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Incidence of Endemic Entomopathogenic Nematodes Following Application of *Steinernema riobrave* for Control of *Diaprepes abbreviatus*[1]

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Abstract: Control of *Diaprepes abbreviatus* by endemic and exotic entomopathogenic nematodes (EPN) was monitored during 2000–2001 in two citrus orchards in central Florida (Bartow and Poinciana). Caged sentinel insect larvae were buried beneath citrus trees for 7 days at 1 to 2-month intervals from April to October each year. At Bartow, the survey occurred in experimental plots that were (i) not treated with commercial EPN, (ii) treated twice annually since 1998 with commercially formulated *Steinernema riobrave*, or (iii) treated twice annually with *S. riobrave* and liquid fertilization (15 times/year) occurred in place of dry fertilizer (3 times/year) used in the other treatments. Four endemic EPN species, in addition to *S. riobrave*, were recovered from the sandy soil at Bartow: *S. diaprepesi*, *Heterorhabditis zealandica*, *H. indica*, and *H. bacteriophora*. Mean insect mortality in control plots was 39.4% (range = 13% to 74%), with seasonal maxima in May to July each year. Endemic EPN were recovered from 55% (range = 22% to 81%) of the cadavers each month. Total numbers of endemic EPN recovered in all plots during 2 years were directly related to the numbers of adult weevils (*D. abbreviatus* and *Pachnaeus litus*) captured in modified Tedder's traps and inversely related to recovery of *S. riobrave*. Insect mortality was higher and cadavers containing endemic EPN were more numerous in untreated control plots than in *S. riobrave*-treated plots, except during months in which *S. riobrave* was applied. In treated plots, endemic EPN were recovered from cadavers at twice the rate of *S. riobrave*. Suppression of endemic EPN in plots treated with *S. riobrave*, combined with inferior persistence by the introduced species, may have attenuated the net efficacy of *S. riobrave* against *D. abbreviatus*. In contrast, *H. indica* was the only endemic nematode recovered from the sandy clay loam soil at Poinciana, where the average mortality of *D. abbreviatus* was 12% (range 3% to 20%) and incidence of *H. indica* did not exceed 8%. Results of these surveys suggest that the regional patterns in the abundance and damage to citrus caused by *D. abbreviatus* in Florida are regulated by endemic EPN and other soilborne enemies of the weevil.

Key words: Abbott's formula, biological control, competition, entomopathogenic nematodes, *Heterorhabditis*, natural control, seasonality, *Steinernema*.

Larvae of the *citrus* root weevil, *Diaprepes abbreviatus* L., are subterranean pests that feed on the fibrous and major roots of citrus trees, wounding the root cortex and creating infection sites for *Phytophthora* spp. (Graham et al., 2003). The resulting pest-disease complex, which debilitates and even kills trees, causes annual losses of $75 million in the citrus industry in Florida (McCoy, 1999). *Diaprepes abbreviatus* is present in all of the citrus-producing regions of Florida, but its abundance and damage to citrus are often more severe in orchards on fine-textured and poorly drained soils than in those on well-drained, coarse-textured soils (McCoy et al., 2002). The incidence and virulence of *Phytophthora* spp. are also favored by those soil conditions that favor *D. abbreviatus* (Graham et al., 2003). Citrus-growing soils in the central ridge region of Florida are uniformly high in sand content (>95%), whereas those nearer the coasts and in the south-central parts of the state (flatwoods regions) vary in soil texture.

Due partly to an absence of effective chemical pesticides, Florida citrus growers have used commercially formulated entomopathogenic nematodes (EPN) for more than a dozen years in their attempts to manage *D. abbreviatus* (Adair, 1994; Bullock et al., 1999; Duncan and McCoy, 1996; Duncan et al., 1996; McCoy et al., 2000, 2001; Stansly et al., 1997). Suppression of *D. abbreviatus* by introduced EPN is greater in coarse as opposed to fine soil texture (Duncan et al., 2001a; McCoy et al., 2002). Several EPN species endemic in Florida are widespread in Florida citrus orchards (Beavers et al., 1983) and cause high mortality of *D. abbreviatus* larvae at some sites (Duncan et al., 1999; McCoy et al., 2000). There is no information regarding soil texture and the incidence of endemic EPN in Florida; however, studies in Hawaii and China revealed direct relationships between the incidence of endemic EPN and the proportion of sand in soil (Hara et al., 1991; Zhang et al., 1992).

A study of the profitability of using commercially formulated *Steinernema riobrave* Cabanillas, Raulston & Poinar to manage *D. abbreviatus* revealed seasonality in natural mortality of *D. abbreviatus* (Duncan et al., 1999). Although EPN-induced epizootics appear to be the exception (Ehlers and Hokkanen, 1996), natural mortality of *D. abbreviatus*, due mainly to several endemic EPN species, exceeded 50% per week in summer. There is a need to better understand patterns of natural mortality in *D. abbreviatus* larvae to effectively intervene in the life cycle and to identify potential biocontrol agents. Moreover, the introduction of exotic EPN for biological control may suppress population densities of some endemic EPN species (Ehlers and Hokkanen, 1996; Millar and Barbercheck, 2001). In systems with economically important levels of natural pest control, competition

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between endemic and introduced EPN could attenuate the net efficacy of EPN treatments because the persistence of introduced EPN is often ephemeral (Duncan and McCoy, 1996; Kaya and Koppenhofer, 1996; McCoy et al., 2001).

