove.

In trial one, 40 single tree plots were arranged on two row beds in a completely randomized design with four experimental treatments and 10 replications. Beds were separated by a drainage ditch ~7.6 m in width. In trial two, 48 single tree plots were arranged on two row beds in a completely randomized block design with eight experimental treatments and six replications. In both trials, an in-row variable tree buffer was also established to prevent treatment interference.

### Nematode Viability and Application

Two species of entomopathogenic nematodes, *S. riobrave* and *H. indica*, formulated as a water-dispersible granule (WDG) and a paste, respectively, were used in these field trials. Regardless of formulation, nematodes were kept cool (~20°C) both in storage and in the field prior to tank mixing. Within 2-3 h of field application, the viability (nematode mobility) of each preparation was determined microscopically by counting the number of mobile and dead infective juveniles (IJ's) in a fixed number of fields at 60× magnification. Samples of nematode preparations used for viability determination were held for a minimum of 2 h with and without aeration before counting. In both trials, viability of the water-dispersible granules of *S. riobrave* averaged only 57.1 and 46.2%, respectively, and therefore, an adjustment in quantity of preparation was made to achieve the desired field rate for experimentation. In trial

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two, the viability of the paste formulation of *H. indica* averaged 94.7% and no adjustments were necessary to achieve the desired field rate.

Since the WDG formulation of *S. riobrave* used in trial one had poor viability, infectivity (virulence) of the preparation was compared to an *in vivo* laboratory culture that was passed through *Diaprepes* six times and an untreated control. The bioassay procedure was identical to that described by Shapiro & McCoy (2000a). Eighth instar *D. abbreviatus* were obtained from the USDA-ARS Horticultural Laboratory (Fort Pierce, FL). One laboratory assay was performed in 50-dram plastic containers filled with Candler sand with soil moisture of about 8% by weight. A single larva was placed on the bottom of each container prior to adding sand, then 500 IJ's were applied to the soil surface. The experiment was arranged in a randomized design with each treatment replicated 10 times. The experiment was conducted at 24°C for 10 d post-inoculation. Nematode parasitism was confirmed via microscopy. Control parasitism was 0%, *in vivo* culture parasitism 40%, and WDG parasitism 80%, suggesting that the viable nematodes in the preparation were highly infectious.

For nematodes used in trial two, a similar laboratory assay was conducted and compared the WDG formulation of *S. riobrave*, the paste formulation of *H. indica*, an *in vivo* laboratory culture of *S. riobrave* that had been passed through *Diaprepes* 16 times, and an untreated control. In this case, 30 replicates were conducted per treatment. Larval mortality in the control was 3.3%, whereas it was 63.3% for the paste formulation, 50.0% for the WDG, and 53.3% for the *in vivo* culture.

In trial one, *S. riobrave* was applied to the soil beneath 10 trees at rates 0, 22, 54, and 108 IJ's/cm$^2$ on 16 April 2000, from 3:30-6:00 p.m. under overcast skies. Water-dispersible granules were pre-mixed in 1 liter of water and the appropriate volume of nematode suspension then added to the tank of a 50-liter electric field sprayer (Chemical Containers, Lake Wales, FL) equipped with a hand-held spray boom with two flat-fan nozzles set apart by 30.5 cm (R and D Sprayers, Opelousas, LA). Nematodes were uniformly applied in 1.9 liters of water per tree at 20 psi. Irrigation was applied to all plots prior to nematode application to assure adequate soil moisture to a depth of 30 cm. Soil temperature ranged from 22-24°C at a depth of 10 cm. Irrigation was applied again for 3 h the following day.

In trial two, *S. riobrave* and *H.indica* were applied at 0, 11, 54, and 108 IJ's/cm$^2$ on 5 October 2000, from 6:00-9:00 p.m. under clear skies. Soil moisture and temperature fell within the range of those for trial one. Both WDG and paste on sponge formulations of *S. riobrave* and *H. indica*, respectively, were pre-mixed in 1 liter of water and the appropriate volume of nematode suspension then added to the spray tank. Prior to and after nematodes were applied to the soil, irrigation was applied for about 3 h to assure soil moisture in the top 30 cm and again for 1 h the following day.

