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Competition Between Entomopathogenic and Free-Living Bactivorous Nematodes in Larvae of the Weevil *Diaprepes abbreviatus*[1]

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Abstract: Field and laboratory experiments were conducted to determine the degree to which free-living, bactivorous nematodes (FLBN) are able to competitively displace entomopathogenic nematodes (EPN) from insect cadavers. Two hundred larvae of the insect *Diaprepes abbreviatus* were buried at regular intervals during 2 years in experimental plots that were untreated or treated twice annually with *Steinernema riobrave.* Larvae were recovered after 7 days, and nematodes emerging from cadavers during the next 30 days were identified. The monthly prevalence of FLBN was directly related to that of *S. riobrave* ($r = 0.38$; $P = 0.001$) but was not related to the prevalence of the endemic EPN, *S. diaprepesi, Heterorhabditis zealandica, H. indica,* or *H. bacteriophora* ($r = 0.02$; $P = 0.80$). In a second experiment, treatment of small field plots with *S. riobrave* increased the prevalence of insect cadavers in which only FLBN were detected compared to untreated controls (30% vs. 14%; $P = 0.052$), and increased numbers of FLBN per buried insect by more than 10-fold. In the laboratory, sand microcosms containing one *D. abbreviatus* larva were treated with (i) the FLBN, *Pellioditis* sp.; (ii) *S. riobrave;* (iii) *S. riobrave* + *Pellioditis;* or (iv) neither nematode. Insect mortality was higher in the presence of both nematodes (57%) than when *S. riobrave* was alone (42%) ($P = 0.01$). An average of 59.2 *Pellioditis* sp. g^{-1} insect body weight emerged in the presence of *S. riobrave,* whereas 6.2 nematodes g^{-1} insect were recovered in the absence of the EPN ($P = 0.01$). *Pellioditis* sp. reduced the number of *S. riobrave* per cadaver by 84%; ($P = 0.03$), and per available insect by 82% ($P = 0.001$), compared to *S. riobrave* alone. Population size of *S. diaprepesi* was not affected by *Pellioditis* sp. in experiments of the same design. Faster development ($P = 0.05$) and nutrient appropriation within the insect cadaver by *S. diaprepesi* compared to *S. riobrave* may increase the fitness of the former species to compete with *Pellioditis* sp. The results of these studies demonstrate the potential of FLBN to regulate population densities of EPN and to dampen estimates of EPN-induced mortality of insect pests in the field.

Key words: free-living nematodes, microbivorous nematodes, *Pellioditis, Steinernema diaprepesi, Steinernema riobrave,* Steinernematidae.

Entomopathogenic nematodes (EPN) in the genera *Steinernema* and *Heterorhabditis* are obligate symbionts with bacteria in the genera *Xenorhabdus* and *Photorhabdus,* respectively. After penetrating into the haemocoel of a suitable insect host, the nematodes release the bacteria from the anterior portion of the intestine. Bacterial septicemia kills the insect, and the EPN complete the life cycle by feeding on the bacteria and bacteria-conditioned insect tissues (Bedding et al., 1993). Phylogenetic evidence suggests that EPN likely evolved from a trophic state of free-living bactivory to one of parasitic bactivory (Blaxter et al., 1998; Poinar, 1993). Competition between EPN and free-living bactivorous nematodes (FLBN) can be inferred from their shared feeding habits, the entomoparasitic behaviors of some species of FLBN (Gerber and Giblin-Davis, 1990), and the relatively high frequency with which FLBN develop either alone or concomitantly with EPN within cadavers of insects (Duncan et al., 2003; McCoy et al., 2002). Competition within and between species of EPN (Koppenhöfer et al., 1995; reviewed in Kaya and Koppenhöfer, 1996) has been the subject of a great deal of research, but there are few reports of the potential of FLBN to compete with and regulate population densities of EPN (Peters, 1996). Because the persistence of EPN can be affected by intra and interspecific competition within the insect host (Kondo, 1989; Selvan et al., 1993), variation in the fitness of commercially formulated EPN species to compete with FLBN may be of practical significance.