In this paper we report results of temporal surveys in two citrus orchards in central Florida. The survey in one of the orchards occurred in experimental plots that were treated or not treated twice annually with *S. riobrave*. The study was conducted to (i) investigate seasonality in the activity of endemic EPN species against *D. abbreviatus* at two locations, (ii) determine whether application of commercial EPN affects the abundance of endemic EPN, and (iii) determine whether net efficacy differs among plots treated or not treated with introduced EPN.

Materials and Methods

Study sites: Two orchards in central Florida were surveyed for EPN activity. The first orchard (near Poinciana in a region characterized as flatwoods) consisted of mature Hamlin orange trees on Swingle citrumelo rootstock on two-row formed beds with spacing of 6.1 m within and 8.5 m between rows on a bed. The soil texture was a sandy clay loam (69:12:19 sand:silt:clay; pH 6.4). Part of the orchard was abandoned due to extensive damage by *D. abbreviatus* and *Phytophthora nicotianae*. In 2000, the survey was conducted in the abandoned part of the orchard, which was not irrigated. In 2001, the survey site was moved to the managed part of the orchard, which was irrigated with a microsprinkler beneath each tree.

The second orchard (near Bartow on the central ridge) contained mature Hamlin and Valencia orange trees on Carrizo citrange rootstock. The orchard was naturally infested with *D. abbreviatus*, *P. nicotianae*, *Tylenchulus semipenetrans* Cobb, and *Pachnaeus litus* L., another weevil with subterranean larvae that feed on citrus roots. Soil texture was a fine sand (97.6:1.5:0.9 sand:silt:clay; pH 6.7). Trees were spaced (without beds) 4.5 m within and 8.1 m between rows and were irrigated with microsprinklers. The survey at the Bartow site was conducted in experimental treatments that were initiated 2 years previously in April 1998. Twelve plots (ca. 0.67 ha each; four replicates) received one of three treatments: (i) fertilized four times annually with dry fertilizer; (ii) fertilized with dry fertilizer, treated with a single foliar spray of carbaryl in April 1998, and treated twice annually thereafter with a commercial formulation of *S. riobrave* (Biovector 355, Certis Corp., Columbia, MD) at a rate of 20 *S. riobrave* per cm^2 soil surface; or (iii) treated with nematodes as in treatment 2, but fertilized 15 times annually with liquid fertilizer. Nematode and liquid fertilizer treatments were applied via the irrigation system. Total annual amount of fertilizer applied in dry or liquid formulations was 239:29:291 (N:P_2O_5:K_2O) kg/ha. Nematode applications occurred in May and September 1998, and in June and either August or September 1999–2001.

Survey methods: Cylindrical cages containing autoclaved sandy soil (10% moisture) and a single larvae of *D. abbreviatus* (reared on artificial diet for 3 to 5 weeks) were buried 25 to 30 cm deep beneath the tree canopies. Cages were made of 225-mesh stainless steel cylinders (7 × 3-cm diam.) secured at each end with polypropylene snap-on caps (McCoy et al., 2000). The survey was conducted from spring through autumn in both 2000 and 2001. Cages were buried approximately bimonthly in 2000 and monthly in 2001. Seven days after burial, cages were removed from soil and insect larvae were rinsed and placed on moistened filter paper in individual parafilm-sealed petri dishes for observation. Mortality of insects were recorded from 0 to 48 hours after removal from soil. Insect cadavers were monitored for up to 30 days, and emerging EPN were identified to species. For initial determination of species, nematodes collected from cadavers of *D. abbreviatus* were reared in *Galleria mellonella* (L.) and different stages were collected and processed for identification using both morphological and molecular sequencing methods as described by Nguyen and Duncan (2002). Sequences of nematodes collected were compared with those from Genbank for *H. zealandica* Poinar (AF029705), *H. indica* Poinar, Karunahar & David (AF029710), and *S. diaprepesi* Nguyen & Duncan (AF440764), using the program Bestfit (Wisconsin Package Version 10.3, Accelrys Inc., San Diego, CA).

Ninety cages were buried on each date at the Poinciana site. Six cages were buried beneath the same 15 trees on each date in an L-shaped pattern. Single cages were placed in a straight line at the center of the bed (between the two rows), at the canopy dripline, and within 10 to 25 cm of the tree base. At a right angle, a cage was placed midway between the trunk and the dripline, at the dripline, and midway between trees in the row. The pattern was revolved 180° on each successive sampling date. The same procedure was used when the survey site in the Poinciana orchard was moved from unmanaged to managed trees. Two hundred cages were buried on each date at the Bartow site. One hundred cages (25/plot) were buried in plots not treated with nematodes and 50 cages (either 12 or 13/plot) were buried in each of the two treatments in which nematodes were applied. In all treatments, cages were buried one per tree, using different locations under the same trees on each date. In months during which nematodes were applied, cages were buried in the morning and nematodes were applied in late afternoon of the same day.

Abundance of the adult weevils in each grove was estimated weekly with modified Tedder's traps (Duncan et al., 2001b). A single trap was located beneath 12 trees in each plot at Bartow (144 traps total). One hundred randomly selected trees were monitored at Poinciana (McCoy et al., 2002).

