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Entomopathogenic Nematodes and Other Natural Enemies as Mortality Factors for Larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae)

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Parasitism and persistence of three species of entomopathogenic nematodes, *Steinernema riobrave, Heterorhabditis bacteriophora,* and *H. indica,* were evaluated as biopesticides against larvae of *Diaprepes abbreviatus* in a mature citrus grove using different soil sampling methods. In three separate tests, commercial formulations of different nematode species were applied with herbicide delivery equipment at rates from 11 to 216 infective juveniles (IJs)/cm² to the soil beneath the tree. The prevalence of parasitism and/or predation by either commercially applied nematodes or indigenous natural enemies associated with weevil larvae in the soil was measured using larvae-baited screened cages placed in the soil before and after nematode application. The results showed that ant predation and nematode parasitism were the dominant mortality factors of caged, 6th instar *D. abbreviatus* during 7 days exposure to field soil. Entomopathogenic fungi and bacteria were incidental. Inundative applications of different rates of entomopathogenic nematodes showed a positive quadratic relationship between number of nematodes applied per given area (log scale) and parasitism of caged larvae. Higher rates (>54 IJs/cm²) were required to achieve levels of parasitism significantly greater than that in the controls. Nematode parasitism of *D. abbreviatus* larvae was similar in caged versus no-cage comparisons conducted in the greenhouse. Nematode numbers in the soil declined over time, reaching pretreatment levels at 14 days posttreatment based on a modified Baermann sampling procedure. © 2000 Academic Press

Key Words: **ant predation; biological control; *Diaprepes;* entomopathogenic nematodes; *Heterorhabditis; Steinernema.***

INTRODUCTION

The *Diaprepes* root weevil, *Diaprepes abbreviatus* L., infests ≈60,000 ha of commercial citrus (18% of the total acreage) found in 20 Florida counties (*Diaprepes* Task Force, 1997). This weevil is native to the Caribbean region where it is an economic pest of many agricultural crops including citrus (Wolcott, 1936). With the gradual spread of *D. abbreviatus* within citrus-growing areas of Florida during the past 30 years, many growers have experienced a devastating effect on the tree caused by larval feeding injury to the roots, particularly in groves where trees are planted in poorly drained soils (McCoy, 1999). At the same time, various controls for both larvae and adults have been marginally effective compared to the earlier use of persistent organochlorine soil pesticides that are now banned due to environmental concerns (Duncan *et al.,* 1999; McCoy, 1999).

Currently, adult *D. abbreviatus* that feed and oviposit on the leaves of citrus and alternate host plants are suppressed on the tree during their time of greatest seasonal abundance with foliar chemical sprays (Bullock *et al.,* 1988). In addition, an ovicide and/or spray oil can be applied to the foliage to reduce viable egg production and the subsequent number of larvae entering the soil (Schroeder *et al.,* 1976; Schroeder, 1996).

At egg hatch, neonates drop from the tree to the soil surface where they enter the soil to feed on the fibrous roots of the citrus tree. As larvae increase in size, they also feed on the bark of the larger roots (Quintela *et al.,* 1998; Rogers *et al.,* 2000). Larval feeding on the bark causes lesions on the roots that are subject to invasion by various root-rotting fungi such as *Phytophthora nicotianae* Dastur and *P. palmivora* (Butler) (Graham *et al.,* 1996). At the soil surface, soil-applied insecticides can be used as a chemical barrier to prevent neonate entry into the soil (Knapp, 1999). The best way to control the more injurious mid to late instars found on the lateral and crown roots appears to be with

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entomopathogenic nematodes as a soil biopesticide (Duncan *et al.,* 1999; McCoy, 1999). Laboratory and field research conducted during the last 10 years has confirmed the potential effectiveness of entomopathogenic nematodes for larval control (Schroeder, 1990; Downing *et al.,* 1991; Duncan and McCoy, 1996; Duncan *et al.,* 1996; Bullock *et al.,* 1999; Shapiro *et al.,* 1999; Shapiro and McCoy, 2000). However, rates of 108 infective juveniles (IJs)/cm^2 or greater appear to be a requirement to reduce larval numbers by ~90% or greater (Bullock *et al.,* 1999; Duncan *et al.,* 1999). These higher rates are not cost effective on mature trees with large under-canopy area and exceed current grower recommendations of 2 million IJs/tree (Knapp, 1999). In addition, Shapiro *et al.* (1999) have shown in the laboratory that both temperature and age of host affect susceptibility of the larvae to nematodes and that the virulence of different nematode species to the host differs inherently.

Neither researchers nor growers are convinced that the current level of pest suppression by various nematode control agents is adequate for management of *D. abbreviatas* and other citrus root weevils. There are no published studies on the profitability of these control agents in either short- or long-term weevil control programs (Duncan *et al.,* 1999). Moreover, the research constraints to answering questions of efficacy and profitability are enormous. For example, no method(s) of directly assessing population density of weevils in soil is efficient unless tree destruction is employed and, even then, the variability in host density from tree to tree requires high replication (Duncan and McCoy, 1996).