### Field Efficacy

In trial one, larval suppression in the soil rhizosphere was determined at 23 ± 1 d post-treatment using a tree removal-soil sampling procedure. Initially, trees were topped using a chain saw, then the roots along with the surrounding soil were removed using a backhoe. Most of the soil adhering to the roots was removed by shaking and/or probing with a shovel. The soil within the root crown was generally wet and compact, while the surrounding soil was moist and easy to process. Using a shovel, soil from the roots and beneath the tree was then placed in buckets for subsequent sieving. Approximately 0.4 m$^3$ of soil was collected per tree to a depth of 30 cm according to the procedures of Duncan & McCoy (1996). All developmental stages of *D. abbreviatus* except larvae less than fifth instar were visually detectable and recovered from the soil using a motor-driven shaker and 0.64-cm mesh sieve. The number of larvae, pupae, and adults were recorded per tree. All larvae exhibiting normal behavior were recorded as live. Each dead larvae was placed in a disposable Petri dish (50 × 9 mm) on a moistened filter paper. Cadavers were examined microscopically every other day for 7 d to detect characteristic changes typical of bacterial, fungal, or nematode infection. Differences in mean larval population density between treatments were tested on square root transformed data by analysis of variance.

In trial two, larval suppression in the soil rhizosphere was determined at 26 ± 1 d post-treatment using the previously described sampling procedure. Soil moisture in relation to the tree was similar to trial one, except where the sandy layer appeared at the surface in Block F causing low soil moisture within a small area within the grove. The experiment was analyzed using a three-way analysis of variance for nematode species, application rate, and block (SAS Institute, Cary, NC). Larval counts were transformed using a square root transformation prior to analysis. Because of the obvious difference in soil texture for Block F, data were analyzed with and without the block included.

In addition to estimating differences in native populations of *Diaprepes* among treatments by tree removal, larval-baited traps were used to measure post-treatment parasitism by nematodes. Our intention here was to determine if levels of larval parasitism by nematodes were comparable to changes in wild larval populations.

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In the field, a hand held auger was used to make a circular hole in the soil beneath the tree canopy midway between the trunk and canopy margin to a depth of 30 cm for insertion of a baited cylindrical cage (McCoy et al. 2000). The cage made from an in-line liquid filter (7 × 3 cm diam.) with stainless steel screen (mesh size 225) was filled partially with excavated soil. Then a single 8th instar larva of *Diaprepes* produced on synthetic diet in the laboratory was placed in the cage in the soil. Additional soil was then added to fill the cage before capping. In trial one, four traps per tree were buried at the compass points beneath 10 trees (n = 40) where they remained for 7 d. Within 12 h of retrieval from the soil, each cage was opened and recovered larvae examined for nematode infection. Healthy and dead larvae were processed and diagnosed for parasitism in the manner described for wild larval collections. This procedure was conducted at 1, 2, and 3 weeks post-treatment. In trial two, two traps per tree (n = 12) were buried in the above manner and the procedure was conducted at 1 week pre-treatment and 1, 2, and 3 weeks post-treatment. For each field test, a contingency table analysis using Chi-square test (SAS Institute, Cary, NC) was performed to compare statistically the effect of field rates of nematodes on larval survival and parasitism in the soil.

## RESULTS

### Trial One

In the spring trial, the density of larvae (> 5th instar) recovered from the soil (0.4 $m^3$/tree) via sieving was variable but quite high, ranging from 11 to 73 with a mean of 35.8 ± 20.5 in the control (Table 1). A few scattered pupae, usually encased in soil, and adults were also recovered at the time of tree extraction. Although larval location in the soil was not quantified, they were more prevalent in the root crown in close proximity to the roots, often lodged in compact soil surrounding the crown roots or in association with moist soil at any depth to 30-40 cm. However, all developmental stages were recovered from dry soil. Dead and diseased larvae and adults were rarely observed. However, the sieving process was most likely destructive to cadavers. Arthropod predators were rare with only an occasional fire ant mound detected. The total root system on all trees was severely damaged by larval feeding over time, and many trees were infected with root rot diseases. Trees were virtually devoid of fibrous roots.

As shown in Table 1, there was no significant difference ($P$ = 0.316) in larval density between the different rates of Bio Vector 355 and the control according to a one-way analysis of variance. The greatest number of late instar larvae were recovered from the highest rate of nematodes. Larval density among trees was variable (pooled S.D. = 1.337).

No difference in larval survival of caged 8th instars of *D. abbreviatus* was found among treatments after exposure for 1 week at 7 d ($P$ = 0.24) (Table 2), 14 d ($P$ = 0.09), or 21 d ($P$ = 0.31) post-application. From all treatments, 34, 20, and 9 larval cadavers, respectively, were recovered from baited cages buried for 7 d at 4, 14, and 21 d post-application. All remaining larvae recovered from the different treatments were healthy. Nematodes recovered from cadavers were identified as bacterial feeding rhabditids only.

