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Estimating Sample Size and Persistence of Entomogenous Nematodes in Sandy Soils and Their Efficacy Against the Larvae of *Diaprepes abbreviatus* in Florida[1]

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Abstract: In two studies to estimate sampling requirements for entomogenous nematodes in the field, highest persistence of *Heterorhabditis bacteriophora* after application occurred beneath the canopies of mature citrus trees. Nematode persistence declined with distance from the center-line of the tree row toward the row-middles. Immediately after nematode application to soil, 32 samples (15 cm deep, 2.5-cm diameter) beneath a single tree were required to derive 95% confidence intervals that were within 40% of mean nematode population density. The estimated probability of measuring the mean density within 40%, using 32 samples, declined to 88% at 2 days post-application and to 76% at 7 days. The persistence in soil of *Steinernema carpocapsae, S. riobravis,* and two formulations containing *H. bacteriophora* and their efficacy against the larvae of *Diaprepes abbreviatus* were compared in a grove of 4-year-old citrus trees. Within 6 days, the recovered population densities of all nematodes declined to <5% of levels on day 0. The recovery of *H. bacteriophora* during the first 2 weeks was lower than that of the other two species. *Steinernema riobravis* and both formulations of *H. bacteriophora* reduced recovery of *D. abbreviatus* by more than 90% and 50%, respectively. *Steinernema carpocapsae* did not affect population levels of the insect.

Key words: control, *Diaprepes abbreviatus,* efficacy, entomopathogenic nematode, *Heterorhabditis bacteriophora,* sampling, *Steinernema riobravis,* survival.

Diaprepes abbreviatus (L.) is one of five species of root weevils whose larvae are destructive to the roots of citrus trees, various ornamental plants, and other important agricultural crops in Florida (37). On citrus, the female oviposits between leaves. As the eggs hatch, the neonate larvae drop to the ground, burrow into the soil, and feed on all root parts for approximately 1 year before leaving the soil as adults (34). Larval feeding on the roots of citrus can cause severe tree decline and mortality. Since its discovery in Florida (36), this weevil has spread to 15 major citrus-growing counties within the state and is considered an important long-term threat to agriculture (22).

In a soil survey of commercial citrus groves and ornamental nurseries, Beavers et al. (3) found indigenous strains of entomopathogenic fungi and nematodes that were infectious to the larvae of *D. abbreviatus.* Two species of nematodes recovered in the soil survey, *Steinernema carpocapsae* (Weiser) and *Heterorhabditis heliothidis* (Kahn, Brooks & Hirschmann), belong to the families Steinernematidae and Heterorhabditidae, which contain strains of species being developed commercially and applied as biological insecticides against a number of soil-inhabiting pests throughout the world (18).

During the past decade, many laboratory and commercially produced nematode preparations of different species and (or) strains from the above families have been evaluated in the greenhouse and the field and have been found to have potential as microbial control agents for larvae of *D. abbreviatus* (2,8,25–31). Although no data have been presented on natural levels of parasitism of *Diaprepes* larvae by entomogenous nematodes, applications of 100 *S. carpocapsae* (26) or *H. bacteriophora* (8) per cm^3 soil reduced adult *D. abbreviatus* emergence from treated soil compared to untreated soil by approximately 70% in the following year. In 1989, a commercial formulation of *S. carpocapsae,* under the trade name BioVector (biosys Corp., Palo Alto, CA) was registered to control root weevil larvae in Florida citrus groves. In

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August 1994, the nematode in the commercial preparation BioVector was replaced with a new species, *S. riobravis* (Cabanillas, Poinar & Raulston), and labeled as BioVector 355. This paper presents the first data on the efficacy of *S. riobravis* against the larvae of *D. abbreviatus* in the field.

Soil texture (20,24), soil temperature (23), soil moisture (7,24), ultraviolet radiation (13), and natural antagonists (15) are important abiotic and biotic factors that affect the persistence of nematodes in the soil (14). The effects of these factors on the nematode populations cannot be studied in the field unless a sampling method is devised to monitor nematode persistence. Quantitative sampling of entomopathogenic nematodes, subsequent to artificial application, to estimate persistence and spatial patterns has not been studied in citrus orchards affected by subtropical climatic conditions. Most published research employs less quantitative nematode-host bioassay methods (4,32). Our study addressed both nematode sample size and an in vitro nematode-host bioassay using *Galleria mellonella* to estimate persistence and efficacy in the soil of *S. carpocapsae, S. riobravis,* and *H. bacteriophora.* In addition, we will report, for the first time, the use of a destructive tree-soil sampling procedure for measuring the efficacy of entomopathogenic nematodes against *D. abbreviatus.*

Materials and Methods

Estimating sample size: Soil beneath two 15-year-old grapefruit trees on Volkamer lemon rootstock was treated at a rate of 2 $\times$ 10^6 nematodes/m^2 with infective third-stage *Heterorhabditis bacteriophora* commercially formulated in an inert clay carrier (Otinem, Ecogen Australia Pty. Ltd.). One tree was treated on 13 February 1994 (experiment 1), the other on 28 February 1994 (experiment 2). A rectangular area (4.6 m $\times$ 7.0 m) centered on the tree was sprayed with 2 liters of water-nematode suspension using a pressurized sprayer with a flat-fan nozzle. Soil was irrigated to field capacity on 28 February 1994 and irrigated by hand for 10 minutes immediately following treatment on both dates using a garden hose spray attachment. Soil type under both trees was Astatula fine sand (92% sand, 2% silt, 6% clay).