The objectives of this study were to determine parasite efficacy and persistence in the field of three species of commercially produced entomopathogenic nematodes, *Steinernema riobrave* Cabanillas, Poinar and Raulston, *Heterorhabditis bacteriophora* Poinar, and *Heterorhabditis indica* Poinar, Karunaker and David, as biopesticides against *D. abbreviatus* in a mature flatwoods citrus grove using different field rates and soil sampling methods. Also, the prevalence of parasitism and predation of indigenous natural enemies to weevil larvae in the soil was measured after nematode application.

MATERIALS AND METHODS

Experimental Site

In 1998–1999, three field trials were conducted in a 12-year-old commercial planting of mixed orange varieties grafted to Swingle citrumelo rootstock, located near Fort Pierce, Florida. The grove was planted as two row beds at a setting pattern of 3.0 × 7.6 m. Soil type was a Pineda sand (alfisols) with a low to moderate organic content and a pH of 6.9. The grove was equipped with low-volume under-tree micro-jet sprinkler irrigation. At the time of the tests, infestations of *D. abbreviatus* and *Pachnaeus litus* (German) were evident in the grove.

Eighty plots were arranged on double-row beds separated by a drainage ditch 7.6 m in width. Each plot was composed of 6 trees in row with a total of 12 trees/plot (~93 m^2). Only the 4 center trees per plot were sampled, although the whole plot received treatment. A 5-tree buffer was maintained at the end of each planting bed. Plots were arranged in a completely randomized design with eight experimental treatments and 10 replications. In tests 2 and 3, where fewer than eight treatments were applied, unused plots were still treated with nematodes.

Nematode Culture and Application

Three species of entomopathogenic nematodes, *S. riobrave, H. bacteriophora,* and *H. indica,* as commercial products formulated as a liquid, a water-dispersible granule, a paste, or on a sponge, were used in these inundative field studies. In all trials, regardless of formulation, nematodes were kept cool (~15°C) both in storage and in the field prior to tank mixing. Prior to field application, the viability of each nematode preparation was determined microscopically by counting the number of motile and dead infective juveniles in a fixed number of fields of view at 60× magnification. Immobile nematodes exhibiting movement when probed with a dissecting needle were viable. In trials 1 and 2, nematode viability was used to adjust for the desired field rate selected for experimentation. In trial 3, the field rate was calculated based on the number of nematodes designated on the label. See Table 1 for a summary on nematode viability and other factors relating to the field trials.

In trial 1, *S. riobrave* were applied at 0, 11, 22, and 54 IJs/cm^2 and *H. bacteriophora* and *H. indica* at 11 and 22 IJs/cm^2 on June 2 and 4, 1998. Sponge-formulated heterorhabditid nematodes were released into a pail containing 15 liters of water prior to tank mixing. The suspension of nematodes was then added to the spray tank in 378.5 liters of well water with a pH of ~7 during sprayer pump agitation. Nematodes were applied using a tractor-mounted herbicide applicator with twin 1.5-m booms in approximately 153 liters of water/ha at a ground speed of 2.4 kmph. Each boom configuration had four fan nozzles (No. 10) located 30 cm apart to allow for coverage of the soil from the edge of the tree canopy to the trunk and one flood jet nozzle (No. 8-OC) mounted on the tip of each boom to cover the area (0.8-m strip) extending from the tree trunk toward the outer (opposite to applicator) margin of the tree. The filters were removed from all spray nozzles during application. The liquid formulation of *S. riobrave* was poured directly into the sprayer holding

TABLE 1

Summary of Data on Nematode Preparations and Field Application for Three Trials in 1998–1999

Trial No.	Nematode species	Type of formulation	% IJ viability[a]	Application time: Date	Hour	Conditions
1	*S. riobrave*	Liquid	74–79	6/4/98	1700–1900	Scattered clouds 32°C
	H. bacteriophora	Sponge	92–93	6/2/98	1700–2000	Scattered clouds 32°C
	H. indica	Sponge	75–78	6/2/98	1700–2000	Scattered clouds 32°C
2	*S. riobrave*	Water-dispersible granule	42.2	10/1/98	1400–1800	Cloudy 29.4°C
3	*S. riobrave*	Liquid	83–87	6/15/99	1400–1700	Partly cloudy 30.5–32°C
	H. indica	Paste	92–95	6/16/99	1400–1700	Partly cloudy 30.5–32°C

[a] Percentage based on five counts/treatment before and after spraying.

tank during pump agitation. The sponge formulation of *H. bacteriophora* was prepared for spraying using the procedure described above.