### Trial Two

The density of larvae (> 5th instar) recovered from the soil via sieving in the fall trial was also high, but numerically lower than the spring. Larval recovery from all treatments ranged from 5 to 50 per tree with a mean of 17.8 ± 10.1 (n = 50). Interestingly, the different developmental stages of *D. abbreviatus* recovered from the soil in the fall trial were similar to those found in the spring, that is, mostly mid-instar larvae, a few scattered pupae, and adults. Root systems of all trees sam-

TABLE 1. LARVAL, PUPAL, AND ADULT RECOVERY OF *DIAPREPES ABBREVIATUS* FROM THE RHIZOSPHERE AFTER 25 D EXPOSURE FOLLOWING TREATMENT WITH DIFFERENT RATES OF *STEINERNEMA RIOBRAVE* (BIO VECTOR 355) IN THE SPRING AT POINCIANA, FLORIDA.

| Treatment | Rate IJ'/$scm^2$ | Live insects recovered from all trees | | | Mean no larvae/0.4$cm^3$ ± SD[A] |
|---|---|---|---|---|---|
| | | Larvae | Pupae | Adults | |
| Control | — | 358 | 7 | 7 | 35.8 ± 20.5 |
| Bio Vector | 22 | 443 | 17 | 8 | 44.3 ± 14.1 |
| Bio Vector | 54 | 375 | 13 | 7 | 37.5 ± 14.1 |
| Bio Vector | 108 | 485 | 5 | 4 | 48.5 ± 12.3 |
| | | | | | F = 1.22, $P$ = 0.316 |

[a]Means based on 10 single tree replication/treatment.

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TABLE 2. CONTINGENCY TABLES, CHI-SQUARE ANALYSIS FOR SURVIVING CAGED 8TH INSTAR LARVAE OF *DIAPREPES ABBREVIATUS* AFTER 1 WEEK EXPOSURE TO FIELD SOIL TREATED WITH DIFFERENT RATES OF *STEINERNEMA RIOBRAVE* (BIO VECTOR 355) IN THE SPRING AT POINCIANA, FLORIDA.

| Treatment | Rate IJ's/cm$^2$ | Mean % larval survival, days post-treatment[a] | | |
|---|---|---|---|---|
| | | 7 | 14 | 21 |
| Control | — | 50.0 | 72.4 | 90.4 |
| Bio Vector | 22 | 62.1 | 85.5 | 78.3 |
| Bio Vector | 54 | 45.6 | 64.9 | 85.5 |
| Bio Vector | 108 | 45.0 | 70.7 | 80.0 |
| Chi-square value | | 4.20 | 6.42 | 3.59 |
| Probability | | 0.24 | 0.09 | 0.31 |
| | | n.s. | n.s. | n.s. |

[a]Means based on 15 single tree replicates per treatment; 4 cages/replicate.

pled were severely damaged by weevils and showed root rot symptoms.

The effect of the different rates of Bio Vector 355 and Grubstake™ 100 on the reduction of larvae of *D. abbreviatus* is presented in Fig. 1. The overall analysis of variance produced highly significant results (3-way ANOVA, F = 3.56, df = 12, 35, $P$ = 0.0016). However, neither the main effect for nematode species nor for application rate was significant (nematode species, F = 2.83, df = 1, 35, $P$ = 0.1013; application rate, F = 1.43, df = 3, 35, $P$ = 0.2510), whereas the main effect for blocks was highly significant (F = 6.98, df = 5, 35, $P$ = 0.0001). The species by dose interaction was also not significant (F = 0.24, df = 3, 35, $P$ = 0.8707).