In experiment 2, 100 cores of soil (2-cm d $\times$ 30 cm deep) were removed from the treated area at 0, 2, and 7 days following treatment and divided into portions from 0–15 cm and 15–30 cm depths. Sampling was identical in experiment 1, except that on day 7 following treatment, four composite samples (each consisting of 16 cores of soil) were collected to estimate nematode population density. Cores were removed at grid intersections located by extending a string at 10 equidistant intervals along each side of the rectangular area. The grid was shifted 15 cm on successive sides of the treated area after each sample date to avoid resampling the same points.

To detect migration of nematodes from the treated area, 15 cores of soil were also taken in a line parallel to and 1 m distant from each edge of the treated area on each sample date. The 15 cores along each side were pooled into single samples. To monitor background levels of heterorhabditid nematodes, 15 cores of soil were also taken from beneath four untreated control trees on each sample date and pooled into a single sample per tree.

Nematodes were collected from soil on modified Baermann funnels for 48 hours. All of the soil (47 cm^3) in single core samples was processed. Composite samples were mixed, and three 50-cm^3 subsamples per sample were processed.

Means and variances of nematode counts (n = 100) at each depth, on each sample date, in both experiments were used to estimate parameters for Taylor's Power Law (33). Sample size to estimate population density with specified precision (d) was estimated from the formula

$$n = \left(\frac{z}{d}\right)^2 a\bar{x}^{b-2} \qquad (1)$$

where n = number of cores of soil, z = the

standard normal variate (1.96 for $P = 0.05$), d = the half-length of a confidence interval expressed as a proportion of the mean ($\bar{x}$), and a and b are parameter estimates from Taylor's Power Law (11). To estimate the probability that mean estimates were within specified levels of precision, for arbitrary values of n and d, Equation 1 was rearranged as

$$z = \sqrt{\frac{nd^2}{a\bar{x}^{b-2}}} \qquad (2)$$

and the probability for a given value of z was obtained from a table of areas beneath the normal curve (11).

Nematode persistence and efficacy against citrus root weevil larvae: Three species of entomopathogenic nematodes, *Steinernema carpocapsae* (BioVector), *Steinernema riobravis* (BioVector 355), and *Heterorhabditis bacteriophora,* were applied at defined rates as a soil drench beneath the canopy of 4-year-old Hamlin orange trees on Swingle citrumelo rootstock planted in an Astatula fine sand. At the time of the test, all citrus trees showed severe tree decline from *D. abbreviatus* larval injury. They had an average of 16 late instar larvae per tree based on sampling conducted 2 weeks before the test. The trees had received routine fertilization twice per year, regular irrigation, and herbicide applications for weed control. No pesticides were applied to the soil.

Three rows consisting of 48 trees, each approximately 2 m high with canopies 2 m in diameter, were selected for the study. All treatments, including an untreated control, were randomized as single tree plots and replicated 10 times except for two treatments containing *H. bacteriophora,* which were limited in quantity. These treatments were replicated four times. Prior to treatment, weeds and debris found beneath the tree canopy were removed and soil barriers were constructed at the dripline of each tree to confine irrigation water applied manually from a truck-mounted tank. One day before treatment, the soil within each ring (about 1.5 m d) received approximately 115 liters of water. Before nematode application, all trees received 38 liters of water to assure uniform soil moisture to a depth greater than 60 cm.

A commercial polymer base formulation (BioVector, biosys Corp., Palo Alto, CA) containing infective juveniles of *S. carpocapsae* with an estimated viability of 97%, based on pre-treatment motility counts in the field, was applied at rates of 2.7 and 5.4 million nematodes/tree. Likewise, an experimental polymer base formulation (biosys Corp., Palo Alto, CA) containing infective juveniles of *S. riobravis* with an estimated viability of 95% was applied at a rate of 2.1 million nematodes per tree. Two experimental preparations, a liquid and granular formulation (Ecogen Corp., Langhorne, PA), containing infective juveniles of *H. bacteriophora* with an estimated viability of 81 and 76%, respectively, were applied at rates of 4.5 and 3.1 million nematodes per tree, respectively. All treatments were applied in 7.6 liters of water using a sprinkling can on 6 April 1994, in late afternoon under partially cloudy skies at an ambient temperature of 31 C. Following field application, an additional 8 liters of water was added to each plot to aid in the vertical movement of the nematodes into the soil. Thereafter, 57 liters of water was applied to each plot on days 5, 8, 12, 14, 19, and 23 post-treatment.