In trial 2, only *S. riobrave* was applied at 0, 22, 54, 108, and 216 IJs/cm^2. Due to low viability, additional clay product was mixed in 15 liters of water to achieve the appropriate rate prior to placement in the spray tank. Application was made on October 1, 1998 to the same experimental plots used in trial 1 (Table 1). Application procedures were the same as described in trial 1.

In trial 3, the liquid formulation of *S. riobrave* was applied at 0, 22, 54, and 108 IJs/cm^2 and a paste formulation of *H. indica* at 11, 22, and 54 IJs/cm^2 on June 15, 1999. Application procedures were identical to other trials, except that ground speed during application was 3.2 kmph (Table 1).

Prior to and after nematodes were applied, irrigation was applied for 2–3 h to assure soil moisture at 12–15% in the top 30 cm, assist nematode penetration into the soil, and stabilize soil temperature on the soil surface during application.

Nematode Parasitism

To estimate parasitism by nematodes and larval infection or predation by soil-borne microbes and ant predators, respectively, a single 6th instar larva of *D. abbreviatus,* produced on synthetic diet in the laboratory, was confined to a screened cage constructed from a plastic vial (5.9 ml by vol) and galvanized steel screen (2 mm mesh). The cylindrical cage was 7.5 cm in length and 2.0 cm in diameter. In the field, a hand-held auger was used to systematically make a single hole in the soil beneath the canopy of four trees, midway between the truck and the canopy margin to a depth of 20 cm for the insertion of the traps. Then, each cage was filled with moistened field soil collected from the region of subsequent cage placement, and a larva was added before capping. In trial 3, soil from each plot site was collected using the above procedure and taken to the laboratory the day prior to burial of the cages. The soil was then used to fill the larvae-baited screened cages. Four cages were buried under each of four trees per plot in the appropriate hole. Cages were marked at the soil surface with a colored flag attached to the end of a wire attached to the cage to designate treatment. Cages remained in the soil beneath the trees for 7 days at which time they were replaced with a new cohort introduced in another location in the soil beneath the tree canopy. This procedure was conducted at 0, 1, 2, 3, and 4 weeks posttreatment.

Within 12 h of retrieval from the soil, each cage was opened and the larvae were recovered for diagnosis. All healthy larvae exhibiting normal behavior were recorded as live. Dead larvae exhibiting gross signs of nematode infection (color change or visible nematodes) were recorded accordingly, but in cases in which suspected scavenger nematodes were detected on or in the cadaver, weevel larvae were placed in a disposable petri dish (50 × 9 mm) on a moistened filter paper with a greater wax moth larva (*Galleria mellonella* (L.)). Since wax moth larvae are highly susceptible to nematode parasitism (Kaya and Stock, 1997), they served as indicators to verify nematode infection. Other dead larvae were held at 23°C for 7 days to allow time for the expression of bacterial, fungal, or nematode parasitism. Bacterial and fungal identification was confirmed by the examination of diagnostic structures microscopically. In trial 3, nematodes from 31 randomly selected dead larvae from control plots were identified in the laboratory. Ant predators were observed on the soil surface while placing and recovering cages, particularly where the soil had been disturbed previously. In trials 1 and 2, the screened cages were constructed of standard window screen (2 mm) and were not ant proof, resulting in high larval predation. Cages void of larvae or containing visible larval remains suggestive of predation were identified as victims of ant predation. In trial 3, a similar cage made from an in-line liquid filter (7 × 3 cm diameter) with stainless steel screen (mesh size-225) was used to eliminate ant effects.