Statistical procedures: Insect mortality and nematode species abundance were expressed as proportions of total insects buried each month. Proportions were transformed (arcsin-square root) prior to statistical analysis. Data from the Bartow site were subjected to analysis of variance for a split plot design in which the whole plot (treatment) design was randomized complete block (four blocks), with sample dates as subplots (Steel and Torrie, 1960; PROC GLM, SAS Institute, Cary, NC). The mean square for treatment x block was designated as the treatment error term in the model. Mean separation was by Student-Newman-Keuls test at $P = 0.05$. Efficacy of commercially formulated *S. riobrave* (Bio Vector) was estimated from Abbot's formula, (T − C)/(100 − C) × 100, where T = percent mortality in treated plots and C = percent mortality in control plots (Abbott, 1925) using both unadjusted treatment means and treatment means adjusted by analysis of covariance (PROC GLM, SAS). The covariate in the latter case was mean mortality for each plot during the months in which Bio Vector was not applied. Linear correlations between dependent variables were measured using means and $\log_e$-transformed means (Minitab Inc., State College, PA).

Results

Seasonality of EPN activity in untreated plots: The mortality of buried insects in the untreated control plots at the Bartow site varied with sampling date (df = 11,33; F = 9.14; $P = 0.0001$). Mortality of insect larvae increased between March to July 2000 and decreased in August and October (Fig. 1A). In 2001, mortality increased from April until May to June, after which it declined steadily until October when the survey ended. Average monthly mortality was higher in 2001 (48.7 ± 7.5%; mean and standard error) than in 2000 (26.2 ± 5.5%; t = 2.4, df = 9, $P = 0.05$).

Five entomopathogenic nematode species were isolated from the Bartow site: *S. diaprepesi, S. riobrave,* Cabanillas, Poinar & Raulston *H. zealandica, H. indica,* and *H. bacteriophora* Poinar. The percentage of insects buried in control plots in which EPN species reproduced followed trends similar to those for insect mortality. Prevalence of EPN was higher in 2001 (31.3% ± 5.3%) than in 2000 (10.6% ± 2.6%; t = 3.6, $P = 0.01$) (Fig. 1B). Reproduction by EPNs occurred in 63.8% ± 4.4% of cadavers each month in 2001, which was higher than in 2000 (43.5% ± 7.6%; t = 2.4, $P = 0.05$).

Recovery of *S. diaprepesi* (27%) and *H. zealandica* (22%) from cadavers accounted for most of the mortality that could be attributed to EPNs, although *S. riobrave* (4%), *H. indica* (3%), and *H. bacteriophora* (1%) also were recovered (Fig. 1A,B). Unidentified rhabditid nematodes also emerged in large numbers from between 3% to 22% (mean = 9.8%) of the insect cadavers recovered from control plots each month (data not shown).