Rainfall and soil moisture were monitored throughout the trial (Fig. 1). Soil temperatures (15-cm depth) ranged from 24 to 28 C during the experiment.

Two soil samples, each comprised of 16 cores (1-cm d × 15-cm deep), were collected from each plot on 0, 5, 12, and 21 days post-treatment. Samples were collected by removing four equidistant cores of soil from each of four equally spaced lines radiating from the trunk to the dripline. The two samples per plot were mixed and three, 50-cm^3 subsamples were extracted on modified Baermann funnels for 48 hours. The three subsamples were combined before counting the entomogenous nematodes.

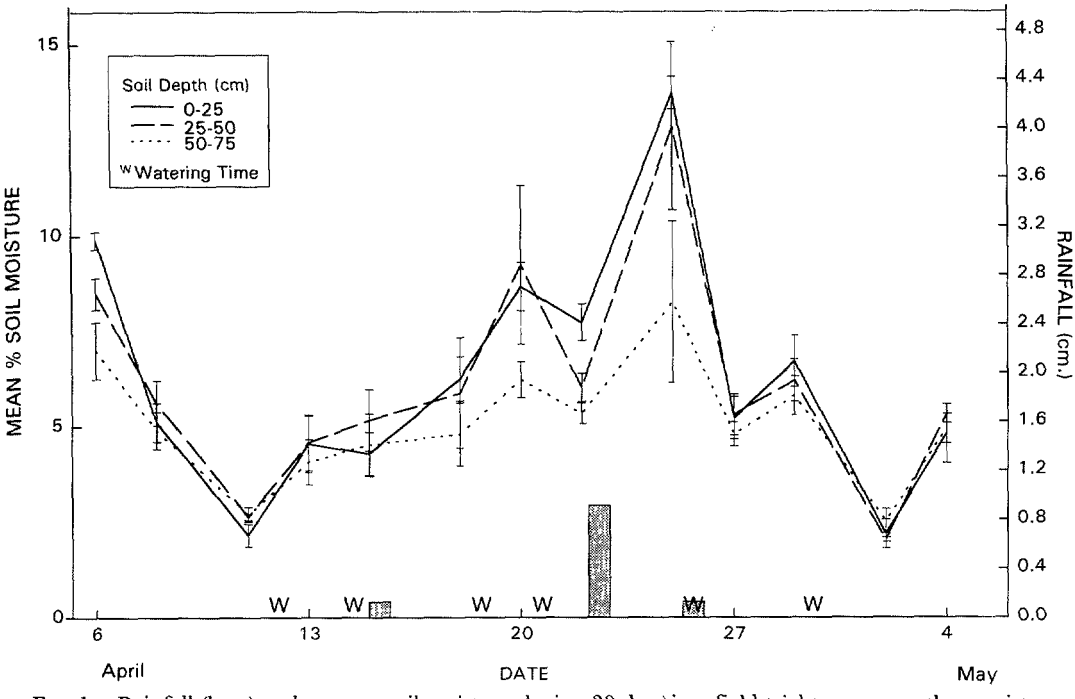

FIG. 1. Rainfall (bars) and average soil moisture during 30 days in a field trial to measure the persistence of entomogenous nematodes and their efficacy against larvae of *Diaprepes abbreviatus*.

Taylor's Power Law was fitted to the means and variances of nematode counts for each treatment on each sample date to estimate experimental replication requirements. Equations 1 and 2 were used to derive estimates of treatment replication requirements and to investigate sampling precision associated with treatment replication of arbitrary number and precision.

Nematode persistence during the first 5 days of the experiment was estimated by dividing the numbers of nematodes recovered on day 5 post-treatment by recovery on day 0. Analysis of variance was performed on transformed (arcsin square root) data, and differences in treatment means were evaluated using Tukey's honestly significant difference procedure. Since there was no difference in persistence between the two rates of *S. carpocapsae,* those data were pooled for comparison with the other treatments. Differences in persistence at subsequent times were not evaluated because after day 5 entomogenous nematodes were not detected in the majority of plots of all treatments.

To estimate biological activity of the different nematodes in the field over time, soil was taken from the same sample collected to estimate nematode population density. After mixing, 70 cm^3 of soil (5–10% moisture content) was placed in each of 10 standard petri dishes, and two *D. abbreviatus* and two *G. mellonella* larvae were added per dish. After a 6-day exposure time, larval mortality was determined. To confirm parasitism, both live and dead larvae were dissected in water to release juvenile and(or) adult nematodes. Nematodes were then identified to genus.