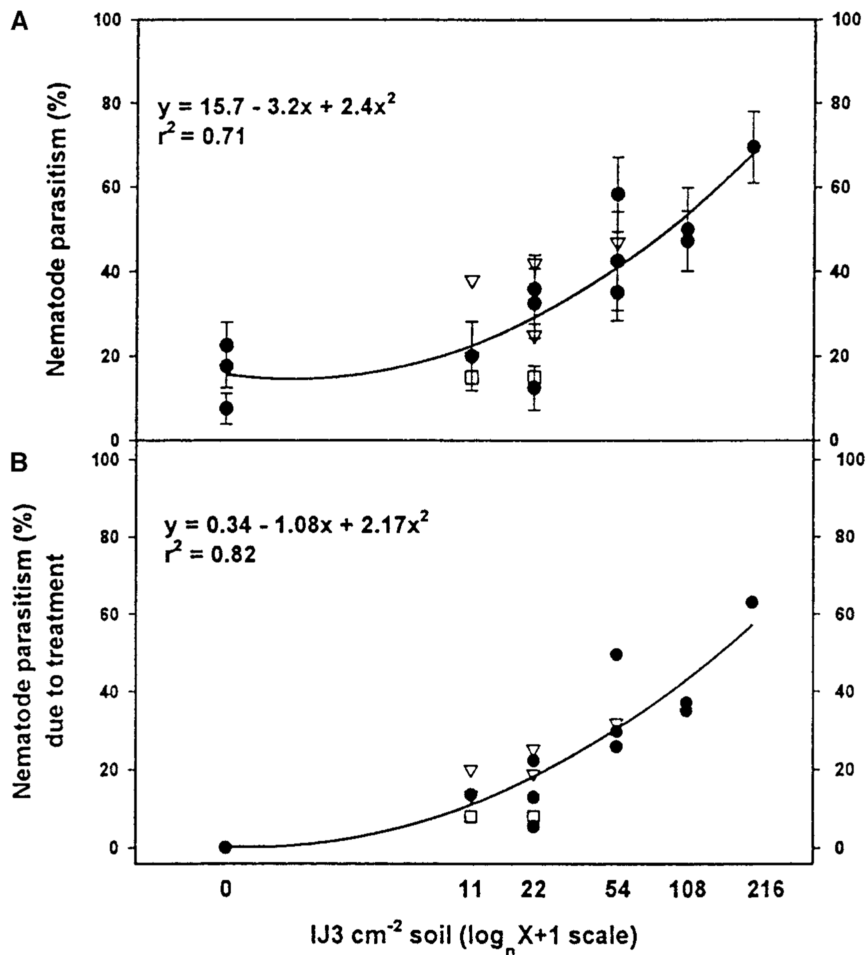

FIG. 1. Relationships, 1 week following application of different species of entomopathogenic nematodes, between application rate and (A) nematode parasitism of buried larvae of *Diaprepes abbreviatus* and (B) adjusted nematode parasitism of *D. abbreviatus* due to treatments. Data in A were corrected by Abbott's formula and redrawn in B. Data are from three experiments in the field using *Steinernema riobrave* (●), *Heterorhabditis bacteriophora* (□), and *H. indica* (▽). Standard errors of means are shown for the *S. riobrave* treatments. Regressions were performed on transformed ($\log_n \times + 1$) independent variables.

Larval Parasitism by Nematodes with and without Cages

To determine whether cages hinder larval parasitism, a test was conducted in the greenhouse using *S. riobrave, H. indica,* and a noninfested control. Fifth instar larvae were placed either in a cylindrical screened cage or in large plastic pots (19 liters) at a depth of 10 cm. Each treatment with or without cages was replicated four times in space and twice in time. About 36,000 IJs/pot (54 IJs/cm^2) of each species were applied per pot except for the control. Larval mortality was recorded after 7 days.

Nematode Persistence

Soil samples were taken in the field plots at 2, 7, 14, and 21 days after nematode application in a manner similar to that described by Duncan and McCoy (1996) and Duncan *et al.* (1996) to confirm establishment and persistence of each nematode species. In each trial, one soil sample was taken randomly at about 60 cm inside the canopy margin to a depth of 20 cm from each of four trees per plot. The four probe samples were pooled in a plastic bag and placed in a cooler to be returned to the laboratory. After thoroughly mixing the bag, a 60-ml (60-cm^3) aliquot was placed on wet filter paper in a modified Baermann funnel positioned over a pan of water. After 72 h, nematodes were recovered from the water trap and were poured into test tubes (70 ml). After decanting off excess water, all nematodes ranging in length from 0.4 to 0.7 mm and appearing like the reference samples were counted microscopically using a gridded petri dish. A known sample of live heterorhabditids and *S. riobrave* was used for reference.

TABLE 2

Parasitism of Caged 6th Instar Larvae of *Diaprepes abbreviatus* after 1 Week Exposure to Field Soil Treated with Different Rates of Three Species of Nematodes, *Steinernema riobrave* (Sr), *Heterorhabditis bacteriophora* (Hb), and *Heterorhabditis indica* (Hi) at Ft. Pierce, Florida (Trial 1)

	Rate	Mean % parasitism ± SE; days posttreatment			
Treatment	IJs/cm^2	7	14	21	28
Control	—	7.5 ± 3.8 a	10.8 ± 5.8 a	17.5 ± 6.5 a	7.5 ± 3.8 a
Sr	11	20.0 ± 8.2 a	20.8 ± 8.3 a	20.8 ± 5.2 a	2.5 ± 2.5 a
Sr	22	12.5 ± 5.6 a	23.3 ± 7.0 a	32.5 ± 9.9 a	10.0 ± 4.1 a
Sr	54	35.0 ± 6.7 a	30.0 ± 7.3 a	39.2 ± 6.2 a	12.5 ± 6.2 a
Hb	11	15.0 ± 10.0 a	17.5 ± 8.4 a	14.2 ± 7.8 a	10.0 ± 5.5 a
Hb	22	15.0 ± 5.5 a	10.8 ± 5.8 a	12.5 ± 4.2 a	7.5 ± 3.8 a
Hi	11	20.0 ± 6.2 a	14.2 ± 6.9 a	10.0 ± 4.1 a	5.0 ± 3.3 a
Hi	22	25.0 ± 8.3 a	15.0 ± 5.5 a	2.5 ± 2.5 a	10.0 ± 5.5 a

Note. Means followed by the same letter in each row are not significantly different at the 5% level of probability using SNK test.