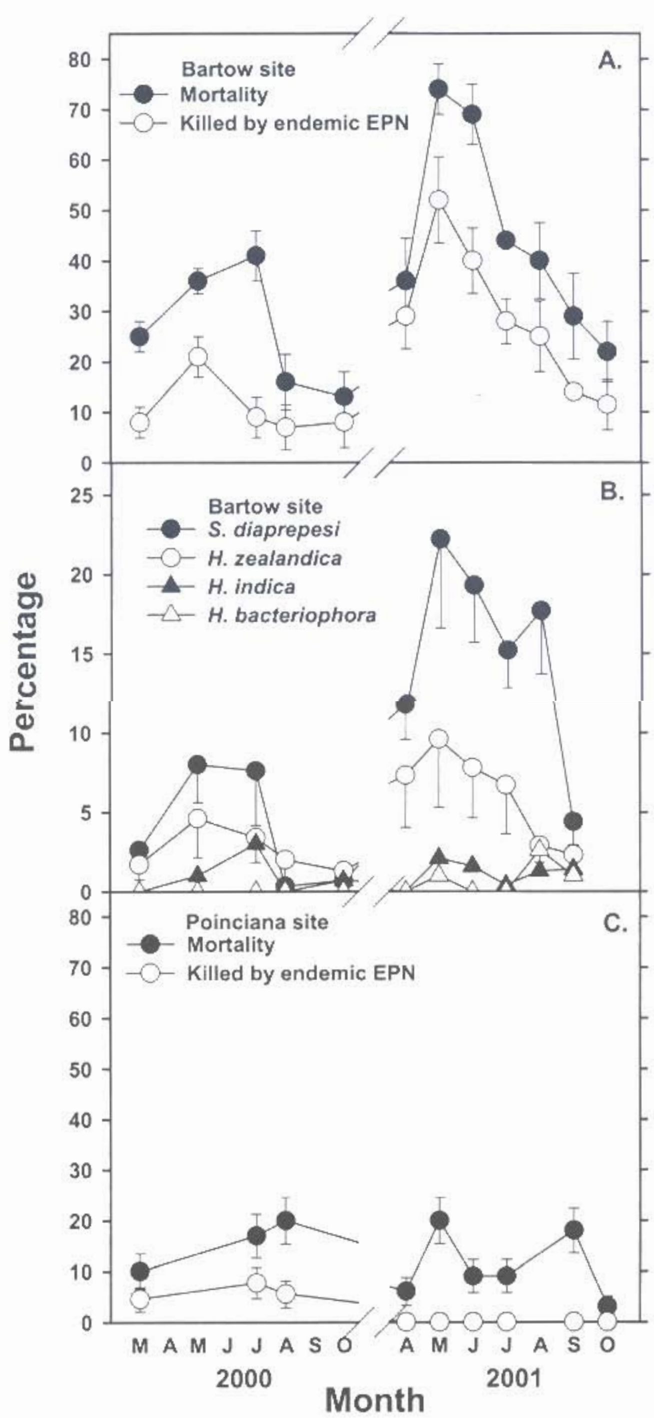

FIG. 1. Percentage of caged sentinel larvae of *Diaprepes abbreviatus* that died during 7 days in the soil and the percentage of sentinel larvae infected by entomopathogenic nematodes at (A) Bartow and (C) Poinciana, and the percentage of sentinel larvae infected by various species of entomopathogenic nematodes at Bartow (B). *Heterorhabditis indica* was the only entomopathogenic nematode species identified at Poinciana. Data are from plots not treated with *Steinernema riobrave*. Standard errors are shown.

Insect mortality at the Poinciana site did not exceed

20% in any month during the survey, and seasonality of mortality was not apparent (Fig. 1C). In contrast to the several species of endemic EPN at Bartow, only *H. indica* was detected at Poinciana, and the incidence of detection did not exceed 8% of the buried insects. The low recovery of EPN at Poinciana precluded detection of spatial patterns in the detection of EPN.

Relationships between endemic EPN, S. riobrave, and insect prey: At Bartow, the average monthly incidence of endemic EPN in all plots during 2 years was directly related to log-transformed numbers of adult weevils (*D. abbreviatus* and *P. litus*) trapped during the survey ($r = 0.76$, $P \leq 0.01$; Fig. 2A). Inverse relationships occurred between the incidence of *S. riobrave* and log-transformed adult weevil abundance ($r = -0.75$, $P \leq 0.01$; Fig. 2B), and incidence of endemic EPN ($r = -0.70$, $P \leq 0.01$; Fig. 3).

Treatment effects on insect mortality and detection of EPN: An interaction between date and treatment occurred

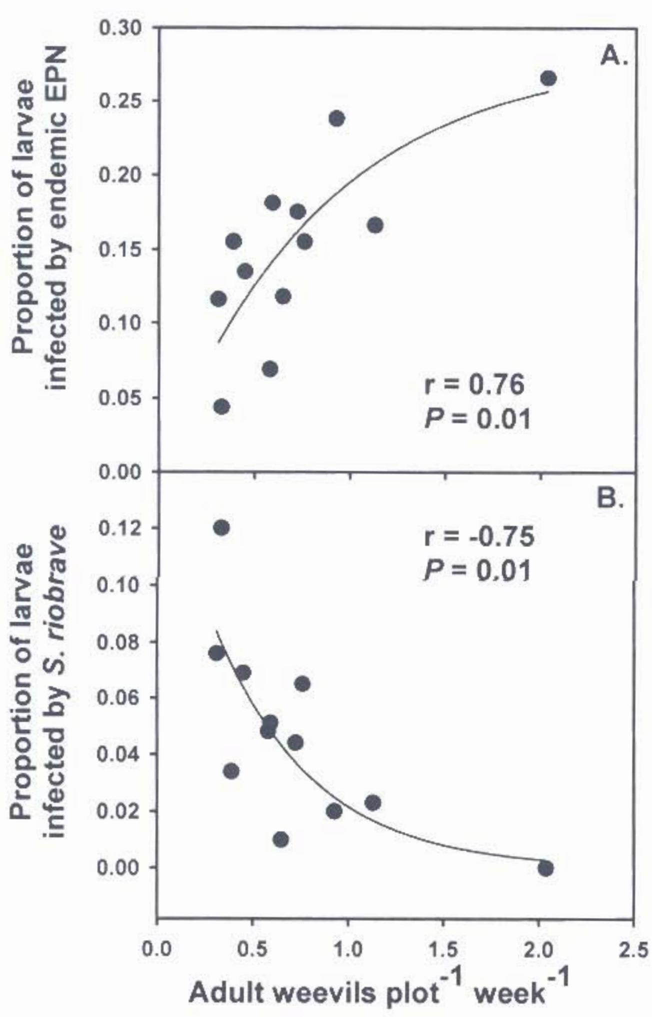

FIG. 2. Linear relationships between the abundance of adult weevils (*Diaprepes abbreviatus* plus *Pachnaeus litus;* $\log_e$-transformed) and the average monthly proportion of sentinel weevils infected by endemic entomopathogenic nematodes (A) and exotic *Steinernema riobrave* (B) during 2 years in 12 research plots at Bartow.