On 9 and 10 May 1994 (33–34 days post-treatment), the *D. abbreviatus* larval population density within the tree rhizosphere to a depth of 60 cm was estimated using a tree removal-soil sampling procedure. On this date, the majority of larvae were 2.0–2.5 cm long (>5th instar) and easily detected in the soil. Trees were uprooted using a front-end loader with a fork head attachment. By vigorously shaking the trees in situ, most of the soil adhering to the roots was removed along with any lar-

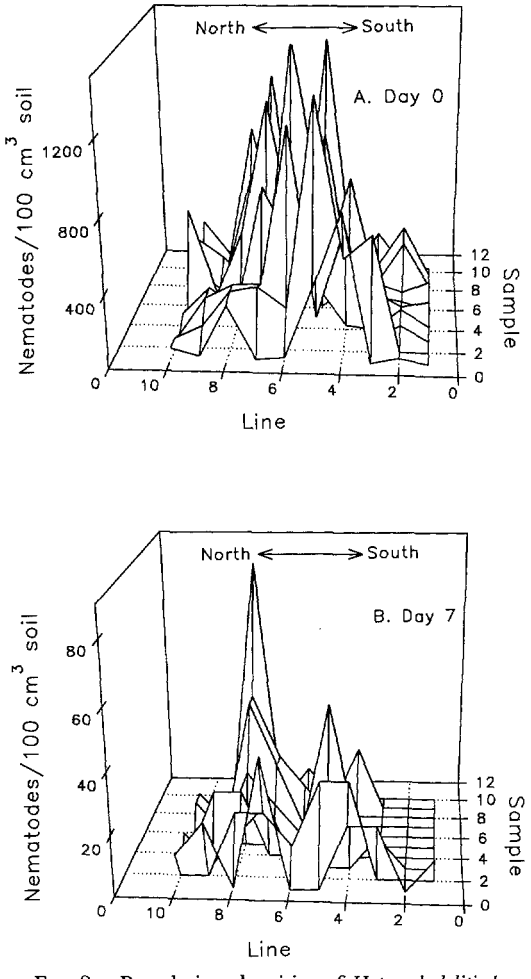

FIG. 2. Population densities of *Heterorhabditis bacteriophora* recovered from 100 individual cores of soil from a grid centered on a mature citrus tree. Nematodes recovered 1 hour after application (A) and 7 days after application (B). Center line of tree row lies between east-west lines 5 and 6.

vae. The tree was then moved into the row middle and the soil lodged in the crown area removed by flushing the root zone with high-pressure water delivered by a handgun sprayer. Larvae from the crown area of the plant were collected on a plastic tarp placed on the ground. To estimate the number of larvae remaining in the upper soil dislodged from roots (30-cm depth) and lower rhizosphere (30–60-cm depth), 0.28 m^3 of soil was randomly collected using a shovel and sieved through a coarse screen to separate the larvae from the soil. The number of larvae per tree was recorded per given volume of soil. Because the quantity of soil lodged within the root crown varied from tree to tree depending on root density, it was impossible to determine the volume of soil extracted from the crown. However, the quantity was at least 20-fold less than the quantity processed in the upper and lower rhizosphere. Differences in larval population density between treatments were tested by analysis of variance and mean separation by Tukey's honestly significant difference procedure.

RESULTS

Estimating sample size and treatment replication requirements: Population density of *H. bacteriophora* was higher under the tree canopy than in the unshaded row-middles in the second experiment (Fig. 2A,B). Immediately following treatment, the average nematode density (10 single cores/line) recovered from each line running parallel to the tree row was approximately seven

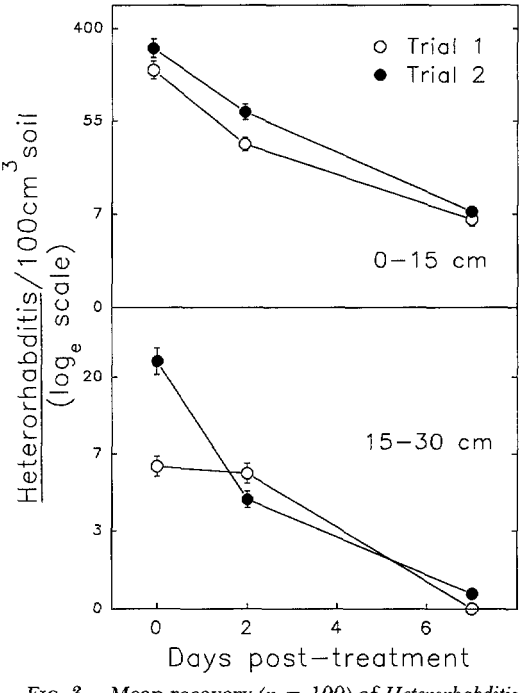

FIG. 3. Mean recovery (n = 100) of *Heterorhabditis bacteriophora* between 0 and 7 days following application to soil beneath a mature citrus tree. Nematodes were recovered from 0–15-cm depth (A) and 15–30-cm depth. Bars represent mean standard error.

times higher ($P = 0.001$) in the middle two lines of samples than in the two southernmost lines and nearly three times greater ($P = 0.01$) than in the two northernmost lines of samples. Linear correlation between $\log_e$ nematode density and distance from the center-line of the row was -0.42 ($n = 100$, $P \leq 0.001$). This pattern persisted throughout the experiment. There were no differences in nematode population densities for sample lines perpendicular to the tree rows. The same trends were clearly evident in the first sample-size experiment (data not shown), although the data were more variable.