Statistical Analysis

For each field test, an analysis of variance (ANOVA) and mean separation (SNK test, $P = 0.05$) were performed to determine the effect of field rates of nematodes on nematode parasitism and other mortality factors (i.e., ants, fungi, bacteria) for each posttreatment sampling date (Proc. GLM; SAS, 1989). A repeated measures analysis (Proc. Mixed; SAS, 1989) was used to analyze nematode parasitism for all sampling dates. The relationship between nematode application rate and larval parasitism at 7 days posttreatment for all field trials combined was determined via regression analysis (Proc. Corr.; SAS, 1989). Dependent variables for control mortality were transformed by Abbott's formula and regressed on nematode rate transformed to $\log_n (x + 1)$. Parasitism of caged versus noncaged *D. abbreviatus* larvae was analyzed using a *t* test (i.e., LSD; SAS, 1989). We used an $\alpha = 0.05$ in all statistical tests.

RESULTS

Nematode Parasitism

When data for all nematode species were pooled, the rate of nematodes applied to soil explained 71% of the variation in parasitism by nematodes of caged 6th instar larvae of *D. abbreviatus* 7 days following treatment (Fig. 1A). Eighty-two percent of the variation in data transformed by Abbott's formula (to remove effects of indigenous nematodes) was explained by nematode rate (Fig. 1B). The relationship between parasitism by nematodes ($\log_n$-transformed) and nematode rate was quadratic and positive.

In trial 1, percentage parasitism of caged 6th instar larvae of *D. abbreviatus* by indigenous nematodes was quite high in the control during the 28-day posttreatment period, ranging from 7.5 to 17.5% (Table 2). When the mean for each of four posttreatment sample dates for the different nematodes was analyzed separately using the SNK test, percentage parasitism was not significantly different from that of the control, ranging from 2.5 to 39.2% (Table 2). When nematode parasitism was analyzed over all sample dates, however, the highest rate of *S. riobrave* caused significantly greater nematode parasitism ($F = 3.17$; $df = 7{,}279$; $P = 0.003$) in *D. abbreviatus* than in the control. The linear relationship between parasitism and nematode rate at 7 days was significant ($r = 0.44$, $P = 0.01$).

In trial 2, percentage parasitism of caged 6th instar larvae of *D. abbreviatus* by indigenous nematodes ranged from 9.3 to 38.0% in the control during the 35 days posttreatment period (Table 3). At 7 days posttreatment, larval parasitism was significantly different ($F = 6.64$; $df = 4{,}33$; $P = 0.0005$) from that of the control at 54 and 216 IJs/cm^2 (Table 3). The highest rate at 216 IJs/cm^2 resulted in 69.4% parasitism. The linear correlation of nematode rate and parasitism was 0.5 ($P = 0.001$). At 2 weeks posttreatment and thereafter, no significant differences were found between treatments and control (Table 3).

In trial 3, percentage parasitism of caged 6th instar larvae of *D. abbreviatus* in the control ranged from 27.5 to 41.6% (Table 4). When each sample date was analyzed separately via the SNK test, differences in nematode parasitism were not detected among treatments, although the linear trend for parasitism and rates was significant ($r = 0.34$, $P = 0.03$) 7 days after application. Nematodes found among the 31 positive samples from the control plots were identified as *S. glaseri* (Steiner), *H. bacteriophora,* and *S. riobrave.*

TABLE 3

Parasitism of Caged 6th Instar Larvae of *Diaprepes abbreviatus* after 1 Week Exposure to Field Soil Treated with Different Rates of *Steinernema riobrave* (Sr) at Ft. Pierce, Florida (Trial 2)

	Rate	Mean % parasitism ± SE; days posttreatment				
Treatment	(IJs cm^2)	7	14	21	28	35
Control	—	17.5 ± 5.3 c	38.0 ± 6.8 a	23.3 ±7.0 a	13.9 ± 6.1 a	9.3 ± 6.3 a
Sr	22	35.8 ± 8.4 bc	52.5 ± 8.7 a	20.0 ± 6.2 a	32.5 ± 8.4 a	7.5 ± 3.8 a
Sr	54	58.0 ± 8.9 ab	42.5 ± 6.5 a	17.5 ± 5.3 a	20.9 ± 9.0 a	10.8 ± 5.8 a
Sr	108	43.8 ± 7.8 abc	41.7 ± 5.9 a	37.5 ± 10.6 a	25.0 ± 10.6 a	12.5 ± 6.7 a
Sr	216	69.4 ± 9.1 a	47.2 ± 7.7 a	19.4 ± 6.9 a	25.0 ± 9.3 a	11.1 ± 4.4 a

Note. Means followed by the same letter in each row are not significantly different at the 5% level of probability using SNK test.