The data were also analyzed after pooling the results for the three application rates within species. Again, the overall analysis of variance produced a highly significant result (3-way ANOVA, F = 5.08, df = 8, 39, $P$ = 0.0002). However, the main effects for nematode species and for application rate failed to reach significance (nematode

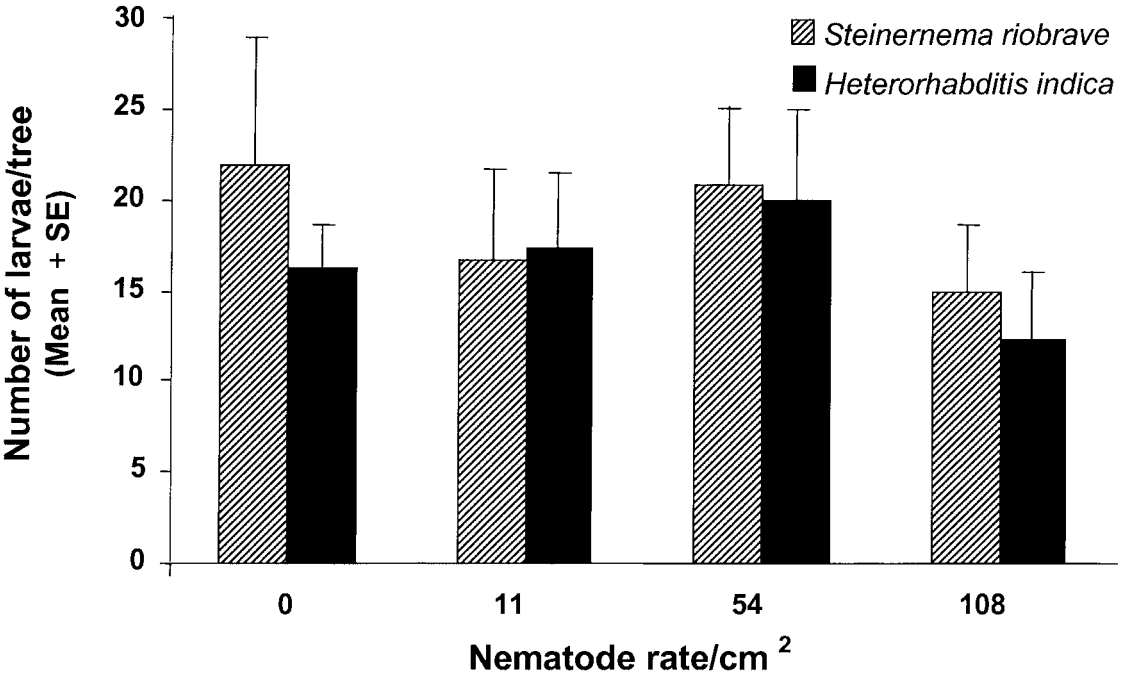



Fig. 1. Live larval recovery of *Diaprepes abbreviatus* from the rhizosphere after 26 d exposure following treatment with different rates of *Steinernema riobrave* (Bio Vector 355) and *Heterorhabditis indica* (Grubstake™ 100) in the fall at Poinciana, FL.

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species, F = 3.06, df = 1, 39, $P$ = 0.0882; application rate, F = 1.68, df = 1, 39, $P$ = 0.2021), whereas the main effect for blocks was highly significant (F = 7.15, df = 5, 39, $P$ = 0.0001). The species by dose interaction was also not significant (F = 0.30, df = 1, 39, P = 0.5874).

As previously mentioned, Block F produced extremely low numbers of larvae (range, 0-26; mean, 6.5) in nearly all treatments. Therefore, we repeated the original analysis but omitted the extreme replicate. In this case, the overall analysis of variance failed to achieve significance (3-way ANOVA, F = 1.88, df = 11, 28, $P$ = 0.0862). However, repeating this analysis without this extreme replicate and after pooling the three lowest application rates produced a significant result (3-way ANOVA, F = 2.98, df = 7, 32, $P$ = 0.0158). Nonetheless, again, the main effect for nematode species was not significant (F = 0.81, df = 1, 32, $P$ = 0.3738) and the main effect for application rate narrowly missed significance (F = 3.45, df = 1, 32, $P$ = 0.0723). The main effect for blocks was highly significant (F = 4.15, df = 4, 32, $P$ = 0.0080). The species by dose interaction were also not significant (F = 0.16, df = 1, 32, $P$ = 0.6960).

In the fall field trial, larval survival of caged 8th instar larvae of *D. abbreviatus* following 7 d exposure to pre-treated soils ranged from 81.8 to 100% (Table 3) and from 66.7 to 100% (Table 4). No significant difference was found among treatments receiving either *S. riobrave* ($P$ = 0.58) or *H. indica* ($P$ = 0.115). Larval survival of *D. abbreviatus* after 7 d exposure to soil treated with different rates of *S. riobrave* at 7, 14, and 21 d post-treatment was significantly lower for the highest rate of *S. riobrave* at 7 d ($P$ = 0.016), while no difference between treatments was found at 14 ($P$ = 0.363) and 21 d ($P$ = 0.279) (Table 3). Larval survival of *D. abbreviatus* following exposure for 7 d to soil treated with different rates of *H. indica* at 7, ($P$ = 0.279) 14, ($P$ = 0.271), and 21 d ($P$ = 0.271) post-treatment was not significantly different from the control (Table 4). Nematodes recovered from cadavers were identified by the junior author. Twelve infected larvae produced *Steinernema* sp. close to *S. riobrave*, 10 infected larvae had *H. bacteriophora*, 6 infected larvae had *Heterorhabditis* sp., and several *Cephalobus* sp. (bacterial feeders) were also recovered.