Population density of *H. bacteriophora* recovered from single cores of soil 0–15-cm and 15–30-cm deep generally declined exponentially during the first 7 days of both trials, creating a wide range of means and associated variances (Fig. 3A,B). Estimated nematode recovery on day 0 was 14% and 28% in the first and second experiments, respectively.

In the first sample-size experiment, no heterorhabditid nematodes were recovered in the composite samples, taken 1 m from the treated area. *Heterorabditis* spp. were recovered from beneath one of the four control trees at densities of 0.0, 2.0, and 6.0 nematodes/100 cm^3 soil on days 0, 2, and 7. In the second sample-size experiment, no heterorhabditid nematodes were detected beneath control trees. *Heterorhabditis* spp. were recovered 1 m distant from the treated areas in three different directions at mean densities of 2.0, 8.0, and 4.0 nematodes per 100 cm^3 soil on days 0, 2, and 7 following treatment.

The parameters of Taylor's Power Law fit to single-core data from both sample-size experiments and both soil depths were $a = 5.64$ and $b = 1.77$ ($r^2 = 0.99$, $P = 0.01$, Fig. 4). From Equation 1, the population density at which 32 cores of soil would result in mean density estimates within 40% of μ, 95% of the time, is approximately 600 nematodes/100 cm^3 soil.

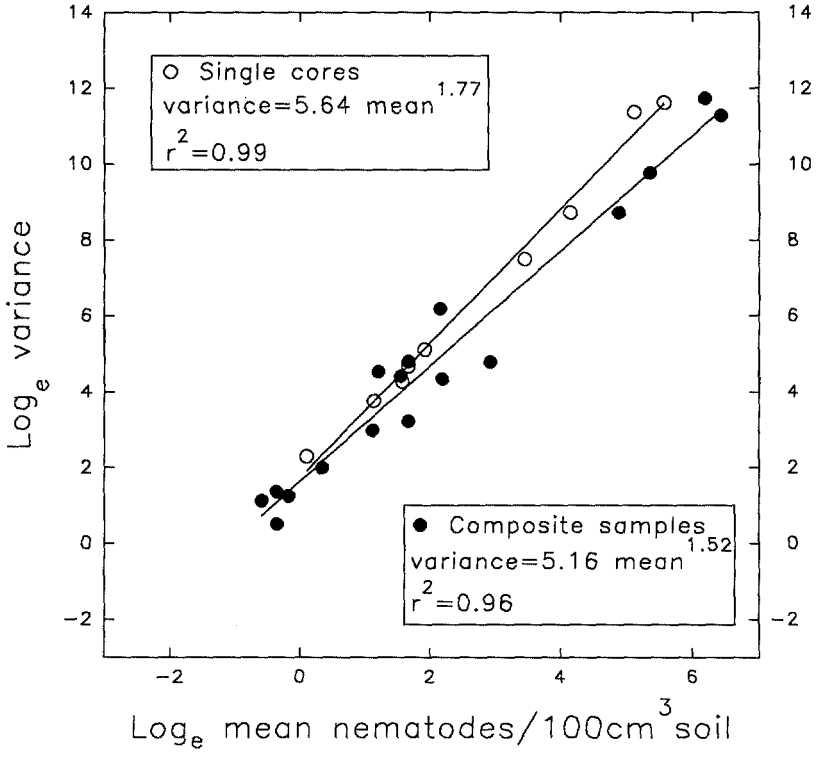

FIG. 4. Fit of Taylor's Power Law ($S^2 = a\bar{x}^b$) to entomogenous nematode count data from single cores of soil and from subsamples of soil samples comprised of 16 single cores of soil.

From Equation 2, the respective probabilities of attaining at least 40% precision from 32 cores of soil for population densities of 600, 60, and 6 nematodes per 100 cm^3 soil is 0.95, 0.88, and 0.76, (Fig. 5A).

In the field trial to measure the persistence and efficacy of entomogenous nematodes against *D. abbreviatis,* the population densities of all steinernematid species declined exponentially during the first 14 days (Fig. 6). *Heterorhabditis bacteriophora* population density declined during the first week in a manner similar to the previous two studies.