Larval Parasitism by Nematodes with and without Cages

In all treatments (control, *H. indica, S. riobrave*), no statistical differences were detected in nematode parasitism in caged *D. abbreviatus* larvae compared with noncaged larvae according to *t* tests. Mean percentage mortality (±SE) for noncaged versus caged larvae were 7.5 (3.7) versus 5.0 (3.3), 64.4 (8.0) versus 57.1 (11.1), and 70 (11.2) versus 74.3 (9.5) for the control, *H. indica,* and *S. riobrave,* respectively.

Nematode Persistence

In trials 1 and 2, the number of nematodes recovered from all plots decreased substantially by 14 days posttreatment (Fig. 2). In trial 1, the average recovery of *H. indica* and *H. bacteriophora,* 7 days posttreatment, was greater than that in the control, and the recovery of *H. indica* was greater than that of *S. riobrave.* In trial 2, 7 days after application, the number of nematodes that were recovered increased significantly ($r = 0.73$, $P = 0.001$) as the rate of application increased.

Comparison of Mortality Factors

In trial 1, ant predation was frequently observed in the larvae-baited cages. In comparison, ant predation caused significantly more larval mortality than other mortality factors. Ant predation increased significantly ($F = 11.54$; $df = 3{,}307$; $P = 0.0001$) over time (Fig. 3A) in a linear manner ($r = 0.54$), reaching ~90% at 28 days posttreatment. According to casual observation, *Solenopsis invicta* Buren appeared to be the dominant species.

In trial 2, mortality due to ant predation and nematode parasitism was greater than other mortality factors for caged larvae in the soil, but not significantly. Ant predation increased in time through 28 days posttreatment (~48%) but declined significantly at 35 days to ~28% (Fig. 3B). In trial 3, ants were excluded from the cages.

In trial 1, on three occasions, the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, was found infecting 2.5–5.0% of the larvae contained in the screened cages. Likewise, *Serratia* sp. was detected infecting about the same number of larvae. Less than 10% of the larvae died of unknown causes.

In trial 2, both *Serratia* and *B. bassiana* were found once infecting 2.8% of the larvae. From 0 to 2.5% of the larvae died of unknown causes. Other mortality factors were not monitored in trial 3.

TABLE 4

Parasitism of Caged 6th Instar Larvae of *Diaprepes abbreviatus* after 1 Week Exposure to Field Soil Treated with Different Rates of Two Species of Nematodes, *Steinernema riobrave* (Sr) and *Heterorhabditis indica* (Hi) at Ft. Pierce, Florida (Trial 3)

	Rate	Mean % parasitism ± SE; days posttreatment				
Treatment	IJs/cm^2	7	14	21	28	35
Control	—	27.5 ± 6.9 a	27.5 ± 6.9 a	25.0 ± 8.3 a	41.6 ± 8.2 a	33.3 ± 8.3 a
Sr	22	38.9 ± 7.3 a	27.5 ± 9.5 a	37.5 ± 10.7 a	45.0 ± 9.7 a	32.5 ± 7.5 a
Sr	54	47.5 ± 10.8 a	22.5 ± 7.9 a	27.5 ± 8.7 a	58.3 ± 9.6 a	27.5 ± 8.7 a
Sr	108	52.5 ± 8.7 a	27.5 ± 7.9 a	40.0 ± 7.6 a	55.0 ± 8.2 a	37.5 ± 6.7 a
Hi	11	37.5 ± 7.7 a	22.5 ± 8.7 a	35.0 ± 6.7 a	46.6 ± 8.4 a	35.0 ± 7.6 a
Hi	22	42.5 ± 8.4 a	32.5 ± 9.9 a	19.4 ± 6.9 a	40.0 ± 5.5 a	44.1 ± 12.6 a
Hi	54	47.5 ± 9.5 a	25.0 ± 9.1 a	45.0 ± 11.7 a	40.0 ± 10.7 a	37.5 ± 4.2 a

Note. Means followed by the same letter in each row are not significantly different at the 5% level of probability using SNK test.