Four EPN species were recovered during a 2-year survey of the prevalence of natural control of buried *Diaprepes abbreviatus* L. sentinel insects in a Florida citrus orchard (Duncan et al., 2003). Entomopathogenic nematodes killed between 8% and 52% of insects buried each month. An additional 5% to 22% of the insects yielded only FLBN that emerged from cadavers in large numbers in much the same manner as EPN. We suspected that the amount of EPN-induced mortality was underestimated due to competitive displacement of EPN by opportunistic FLBN within the insect cadavers. We tested this hypothesis by analyzing the strength of relationships between the seasonal occurrence of FLBN and EPN, and in a series of experiments reported herein.

Materials and Methods

Relationships between FLBN and EPN in the field: The prevalence of endemic and introduced EPN and endemic FLBN in cadavers of *D. abbreviatus* was monitored for 2 years in experimental plots in a citrus orchard near Bartow, Florida. Treatments initiated 2 years before the survey began included (i) untreated control that received dry fertilizer four times per year, (ii) treatment with *Steinernema riobrave* Cabanillas, Poinar, and Raulston in June and September each year and fertilization as in controls, and (iii) treatment with *S. riobrave*

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in June and September and fertigation with liquid fertilizer 15 times per year. Plots were ca. 0.6 ha and treatments were arranged in a randomized complete block design with four replicates. Complete details of the experiment and the survey of endemic EPN are given by Duncan et al. (2003). Briefly, 200 laboratory-reared *D. abbreviatus* larvae were buried approximately bimonthly between May to October 2000 and monthly from April to October 2001. One hundred cages were buried beneath individual trees in control plots, and 50 cages each were buried beneath individual trees in the plots receiving the two nematode treatments. After 7 days, larvae were recovered and cadavers were placed on moistened filter paper in petri dishes sealed with Parafilm. Cadavers were observed periodically for 30 days with the aid of a dissecting microscope, and nematodes that emerged were identified as EPN or "free-living" (FLBN). Third-stage infective juvenile EPN (IJ3) were observed with a compound microscope (×1000) for species determination. Periodically, IJ3 from cadavers were allowed to infect new *D. abbreviatus* to recover adult nematodes to confirm the species identification. Cadavers that were co-inhabited by FLBN and EPN were assigned to the category for the EPN to designate the likelihood that insect mortality was due to infection by EPN. Data for each taxonomic category each month were expressed as percentages of the total number of buried larvae, transformed (arcsin square root), and correlations between the taxonomic categories in plots treated with *S. riobrave* were determined (Minitab Inc., State College, PA). Treatment effects on the percentage of insects producing only FLBN were tested by 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 × block was designated as the treatment error term in the model. Mean separation was by Student-Newman-Keuls test at $P = 0.05$.

A second experiment was conducted in August 2001 at a different site to test the hypothesis that inundation of soil with EPN can increase the prevalence of insect cadavers from which only FLBN are detected. Five pairs of 1-m^2 plots were established by clearing vegetation to expose the soil (Candler fine sand; 97% sand, 2.5% silt, 0.5% clay) in an area between two rows of citrus trees at the Citrus Research and Education Center in Lake Alfred, Florida. Two meters separated plots within a pair, and 5 m separated each pair of plots. Plots were established in the row middle to reduce the prevalence of infections by endemic EPN, which are highest beneath the tree canopy and lowest in row middles (Duncan, unpubl.; Glazer et al., 1996). Ten larvae of *D. abbreviatus* were buried at a depth of 30 cm in each plot (Duncan et al., 2003). The next day, one random plot of each pair was treated with commercially formulated *S. riobrave* (Biovector 355, Certis USA, Columbia, MD) at the rate of 20 IJ3 cm^{-2} soil surface in 1 liter of water using a sprinkling can. Nematodes were incorporated in soil with 4 liters of water following treatment, and controls were treated with 5 liters of water. Seven days later, larvae were recovered and processed as described previously. The experiment was repeated once. In both experiments, treatment differences for the numbers of cadavers from which FLBN emerged was tested with a paired *t*-test. In the second experiment nematodes were counted and a *t*-test was used to detect treatment differences in total EPN and FLBN. The linear relationship between numbers of EPN and FLBN emerging from cadavers was measured by calculating the correlation between transformed ($\log_e x + 1$) data.