Recovery on day 0 was estimated to be

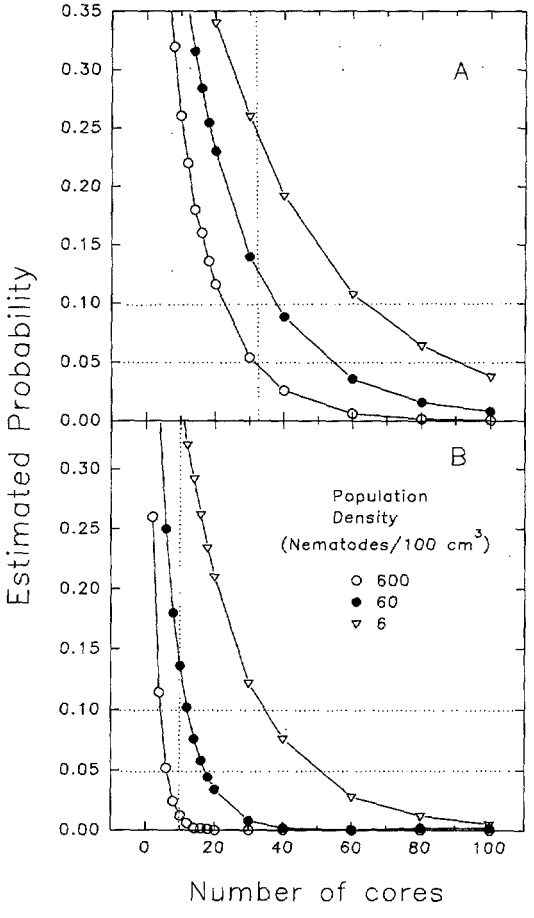

FIG. 5. Estimated probabilities that an estimate of nematode population density is beyond 40% of μ for different sample sizes comprised of counts from single cores of soil from beneath a single tree (A) or 32-core samples collected systematically from beneath randomly chosen trees (B).

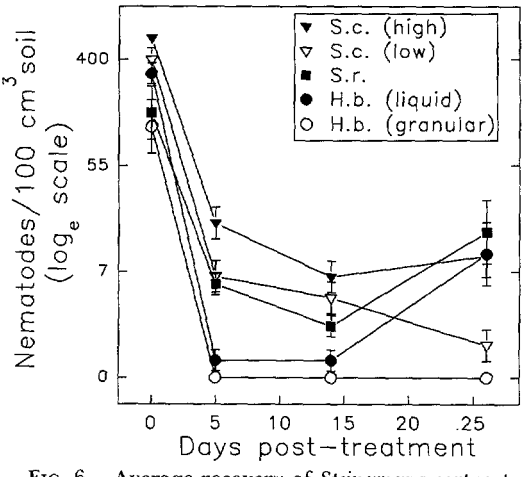

FIG. 6. Average recovery of *Steinernema carpocapsae* (S.c.), *S. riobravis* (S.r.), and *Heterorhabditis bacteriophora* (H.b.) (n = 10 for S.c. and S.r.; n = 4 for H.b.) between days 0–26 following application to soil beneath 4-year-old citrus trees. Bars represent mean standard error.

45% for *S. carpocapsae* (low rate), 31% for *S. carpocapsae* (high rate), 18% for *S. riobravis,* 19% for *H. bacteriophora* (liquid formulation), and 10% for *H. bacteriophora* (granular formation).

Parameter values of Taylor's Power Law fit to these composite-sample data were a = 5.16 and b = 1.52 (r^2 = 0.96, P = 0.01, Fig. 4). From Equation 1, the population density at which 10 replicate plots (32 core samples per plot) would result in mean density estimates within 40% of μ, 95% of the time, is approximately 200 nematodes/100 cm^3 soil. From Equation 2, the respective probabilities of attaining at least 40% precision from 10 replicate plots (32 cores/plot) for population densities of 600, 60, and 6 nematodes per 100 cm^3 soil are 0.99, 0.86, and 0.61 (Fig. 5B).

Nematode persistence: Persistence of *H. bacteriophora* in the 0–15-cm horizon 2 days post-treatment was 18.8% and 23.6% in the first and second sample-size experiments (Fig. 3A). By 7 days post-treatment, persistence in the two experiments was estimated to be 3.5% and 2.4%, respectively. Migration to lower soil depth did not appear to be a major factor in persistence because population densities at the 15–30-

cm depth declined in a manner similar to those in the 0–15-cm depth (Fig. 3B).

Persistence of *S. riobravis* (4.4%) between days 0 and 5 in the efficacy field trial (Fig. 6) was greater ($P = 0.05$) than that of *S. carpocapsae* (2.1%). Survival of *H. bacteriophora* from the liquid formulation (0.2%) was less ($P = 0.01$) than that of either steinernematid species. No *H. bacteriophora* from the granular formulation were detected in any plot on day 5 post-treatment or thereafter. Bioassay data conformed reasonably well with nematode counts from soil samples. Almost no infection of either insect species occurred 14 or 28 days post-infection (Table 1). Parasitism of *G. mellonella* was greater than that of *D. abbreviatus* in all nematode treatments on days 0 and 5 post-treatment. No insects were parasitized in soil from control plots at any time during the experiment.