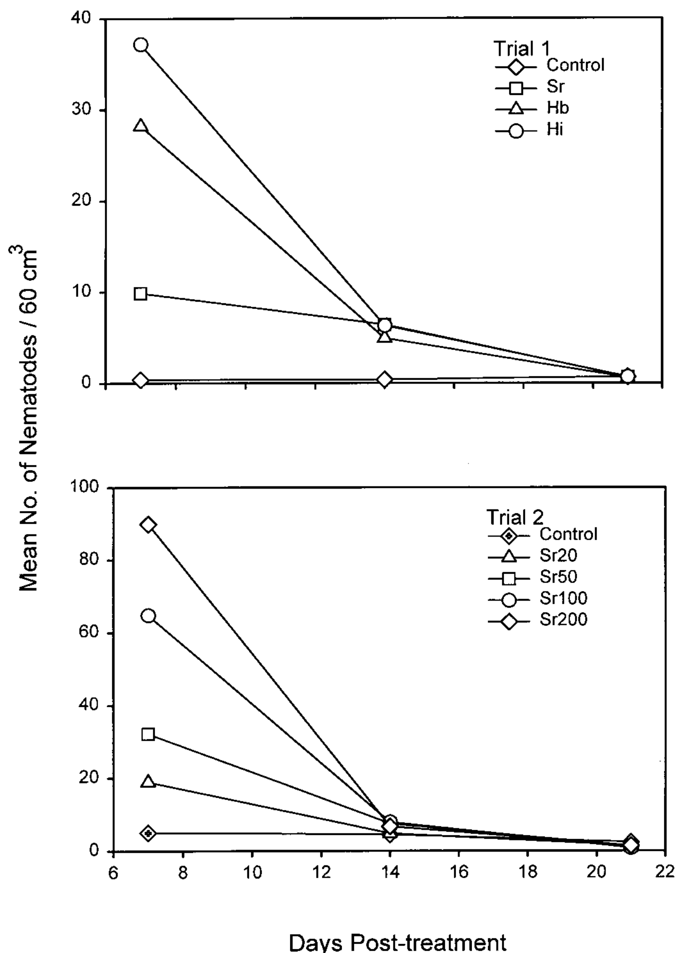

FIG. 2. Mean number of *Steinernema riobrave* (Sr), *Heterorhabditis bacteriophora* (Hb), and *H. indica* (Hi) recovered from the soil at different time intervals postapplication using Baermann nematode extraction for two field trials.

DISCUSSION

In field trials and supplemental experimentation presented herein, we demonstrated that cages are not barriers to infection in the soil by invasive nematodes and other mortality factors. Campbell and Gaugler (1993) demonstrated that active host-searching nematodes, such as *S. riobrave* and *Heterorhabditis* spp. (Lewis *et al.,* 1992; Grewal *et al.,* 1997), are more successful in finding immobile hosts than mobile hosts. Based on those results, we might have expected greater parasitism in caged larvae than in noncaged larvae, but we did not detect differences. Our data suggest that the larvae-baited cage is useful in detecting rate responses among treatments (Fig. 1) and, therefore, has potential as a method alternative to that of destructive sampling of trees and monitoring of adult emergence. These alternative methods are labor intensive and not always feasible in citrus. However, what caged parasitism measured for a relatively short period of time (7 days) might mean, in terms of reduction of natural larval populations, is not known. In other words, 50% parasitism in cages may not equal 50% reduction under the tree. It is reasonable to assume that a caged host cannot avoid the nematode(s) by moving away, resulting in higher parasitism. Alternatively, an exposure time of 1 week will likely result in a lower level of parasitism than one lasting for a longer time.

The effect of inundative applications of different species and rates of entomopathogenic nematodes on caged larvae of *D. abbreviatus* was often not statistically significant, due to high levels of nematode parasitism in the controls (Tables 2–4). Rates of 54 IJs/cm^2 or higher were required to achieve levels of parasitism that were significantly different from that of the controls (Fig. 1). Nematodes identified from the various treatments suggest that *S. glaseri* was native, but *H. bacteriophora* was either native or introduced to the soil of our study site. Since *S. riobrave* is reported only from south Texas, it is unlikely that it is native to Florida. Therefore, *S. riobrave* was introduced the pre-