Competition between FLBN and EPN in the laboratory: We studied the abilities of two species of EPN to compete with *Pellioditis* sp., a free-living rhabditid nematode. *Steinernema riobrave* obtained from Certis Corporation (Colombia, MD) and *S. diaprepesi* Nguyen and Duncan isolated from a citrus orchard near Bartow, Florida, were maintained on larvae of *D. abbreviatus* in petri dishes of autoclaved Candler fine sand at 10% moisture. *Pellioditis* sp. was isolated from a population recovered from a cadaver of *D. abbreviatus* buried in a sample of raw Candler fine sand. The free-living nematode was maintained with its associated bacteria (unidentified) on nutrient agar. Larvae of *D. abbreviatus* (aged 4 to 8 weeks) were maintained axenically in containers of nutrient media.

Petri dishes (60 × 15-mm; 120 in number) were filled with Candler fine sand autoclaved 2 weeks previously and moistened to <10% (wt water:dry wt soil). A single larva of *D. abbreviatus* was added to each dish and factorial treatments were established in each of 30 experimental units: (i) untreated control; (ii) *Pellioditis* sp.; (iii) *S. riobrave;* (iv) *Pellioditis* sp. + *S. riobrave*. Six hundred nematodes of each species in 0.5 ml water were added to the appropriate treatments. *Pellioditis* sp. were added to dishes at the same time as *D. abbreviatus,* and *S. riobrave* were added 7 days later for a final soil moisture of 10%. *Pellioditis* sp. were added prior to *S. riobrave* in the eventuality that prior parasitism of the insect by the FLBN is necessary for infection by both species. Petri dishes were sealed with parafilm after each addition of nematodes and maintained on a laboratory bench at 25 ± 2 °C. Seven days after addition of *S. riobrave,* insect larvae were recovered and cadavers were rinsed in tap water, placed on moistened filter paper in individual petri dishes, sealed with parafilm, and observed for 30 days to determine whether *Pellioditis* sp. and (or) *S. riobrave* would emerge. The experiment was conducted five times. In each experiment, percentage mortality and percentage of larvae yielding *S. riobrave* and (or) *Pellioditis* sp. were determined.

Two additional experiments were conducted essentially as described above, but with modifications. The

insects were weighed prior to use and two additional treatments were used: (v) *S. diaprepesi;* (vi) *S. diaprepesi* + *Pellioditis* sp. Ten days following the first detection of nematodes emerging from insect cadavers, the contents of the petri dish (cadavers and filter paper) were macerated with forceps and scalpel, rinsed into small flasks in 100 cm^3 H_2O, stirred for 2 minutes, and filtered through a sieve (425 μm) to a final volume of 50 ml. Nematodes in a 2-ml aliquot were identified and counted. Numbers of nematodes recovered per insect were correlated with insect body weight ($r = 0.51$, $n = 23$, $P = 0.04$); therefore, the nematodes per cadaver and per available insect were expressed per body weight of the insect. Data were expressed per available insect to illustrate overall effect on the populations, and per cadaver to reveal the effects of competition on reproduction independently of insect mortality rate.

Percentage mortality data for *S. riobrave* and *Pellioditis* sp. for all seven experiments were transformed (arcsin square root) and treatment differences were tested with ANOVA for factorial treatments in an RCB design (blocked by experiment; $n = 7$). Differences in mortality in the two experiments with *S. diaprepesi* alone and combined with *Pellioditis sp.* were analyzed with *z*-tests for binomial data ($n = 30$). The treatment effects on numbers of *Pellioditis* sp. or numbers of *S. riobrave* produced $gram^{-1}$ of available insect or $gram^{-1}$ of cadaver were tested with a two-way analysis of variance.

EPN IJ production: Sixty petri dishes containing Candler fine sand and a larva of *S. diaprepesi* were established as described previously. Four hundred IJ3 of *S. diaprepesi* or *S. riobrave* were added to 30 dishes each. The dishes were then sealed with parafilm and maintained on a laboratory bench at room temperature (24 ± 2 °C). At 4-day intervals for 28 days, dishes were examined and cadavers were transferred to moistened filter paper in sealed petri dishes for observation as described previously. The cumulative percent mortality, cumulative IJ3, and cumulative percentage of ruptured larvae were determined for each observation date. The experiment was conducted three times, and data were pooled to calculate means and standard errors of the response variables ($n = 3$). Comparison of means within experiments were made using *z*-tests for binomial data ($n = 30$).