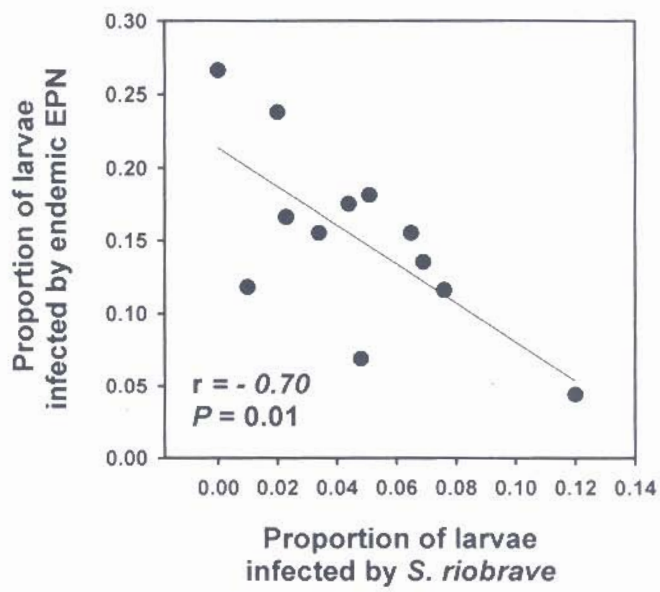

FIG. 3. The linear relationship between average monthly proportion of sentinel weevils infected by endemic entomopathogenic nematodes and exogenous *Steinernema riobrave* during 2 years in 12 research plots at Bartow.

for mortality of larvae buried at the Bartow site ($df = 22.99$ $F = 2.34$, $P = 0.002$). Because application of *S. riobrave* was expected to increase the mortality of larvae only temporarily (Duncan et al., 1996), ANOVA was performed separately for months in which *S. riobrave* was applied, and months without nematode treatment (Table 1). Average larval mortality was 43% and 49% higher in plots treated with *S. riobrave* and *S. riobrave* plus liquid fertilizer ($P = 0.01$), respectively, than in control plots during months in which *S. riobrave* was applied (Table 1; Fig. 4). However, in months when no nematode treatments were applied, average mortality was reduced by 16% and 25%, respectively, in *S. riobrave* and *S. riobrave* plus liquid fertilizer-treated plots ($P = 0.05$) compared to that in control plots.

The detection of EPN in treated and untreated plots also differed between months with and without application of *S. riobrave*. During non-treatment months, numbers of cadavers containing *S. riobrave* from control and treated plots did not differ (Table 1; Fig. 5A). However, the average numbers of cadavers containing *S. riobrave* from plots of both nematode treatments were 10-fold greater ($P = 0.02$) than that from control plots during months when nematodes were applied (Table 1; Fig. 5B). ANOVA revealed no significant effect of treatments on individual species of endemic EPN during either non-treatment or treatment months. However, detection of the entire community of endemic EPN was 34.8% and 41.2% lower in plots treated with *S. riobrave* and *S. riobrave* plus liquid fertilizer, respectively, during the eight non-treatment months (Table 1; Fig. 5A). Treatment effects on detection of endemic EPN were not evident during the 4 months in which *S. riobrave* was

TABLE 1. Effects of sampling date and experimental treatments on the mortality of buried *Diaprepes abbreviatus* larvae, and the detection of endemic entomopathogenic nematodes (EPN) and *Steinernema riobrave* in a citrus orchard in central Florida.

Source	Df	Percentage mortality		Mortality from endemic EPN		Mortality from *S. riobrave*	
		F-statistic	Probability	F-statistic	Probability	F-statistic	Probability
				Months without nematode application			
Block	3	1.74	0.171	6.96	0.001	1.49	0.226
Month	7	22.52	0.001	10.06	0.001	2.51	0.024
Treatment	2	5.37	0.046	5.12	0.050	2.23	0.189
Month × treatment	14	0.85	0.599	0.72	0.751	0.81	0.655
Error	63						
				Months with nematode application			
Block	3	2.04	0.131	1.49	0.238	1.38	0.271
Month	3	24.66	0.001	21.08	0.001	8.53	0.001
Treatment	2	10.43	0.011	0.65	0.554	8.70	0.016
Month × treatment	6	1.58	0.191	2.86	0.027	2.81	0.029
Error	27						

applied, although the trends were similar to those during the non-treatment months (Table 1; Fig. 5B). The combined recovery of both endemic and introduced EPN during non-treatment months was lower ($P = 0.05$) in plots treated with *S. riobrave* and liquid fertilizer, whereas there were no apparent treatment effects on recovery of all EPN during months when *S. riobrave* was applied (Fig. 5A, B).

Treatment effects on efficacy as estimated by Abbott's formula: Treatment efficacy covaried with the estimated prevalence of endemic EPN in each plot (Table 2). The estimated efficacy of nematode treatments from Abbot's formula increased by 45% when mean efficacy on all treatment dates (28.5%) was adjusted using analysis of covariance (41.3%) (Table 3).