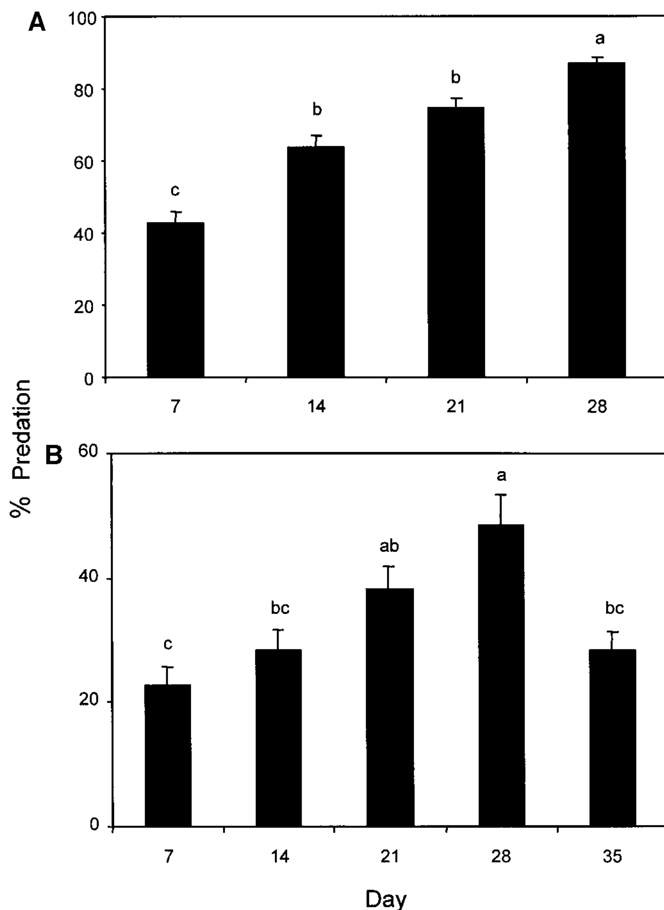

FIG. 3. Percentage predation by various species of ants attracted to cages baited with *Diaprepes abbreviatus* in time for trials 1(A) and 2(B).

vious year, suggesting that it is capable of recycling in this grove. Indigenous entomopathogenic nematodes have been reported from citrus groves using caged larvae of *D. abbreviatus* as indicators (Beavers *et al.,* 1983). Interestingly, these authors reported that the abundance of these nematodes was greatest from June through October, which is the same time that our three field trials were conducted in 1998–1999. These data suggest that when parasitism by indigenous nematodes is very high, the grower will benefit from higher parasitism only when using rates of 54 IJs/cm^2 or more using an inundative application. If indigenous nematodes persist in the summer months, as all data suggest (Beavers *et al.,* 1983; Duncan *et al.,* 1996; Fig. 4), it would appear that the inundative approach offers limited benefit to the grower, particularly when the cost of high rates of nematodes are a consideration. Inundative applications at other times in the year or perhaps in other fields with lower endemic populations may be more beneficial. From another perspective, groves with a history of indigenous nematodes within a season may never require an inundative application. From a research standpoint, if one wants to obtain clear differences between treatments including the control, an appropriate nematicide will be required to reduce the indigenous nematode population.

The mean number of nematodes/60cm^3 of soil recovered at 2 days posttreatment was significantly higher in the treatments than in the controls in both trials using the Baermann extraction method; however, nematode recovery was virtually the same in all treatments at 14 days posttreatment. This suggests that nematodes experienced rapid death in the soil or were washed out quickly from the top 0–20 cm of soil. This rate of persistence in Pineda sandy soil common to coastal Florida agrees with the findings of Duncan *et al.* (1996) and Duncan and McCoy (1996) for astatula fine sandy soil common to the interior ridge. Both soils are sandy loam type with low organic matter and clay. In terms of persistence in the upper soil horizon, Duncan and McCoy (1996) showed that, 7 days following application, the number of nematodes for two species declined by >99% at <1 cm depth, by 69–76% at 1–3 cm depth, and by 64–76% at 3–15 cm depth.

Numerous species of ants are the predominant predators of the larvae of *D. abbreviatus* on and in the soil beneath the tree of citrus groves in Florida (Whitcomb *et al.,* 1982) and regions of the Carribean (Jaffe *et al.,* 1990). Field observations by several investigators indicated that larval mortality is extremely high on the surface and in the soil after larval entry (Whitcomb *et al.,* 1982). In disturbed situations, *S. invicta,* 1 of 30 species of ants reported from citrus, increases population levels more quickly than other species (Lemke and Kissam, 1988; Tedders *et al.,* 1990). In this study, ant foraging, mainly by *S. invicta* and *Brachymyrmex obsurior* Forel, on larval-baited cages appeared to be high (40–85%) in both trials 1 and 2 where ant proof cages were not used as detection units. In fact, percentage predation increased in time, suggesting that our disturbance of the soil when collecting and burying the cages stimulated ant foraging (Figs. 3A and 3B). Foraging ants were observed in association with larval cadavers, suggesting that larvae infected with nematodes were consumed, on occasion, by ants. Baur *et al.* (1998) reported foraging ants as scavengers of steinernematid-killed and, to a lesser extent, heterorhabditid-killed hosts. This activity may reduce chances of nematode recycling and may have reduced our estimate of nematode parasitism. However, comparison of the data from trials 1 and 2 where ants were not excluded to trial 3 with ant exclusion show overall similarity in nematode parasitism (Figs. 3A and 3B).

In conclusion, it would appear that indigenous nematodes and ant predators in some citrus groves are the key natural enemies of *D. abbreviatus* larvae in the soil. An understanding of the economic benefits of biological control by these organisms will require long-term evaluation of tree growth and productivity.

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