Results

Relationships between FLBN and EPN in the field: The average monthly percentage of buried insects from which only FLBN were detected was 9.8% (range = 3.0% to 22.3%). FLBN alone were detected emerging from between 8.3% and 36.2% of the cadavers each month (mean = 27.3%). The prevalence of buried larvae from which mixed populations of EPN and FLBN were detected each month ranged from 3% to 11%. All species of EPN detected in the survey were sometimes recovered concomitantly with FLBN, and there were no significant differences among species in the prevalence of mixed infections.

The greatest numbers of cadavers producing only FLBN occurred during months when commercially formulated *S. riobrave* were applied in the orchard (Fig. 1A). The percentage of weevils infected with *S. riobrave* during the survey was directly related to the percentage of weevils infected with FLBN ($r = 0.38$; $P = 0.001$). Four species of endemic EPN were encountered during the survey: *S. diaprepesi, Heterorhabditis zealandica* Poinar, *H. indica* Poinar, Karunakar & David, and *H. bacteriophora* Poinar. Despite a wide range in the detection of endemic EPN, there were no significant relationships be-

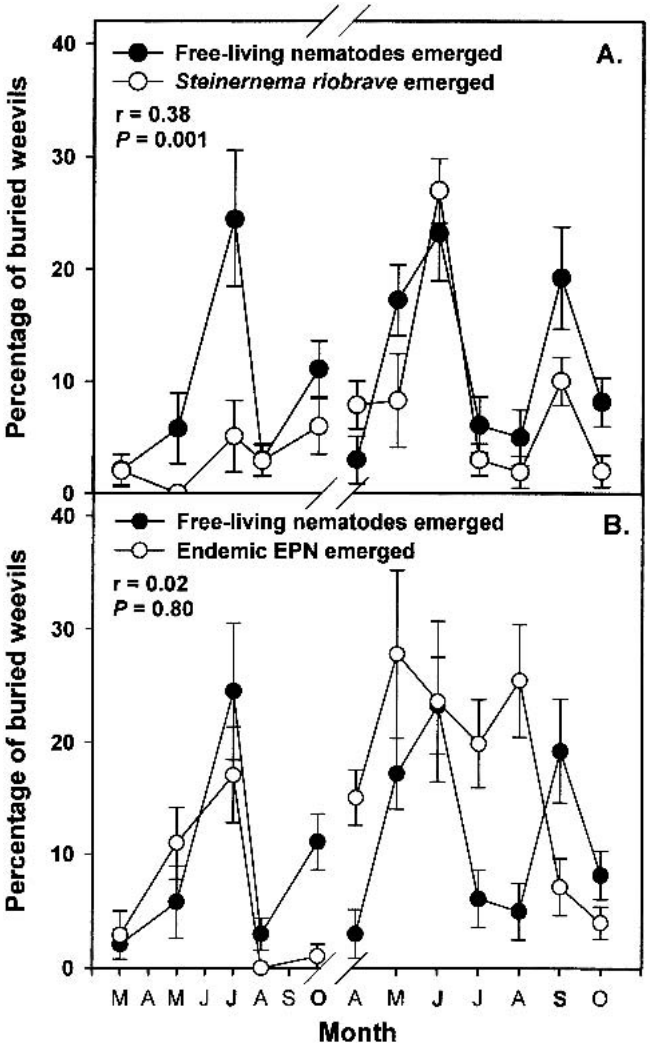

Fig. 1. Seasonal prevalence of *Steinernema riobrave* and unidentified free-living bactivorous nematodes (A) and endemic entomopathogenic nematodes and unidentified bactivorus free-living nematodes (B) in plots treated periodically with *S. riobrave* in a Florida citrus orchard. Prevalence was measured as the percentage of larvae of *Diaprepes abbreviatus,* buried for 7 days, from which nematodes emerged ($n = 100$ larvae per month). Data were transformed (arcsin-square root) for correlation analysis, but untransformed means and standard errors are shown in the figure. Months in which treatments were applied are shown in bold on the x-axis.

tween detection of FLBN and endemic EPN either as individual species (not shown) or collectively (Fig. 1B; $r = 0.02$; $P = 0.80$).