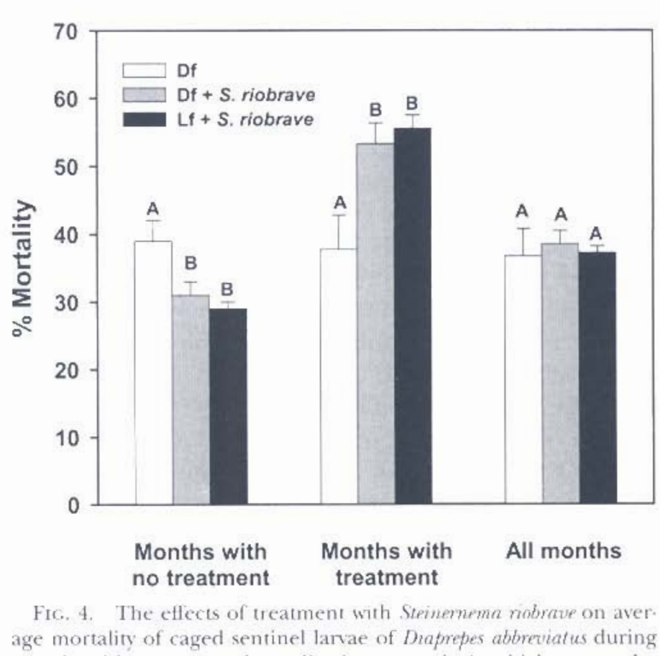

FIG. 4. The effects of treatment with *Steinernema riobrave* on average mortality of caged sentinel larvae of *Diaprepes abbreviatus* during months without nematode applications, months in which nematodes were applied, and over all months at the Bartow site. Df = dry fertilizer; Lf = liquid fertilizer. Thin bars are standard errors of means. Data were transformed (arcsin square-root) prior to ANOVA and mean separation, but untransformed statistics are shown. Means within a group with the same letters do not differ at $P = 0.05$.

DISCUSSION

A pronounced difference in the rates of natural control of sentinel *D. abbreviatus* larvae in these two citrus orchards was consistent with the hypothesis that soilborne natural enemies are important determinants of regional patterns of the abundance of *D. abbreviatus* in Florida. Moreover, inundative treatments with the exotic *S. riobrave* reduced the detection of endemic EPN and apparently dampened the rate of natural control to an extent that may have mitigated the beneficial effects of the treatments.

The average mortality of buried larvae, EPN species diversity, and EPN detection were between 3-fold and 9-fold greater at the Bartow site than at Poinciana. Endemic EPN were a primary natural enemy at Bartow, accounting for more than half of the mortality in untreated plots. Indeed, the role of EPN may be somewhat greater than detected because free-living bactivorous nematodes that emerged from between 2% to 22% of the dead larvae each month are capable of suppressing EPN development in the cadavers (Duncan et al., 2003). The range in the monthly detection of *S. diaprepesi* and *H. zealandica* at the Bartow site (2% to 32%) suggests an endemic 'balanced association' with *D. abbreviatus* (Peters, 1996), in contrast to epizootics of greater amplitude, but irregular frequency, which is only rarely detected in EPN (Akhurst et al., 1992). Other examples of balanced associations between EPN and insects include *S. kraussei* and the false spruce webworm *Cephalcia abietis* (8% to 15% mortality), *S. feltiae*—*S. affinis* and *Bibio* sp. (23% to 68% mortality), *S. riobrave* and *Helicoverpa zea* (average 10% mortality), and *S. carpocapsae* and *H. zea* (22% to 30% mortality) (Bovien, 1937; Mrácek, 1986; Raulston et al., 1992; Sparks et al., 1989). The abundance of adult *D. abbre-*

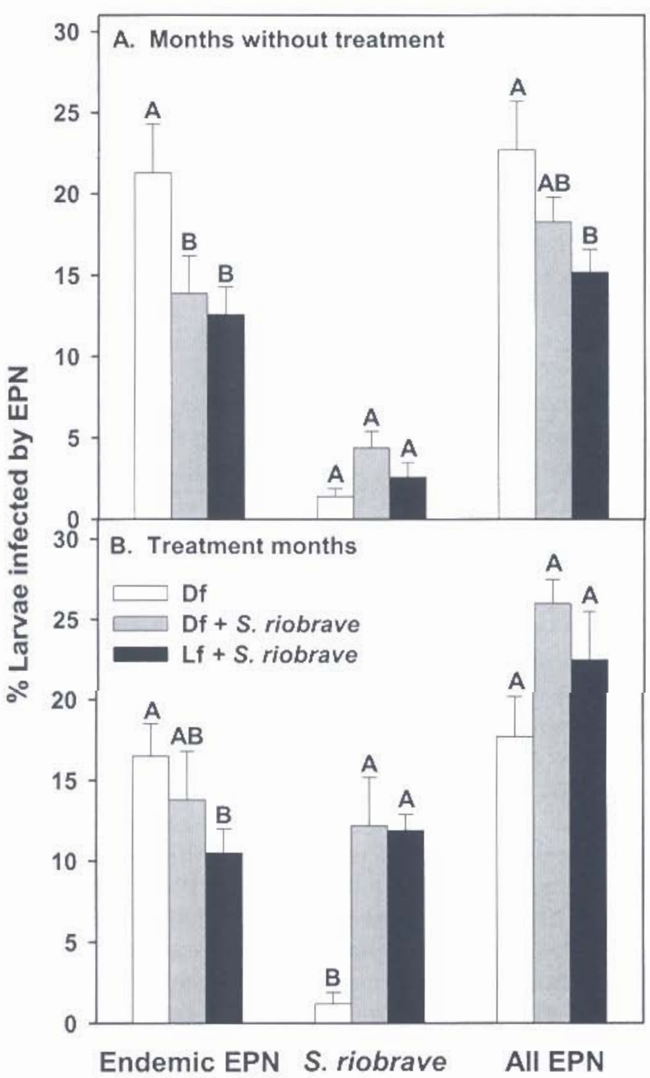

FIG. 5. The effects of treatment with *Steinernema riobrave* on the average percentage of buried sentinel *Diaprepes abbreviatus* weevil larvae infected by endemic entomopathogenic nematodes and (or) *S. riobrave* during months without nematode applications (A) and months in which nematodes were applied (B) at the Bartow site. Df = dry fertilizer; Lf = liquid fertilizer. Thin bars are standard errors of means. Data were transformed (arcsin square-root) prior to ANOVA and mean separation, but untransformed statistics are shown. Means within a group with the same letters do not differ at $P = 0.05$.