## Discussion

Spring and fall applications of *S. riobrave* and *H. indica* at commercial rates (10-20 IJ's/cm$^2$) and higher (54-108 IJ's/cm$^2$) failed to reduce larval populations and increase nematode parasitism appreciably in the soil. When compared to previous field trials, these data further substantiate the broad variability in field efficacy experienced by previous researchers when applying entomopathogenic nematodes as biopesticides to different citrus groves (Duncan et al. 1999, McCoy & Duncan 2000).

Variation in efficacy can be caused by multiple factors relating to the nematode, its host, and the environment (Kaya & Gaugler 1993). A number of contributing factors such as larval age, soil temperature, nematode virulence, culture method, and soil characteristics have been recognized in experimentation with entomopathogenic nematodes as biological control agents of *Diaprepes* in citrus soils (Shapiro & McCoy 2000a, 2000b, 2000c; Shapiro et al. 1999). In addition, sampling methods for assessing nematode efficacy in the field have differed widely among researchers and no doubt have contributed somewhat to the variation in larval control (Bullock & Miller 1994, Duncan & McCoy 1996, McCoy et al. 2000).

Recent field and microcosm experiments, designed to determine the effect of soils of different composition and texture on nematode efficacy, strongly suggest that field failures reported herein were soil-related (Duncan et al. 2001).

TABLE 3. CONTINGENCY TABLE, CHI-SQUARE ANALYSIS FOR SURVIVING CAGED 8TH INSTAR LARVAE OF *DIAPREPES ABBREVIATUS* AFTER 1 WEEK EXPOSURE TO FIELD SOIL TREATED WITH DIFFERENT RATES OF *STEINERNEMA RIOBRAVE* (BIO VECTOR 355) IN THE FALL AT POINCIANA, FLORIDA.

| | | Mean % larval survival, day post-treatment[a] | | | |
|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 |
| Control | — | 100.0 | 100.0 a | 90.9 | 83.3 |
| Bio Vector | 11 | 81.8 | 100.0 a | 83.3 | 100.0 |
| Bio Vector | 54 | 91.7 | 91.7 a | 75.0 | 100.0 |
| Bio Vector | 108 | 90.9 | 63.6 b | 60.0 | 83.3 |
| Chi-square value | | 1.97 | 10.26 | 3.19 | 3.84 |
| Probability | | 0.58 | 0.016* | 0.363 | 0.279 |
| | | n.s. | | n.s. | n.s. |

[a]Means based on 12 cages/treatment.

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TABLE 4. CONTINGENCY TABLE, CHI-SQUARE ANALYSIS FOR SURVIVING CAGED 8TH INSTAR LARVAE OF *DIAPREPES ABBREVIATUS* AFTER 1 WEEK EXPOSURE TO FIELD SOIL TREATED WITH DIFFERENT RATES OF *HETERORHABDITIS INDICA* (GRUBSTAKE™ 100) IN THE FALL AT POINCIANA, FLORIDA.

| Treatment | Rate IJ's/cm$^2$ | Mean % larval survival, day post-treatment[a] | | | |
|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 |
| Control | — | 66.7 | 83.3 | 100.0 | 100.0 |
| Grubstake | 11 | 100.0 | 91.7 | 100.0 | 100.0 |
| Grubstake | 54 | 90.9 | 83.3 | 83.3 | 83.3 |
| Grubstake | 108 | 72.7 | 60.0 | 91.7 | 91.7 |
| Chi-square value | | 5.92 | 3.74 | 3.91 | 3.911 |
| Probability | | 0.115 | 0.291 | 0.271 | 0.271 |
| | | n.s. | n.s. | n.s. | n.s. |

[a]Means based on 12 cages/treatment.