Nematode efficacy against citrus root weevil larvae: The total number of larvae recovered from the rhizospheres of control trees (14.8) was similar to the pretreatment estimate (16.0) using the tree removal-soil sampling procedures described herein (Table 2). More *D. abbreviatus* were recovered from the lower rhizosphere than from the other sites. Since the volume of soil found in the crown was significantly less than the volume processed in the other areas of the rhizosphere, most likely the density of *D. abbreviatus* larvae was greater in the crown area at this time of the year.

Larval parasitism by *S. riobravis* appeared to be significantly higher in all regions of the rhizosphere based on larval recovery compared to the other nematode species (Table 2). Both formulations of *H. bacteriophora* were superior to both rates of *S. carpocapsae* in reducing the total number of larvae in the rhizosphere.

In the crown region, both the liquid formulation of *H. bacteriophora* and *S. riobravis* gave similar results and significantly reduced larval populations. Neither rate of *S. carpocapsae* appeared to have an effect on larval populations when compared to the untreated check.

Discussion

The effect of sampling intensity on the accuracy of population density estimates for entomogenous nematodes, under conditions in these studies, was similar to that reported for other nematode species (11,12). At population densities resulting from standard inoculation rates (in the present study, ca. 600 nematodes per 100 cm^3 soil in the top 15 cm soil), we estimated that 32 cores of soil should achieve population measurements within 40% of μ approximately 95% of the time. When those population densities decline 100-fold, the same sample intensity should produce confidence interval half-lengths of approximately 66% of $\bar{x}$ 95% of the time. While the additional error involved in compositing and subsampling from the single cores was not measured in this study, we determined that 32-core composite samples taken from 10 individual plots resulted in 95% confidence interval half-lengths of approximately 31% of $\bar{x}$ at population densities of 600 nematodes per 100 cm^3 soil and 88% of $\bar{x}$ when populations decline 100-fold. These levels of sampling precision were sufficient to estimate persistence of the nematodes in the field because of the wide range of densities encountered over relatively short time intervals. For field studies in which treatment means are expected to exhibit smaller ranges, sampling requirements can be estimated using the appropriate parameter estimates for Taylor's Power Law.

Exponential population decay rates commonly have been reported for entomogenous nematodes (6,15,23). However, with the exception of one other study also conducted in sandy soil (23), most workers reported higher persistence of entomopathogenic nematodes in soil than measured in our studies. For example, Curran and Heng (6) estimated a minimum of 30 days was required before populations of various *Steinernema* spp. declined by 90% in vitro compared to less than 6 days in this study. Further, using a clay loam soil, they

TABLE 1. Mean percentage mortality and parasitism by different entomogenous nematodes to late instar larvae of *Diaprepes abbreviatus* and *Galleria mellonella* after 4 days exposure to treated field soils in the laboratory.

		Posttreatment sample time in days[a]															
		0				5				14				26			
		Diaprepes		Galleria		Diaprepes		Galleria		Diaprepes		Galleria		Diaprepes		Galleria	
Nematode species	Rate/tree (million)	% Mort[b]	% Par[c]	% Mort	% Par	% Mort	% Par	% Mort	% Par	% Mort	% Par	% Mort	% Par	% Mort	% Par	% Mort	% Par
Steinernema carpocapse	2.7	100	25	100	100	35	25	80	50	20	0	25	0	0	0	100	0
Steinernema carpocapse	5.4	75	5	100	100	35	25	100	75	75	0	10	0	60	0	95	0
Steinernema riobravis	2.1	70	35	100	70	20	0	55	30	35	15	30	0	20	0	95	0
Heterorhabditis bacteriophora (liquid)	4.5	100	37.5	100	50	25	0	75	25	25	0	20	0	10	0	25	0
Heterorhabditis bacteriophora (granule)	3.1	83.3	16.1	100	50	37.5	0	62.5	50	15	0	10	0	30	0	60	0
Control	—	85	0	50	0	20	0	70	0	25	0	25	0	10	0	95	0

[a] Each treatment replicated 10 times; two larvae hosts in 70 cm^2 of field soil.
[b] Mort = mortality, Par = parasitism.
[c] Parasitism based on dissection of both live and dead larvae.

TABLE 2. Effect of different species of entomopathogenic nematodes applied to soil beneath the citrus tree canopy on the recovery of late instar larvae of *Diaprepes abbreviatus* from different locations in the root zone after 30 days.