viatus was inversely related to the detection of EPN and levels of natural control at the two sites. At Poinciana, 31.1 and 73.4 adult weevils per Tedders trap were caught in 2000 and 2001, respectively (McCoy et al., 2002), whereas the corresponding trapping rates at Bartow were only 1.56 and 1.88 weevils, respectively (Duncan, unpubl.). The difference in weevil abundance in these two orchards is consistent with data from an ongoing temporal survey of *D. abbreviatus* in which adult abundance is an order of magnitude higher in three orchards in flatwoods regions compared to three orchards on the ridge (Futch, 2002). Data from the present study suggest that these differences in abundance of *D. abbreviatus* may be due in part to natural enemies of the subterranean stages of the weevil.

TABLE 2. Proportional mortality of *D. abbreviatus* larvae buried in a citrus orchard in central Florida.

Source	Degrees of freedom	F-statistic	Probability
Plot (covariate)[a]	1	5.31	0.030
Date	2	48.97	0.000
Treatment	2	8.97	0.001
Date × treatment	4	2.11	0.108
Error	26		
Total	35		

[a] The covariate was the average insect mortality in each plot during months in which no *S. riobrave* were applied for insect management.

We did not address the cause of different levels of natural biological control in these two orchards. The efficiency of the buried-cage technique may differ in soils of different texture due to the degree of contact between the cage wall and the surrounding soil; however, Duncan et al. (2001a) obtained similar levels of infection by *S. riobrave* applied to plots at Poinciana containing cages buried using this technique, and in 170-liter microcosms of the same soil in which the cages were buried by hand. Alternatively, a number of reports show that the efficacy of introduced EPN (Duncan et al., 2001a; Kung et al., 1990; Molyneaux and Bedding, 1984) and the incidence of endemic EPN (Hara et al., 1991; Zhang et al., 1992) are favored by coarse-textured soils and impaired by soils of fine texture. Several (Adair, 1994; McCoy et al., 2000; Stansly et al., 1997) but not all (Bullock et al., 1999) field studies in flatwoods regions reported limited, if any, measurable efficacy by EPN, whereas significant efficacy has been shown in similar studies on the central ridge (Duncan et al., 1996, 2003; Duncan and McCoy, 1996; Schroeder, 1990). Thus, while there are many possible reasons for differences in the natural control of sentinel *D. abbreviatus* at Bartow and Poinciana, there is a body of evidence supporting the hypothesis that soil texture is a primary cause. In contrast to the sandy clay loam soil texture at Poinciana, sandy soils with >90% sand content are common in the flatwoods regions; however, the average sand particle size tends to be smaller than that on the ridge (Carlisle et al., 1978). Efficacy of *S. riobrave* in fine sandy soil was lower than in coarse sandy soil in laboratory studies (Duncan et al., 2001a). Comparative field studies in a range of sandy soils on the ridge and in the flatwoods are needed to determine the incidence of endemic EPN and the expected profitability of inundative treatment with EPN to manage *D. abbreviatus* on the ridge and in the flatwoods.

The seasonal pattern of EPN detection at Bartow was similar during both years and may correspond to prey abundance, as proposed recently for EPN in a citrus orchard in Israel (Efron et al., 2001). *Diaprepes abbreviatus* teneral adults display a pattern of peak emergence from soil during the spring (Duncan et al., 2001b). Adults lay eggs on citrus leaves, and the neonate larvae

TABLE 3. Estimated efficacy of *S. riobrave* against *D. abbreviatus* larvae from Abbott's formula using unadjusted mean mortality rates and means adjusted from analysis of covariance.[a]

Treatment means		Unadjusted mortality	Abbott	Adjusted mortality	Abbott
July 2000	Dry fertilizer	0.41		0.34	
	S. riobrave – dry fertilizer	0.45	(.06)	0.47	(.20)
	S. riobrave – liquid fertilizer	0.63	(.37)	0.69	(.53)
October 2000	Dry fertilizer	0.13		0.06	
	S. riobrave + dry fertilizer	0.42	(.33)	0.43	(.39)
	S. riobrave + liquid fertilizer	0.25	(.14)	0.30	(.26)
June 2001	Dry fertilizer	0.69		0.62	
	S. riobrave + dry fertilizer	0.87	(.58)	0.87	(.66)
	S. riobrave + liquid fertilizer	0.79	(.32)	0.85	(.61)
September 2001	Dry fertilizer	0.29		0.24	
	S. riobrave + dry fertilizer	0.38	(.13)	0.39	(.20)
	S. riobrave + liquid fertilizer	0.54	(.35)	0.58	(.45)