When soil (entisol type) from a deep sandy ridge grove (Lake Alfred) with a percent sand:silt:clay ratio of 97.6:1.5:0.9 was compared to sandy clay loam soil (alfisol type) from our experimental site (Poinciana) with a ratio of 68.8:11.8:19.4, *S. riobrave* applied at 20 IJ's/cm$^2$ killed 70-80% of the larvae of *D. abbreviatus* buried to a depth of 30 cm in the sandy soil (Lake Alfred), but only 4-17% of the larvae in sandy clay loam soil (Poinciana) suggesting that higher clay soil with finer texture reduced host contact or affected the infection process.

This marked difference in nematode efficacy between sandy and sandy clay loam soils is suggested in the published literature. For example, in two groves on the central ridge with a deep sandy soil (entisol type), *S. riobrave* applied at 108 IJ's/cm$^2$ reduced larval populations by 75-90% after three weeks (Duncan & McCoy 1996, Duncan et al. 1996). Both tests were evaluated in the same manner as these trials using the tree removal/soil sieve methodology. In another series of field trials conducted in a flatwoods grove near Ft. Pierce with Pineda sandy clay loam soil, larval parasitism by *S. riobrave* ranged from 40-45% when rates of 54-108 IJ's/cm$^2$ were applied, suggesting soil-related inhibition (McCoy et al. 2000).

Shapiro et. al. (2000) measured virulence and persistence of *S. riobrave* and *H. bacteriophora* in Marl (high silt + clay), ridge (entisol, sandy), and coastal flatwoods (spodosol, sandy clay loam) soils in the laboratory. Although both nematode species were virulent to *D. abbreviatus* larvae in all soils, both virulence and persistence were greater in the heavier Marl soil and virulence was greater in spodosol compared to entisol soil. These laboratory data appear contradictory to field results. However, physical properties of the soil were not a factor. Shapiro et al. (2000) suggest that the chemical composition of the soils is not a deterrent to parasitism but physical properties of the soil, such as structure, compaction, etc. might contribute to the variation in field efficacy.

In recent microcosm studies comparing the efficacy of *S. riobrave* at a rate of 20 IJ's/cm$^2$ in eight autoclaved soils from citrus groves including Poinciana, Duncan et al. (2001) found that larval mortality of *D. abbreviatus* was positively correlated with the proportion of sand in the soils, but was inversely related to the percentage of fine sand. The strongest correlation with efficacy was with percentage of medium and coarse sand in soils. Both nematode emergence from the cadaver and recycling in cadavers were favored coarse sandy soils.

The importance of soil texture in relation to soil compaction as determined by Duncan et al. (2001) is supported by field observations we made on soil compaction within the tree rhizosphere at the time of tree removal. Soil surrounding the roots was very fine in texture resulting in extreme compaction on the roots and within the rhizosphere. Soil was so compact within the root crown of the tree, it was virtually impossible to remove with a probe. When larvae adjoining the roots were removed with a probe, invariably they were healthy suggesting the soil was so compact nematode penetration of the soil was infrequent.

As previously mentioned in the methods, nematode mobility and viability of WDG formulation of *S. riobrave* was generally lower in these studies compared to formulations from earlier field studies (Duncan & McCoy 1996; Duncan et al. 1996). It might be argued that nematode vigor was a factor in explaining poor field efficacy in these trials. Although this could be true for *S. riobrave*, it cannot explain our results with *H. indica*, where nematode viability was excellent.

Both larvae used in baited cages to measure nematode parasitism (8th instar) and the larval instars recovered from the native soil (6th-10th instar) fall within the age group (i.e., 100 d old) reported by Shapiro et al. (1999) as being least

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susceptible to nematode infection by both *S. riobrave* and *H. indica*. In view of their findings, it is reasonable to assume that host age can influence nematode parasitism in the field.

The results of these field studies supported by the studies of Duncan et al. (2001) pose important implications relating to the biological control of larvae of *D. abbreviatus* with entomopathogenic nematodes in Florida citrus. Foremost, soil characteristics appear to be important determinants of field efficacy. Current nematode products appear most efficacious in deep sandy soils common to the central ridge of Florida; however, efficacy is affected substantially by different soils, particularly the sandy clay loams. The issue of optimal rate appears variable and is likely influenced by host age and edaphic factors. Finally, further research is warranted on nematode species selection and the dynamics of edaphic conditions in relationship to field performance.

## Acknowledgments

This research was supported by the Florida Agricultural Experiment Station, a grant from the Florida Citrus Production Research Advisory Council Grant No. 942-18E and approved for publication as Journal Series No. R-08331.

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