Treatment	Rate (Viable nema/ tree × 10^6)	Mean no. larvae ± SE at different soil depths[a] Upper rhizosphere[b] (0–30-cm depth)	Lower rhizosphere[b] (30–60-cm depth)	Crown[b]	Total
Control	—	2.5 ± 0.8 a	8.4 ± 2.6 a	4.2 ± 1.6 a	14.8 ± 4.0 a
Steinernema carpocapsae	2.7	2.7 ± 0.7 a	6.5 ± 1.7 ab	5.1 ± 1.7 a	14.3 ± 3.3 a
S. carpocapsae	5.4	1.9 ± 0.8 a	4.8 ± 1.0 ab	3.6 ± 1.0 a	10.3 ± 2.5 a
S. riobravis	2.1	0.1 ± 0.1 b	0.7 ± 0.4 c	0.3 ± 0.2 b	1.1 ± 0.6 c
Heterorhabditis bacteriophora granule	3.1	1.8 ± 1.1 a	3.3 ± 0.8 b	1.8 ± 1.0 a	6.8 ± 1.7 b
H. bacteriophora liquid	4.5	1.8 ± 1.2 a	4.3 ± 1.2 ab	0.3 ± 0.3 b	6.3 ± 2.3 b

[a] Means followed by the same letter are not significantly different ($P = 0.05$). All treatments replicated 10 times except *H. bacteriophora* treatments, which were replicated four times.
[b] Volume of soil sampled: Crown—Not determined, Upper rhizosphere—0.283 m^3, Lower rhizosphere—0.283 m^3.

demonstrated a major effect of extraction method on survivorship estimates for some nematode species. As shown by Curran and Heng (6), the proportion of nematodes recovered by sucrose centrifugation from these soils increased somewhat with time following inoculation in laboratory studies (Duncan, unpubl.). However, the use of sucrose centrifugation with these sandy soils did not increase survivorship estimates appreciably in the unpublished studies.

Recovery of heterorhabditid nematodes at depths >15 cm and at 1 m beyond the treatment zone demonstrated that lateral and vertical movement of nematodes out of the sampling zone probably resulted in underestimation of survival in the field. Nevertheless, emigration losses were probably low because vertical and horizontal migration of *H. bacteriophora* and *S. carpocapsae* was found to be negligible during 30 days in soils of similar texture (26). Rather, the negative correlation between nematode recovery and distance from the tree row centerline suggests that physical factors were important sources of mortality.

Poor survival of *H. bacteriophora* in soil outside of the tree canopy may have resulted from more rapid desiccation (9,10, 35), higher soil temperature (23), or increased UV radiation (13) compared with conditions under the canopy. The infestation patterns evident in these studies suggest that requirements for optimum nematode application may differ depending on tree age, particularly in the case of young trees that provide little shade.

Greater persistence of *S. riobravis* and *S. carpocapsae* than *H. bacteriophora* in soil 5–6 days following treatment was consistent with other reports (18,23). *Heterorhabditis bacteriophora* persistence was least in the field trial measuring persistence and efficacy. That result was consistent with the fact that the young trees in the study provided the least amount of shade encountered in any experiment. Higher survivorship of *H. bacteriophora* immediately after treatment in the second compared to the first sample-size experiment was likely due to pre-irrigation of the test site in the second trial (7).

Although no nematode species persisted at high levels in soil, *S. riobravis* effectively reduced the density of *D. abbreviatus,* suggesting that conditions were favorable for parasitism. Molyneux and Bedding (24) demonstrated in laboratory trials that *Heterorhabditis* sp. and *S. glaseri* effectively parasitized *Lucilia cuprina* larvae in fine sand at temperatures and moisture levels encompassing all those encountered in the present work. Nevertheless, in a related study *H. bacteriophora* and *S. carpocapsae* persisted poorly in sandy soil as tempera-

ture increased from 15 C (23). Results of other field trials using *S. carpocapsae* demonstrated significant control of insect larvae in soil that did not persist for more than 8 days (5,21). Thus, a high rate of persistence in soil may not be a major determinant of short-term efficacy.

It is unknown whether repeated inoculation of these nematode species is necessary to maintain measurable insect control. However, the persistence at low levels of all nematode species 26 days post-treatment was consistent with results of other studies in which nematode recycling eventually resulted in long-term pest suppression (16,19).

Entomopathogenic nematode species are known to have different search strategies in the soil (17). Clearly, this was the situation in our study. *Steinernema riobravis* appeared to have the greatest searching ability because it aggressively reduced the higher larval population densities both in the crown and the lower rhizosphere. Efficacy of *S. riobravis* was approximately the same as that reported in a study conducted under similar conditions (9). By comparison, *H. bacteriophora,* which has been reported to infect hosts deeper in the soil (1), was most effective in the crown region near the soil surface. We cannot explain the complete failure of *S. carpocapsae,* particularly in the crown region where one would expect it to be most efficacious.

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