[a] The covariate was the average insect mortality in each plot during months in which no *S. riobrave* were applied for insect management

fall from the canopy to enter the soil. Nigg et al. (2003) and McCoy et al. (2003) reported that from mid-winter until 2 to 3 weeks after adult emergence virtually no neonate larvae fell from the citrus tree canopy at the Poinciana site, whereas there was continuous recruitment of neonate larvae into soil thereafter until early November. Therefore, increased activity of endemic EPN in the spring accords with a density-dependent response to prey availability. Whether density-dependent responses of natural enemies of EPN or other factors are responsible for the decline in EPN detection after mid-summer is unknown. The seasonal patterns observed in this study support the proposal by McCoy et al. (2000) that application of commercial EPN is likely to have the greatest effect when applied to augment the relatively low activity by endemic species in early spring and autumn.

The persistence of endemic species such as *S. diaprepesi* and *H. zealandica* was superior to that of the introduced *S. riobrave*. The average detection of all endemic EPN during months without nematode applications was 15-fold greater than that of *S. riobrave* in control plots and 3- to 5-fold greater in treated plots. Detection of *S. riobrave* in treated plots exceeded that in control plots only during the treatment months when the occurence of endogenous and exogenous EPN was similar. *Steinernema riobrave* is not considered to be endemic in Florida. The recovery of low numbers of *S. riobrave* in untreated plots may have resulted from leakage of valves in the irrigation system during treatments or by migration of infected teneral adult weevils before they died. The ephemeral persistence of *S. riobrave* was consistent with previous reports for this species in Florida citrus (Duncan and McCoy, 1996; McCoy et al., 2000) and with reports for many species of introduced EPN (Kaya and Koppenhofer, 1996).

The treatment differences in this study revealed suppression of endemic EPN by applications of exogenous EPN. Millar and Barbercheck (2001) reported occasional suppression in the prevalence of endemic *H. bacteriophora*, but not *S. carpocapsae*, following the application of *S. riobrave* in a cornfield in North Carolina. A limited opportunity for dispersal by invasive species, and adaptation of endemic populations to local edaphic and biotic conditions, mitigate species displacement by introduced subterranean organisms such as EPN (Ehlers and Hokkanen, 1996; Graham and Mitchell, 1998). Nevertheless, the introduction of an effective biological control organism could affect the abundance of endemic species by reducing the available prey. The detection of endemic EPN at the Bartow site was directly related to the availability of two prey species, *D. abbreviatus* and *P. litus*. Efron et al. (2001) also found a strong positive correlation between numbers of EPN and the insect *Maladera matrida* in citrus in Israel. An opposite (inverse) relationship between *S. riobrave* and prey abundance at Bartow occurred because *S. riobrave* were applied periodically to some plots and thus were not regulated by prey availability. The suppression of prey during treatment months by applications of *S. riobrave* resulted in the inverse relationship between detection of endemic EPN and *S. riobrave*. Moreover, the combination of low persistence by *S. riobrave* and partial displacement of endemic EPN reduced the rate of insect mortality in treated compared to untreated plots during much of the treatment intervals. Thus, partial suppression of endemic EPN has the potential to mitigate the net efficacy of the introduced EPN. Despite significant reduction of *D. abbreviatus* during 7 days following each treatment, the average mortality each month measured during 2 years did not differ among the treatments. This result was consistent with treatment effects on the abundance of adult *D. abbreviatus* and *P. nicotianae* measured during 4 years at Bartow (Duncan et al., 2002). Abundance of adult weevils and the fungus were suppressed during the first 2 years of the experiment, but those differences converged to non-significant levels by the fourth year. The estimated quality of the commercially formulated nematodes declined in the second and third years of the study (Duncan et al., 2002). Thus, whether the loss of treatment efficacy was due to inferior product quality, suppression of endemic EPN, or a combination of these and other factors is unknown.

Abbott's formula is used to correct mortality data for background or natural mortality when estimating treatment efficacy (Abbott, 1925). The suppression of endemic EPN by *S. riobrave* invalidated an assumption of Abbott's formula that natural mortality between treatments is equal. We chose to use adjusted treatment means from analysis of covariance to correct for unequal baseline mortality among treatments. Obtaining independent estimates of natural mortality among treatments adds considerably to the data collection necessary to estimate efficacy in the field. Nevertheless, this approach may be important for long-term experiments in which treatment efficacy is periodically estimated.

In conclusion, the central ridge of Florida may represent a favorable niche for the use of EPN for biological control of *D. abbreviatus*. The rate of natural control of the insect larvae in this region is high, which contributes to reduced damage to the citrus root system. Management tactics that can further reduce the numbers of these larvae have the potential to increase fruit yield enough to be profitable (Duncan et al., 2002). In contrast, the levels of control achieved by EPN against the larger numbers of weevil larvae in flatwoods regions may not be adequate to profitably reduce the effects of root damage. Even under the most favorable conditions, displacement of endemic EPN by non-persistent exotic EPN may reduce the overall effectiveness of this tactic. Additional research is warranted to determine whether augmentation of persistent endemic species, rather than introduction of exotic species, is a means to increase the net efficacy of treatment with EPN.

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