Analysis of variance indicated a treatment effect on the average monthly prevalence of FLBN during the 4 months when *S. riobrave* was applied (df = 2/27; F = 5.22; $P = 0.05$). The prevalence of FLBN was greater in plots treated with *S. riobrave* and fertigation (25.8% ± 4.3% than in control plots that received dry fertilizer and no *S. riobrave* (13.2% ± 2.3%), or plots treated with *S. riobrave* and dry fertilizer (13.0% ± 1.2%). Treatments did not affect the average monthly prevalence of FLBN during non-treatment months (range = 5.4% to 6.5%) df = 2/63; F = 0.39; $P = 0.69$).

In the second experiment conducted at Lake Alfred, Florida, the mortality of *D. abbreviatus* in treated and untreated plots was 88% and 34%, respectively. *Steinernema riobrave* emerged from 60% of the insects that were buried in the treated plots and from 2% of the insects in non-treated plots. *Steinernema diaprepesi* emerged from 14% of insects buried in non-treated plots. More insects from treated plots yielded only FLBN than from untreated plots (30% vs. 14%; $t = 2.75$; $P = 0.052$; data not shown). When the experiment was repeated, mortality in treated and untreated plots was 100% and 16%, respectively. *Steinernema riobrave* emerged from 88% of insects buried in treated plots and 2% of insects buried in untreated plots. Twice as many cadavers from treated plots (12%) contained only FLBN compared to untreated plots (6%), but the difference was not significant ($P = 0.60$). Ninety-two percent of insects buried in treated plots contained FLBN alone or in combination with EPN, in contrast to 14% of insects buried in untreated plots (Fig. 2). The mean

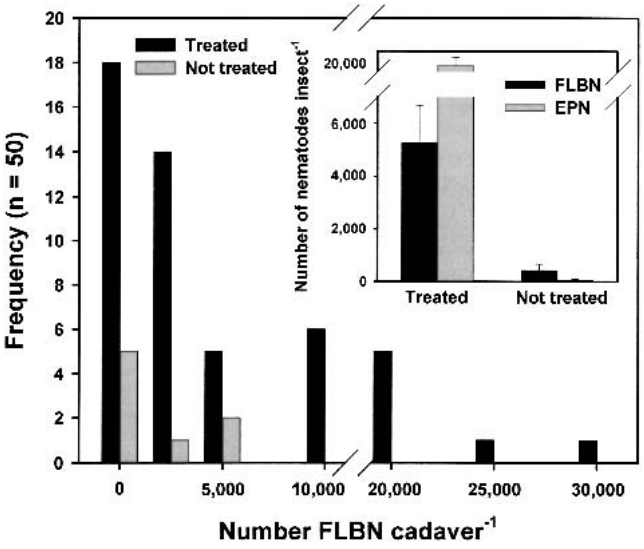

FIG. 2. The frequency of buried insects that contained various numbers of free-living bactivorous nematodes (FLBN) after 7 days in plots treated with 20 *S. riobrave* cm^{-2} soil surface or in untreated plots. Inset shows the means and standard errors for numbers of FLBN and *S. riobrave* recovered per cadaver.

(± standard error) numbers of FLBN recovered per insect buried in treated and untreated plots were 5,268 ± 1,372 and 438 ± 222, respectively ($t = 3.37$, df = 4, $P = 0.01$). Numbers of *S. riobrave* in cadavers from treated plots were inversely related to numbers of FLBN ($r = 0.43$; n = 50; $P = 0.002$).

Competition between FLBN and EPN in the laboratory: Both *Pellioditis* sp. (F = 8.18; df = 1, error df = 18; block df = 6; $P = 0.01$) and *S. riobrave* ($P = 0.0001$; F = 146.1) increased the percentage mortality of *D. abbreviatus,* and there was no interaction ($P = 0.78$) between the two nematode species (Fig. 3A). Average mortality in the *Pellioditis* sp. treatment was 7.6% compared to 3.3% in the untreated controls. When both nematodes were combined, mortality increased to 56.6% ± 5.9% (mean and standard error), compared to 41.7% ± 4.1% in the *S. riobrave* treatment. The mortality in the combination treatment was numerically greater than that for *S. riobrave* alone in six of the seven experiments. The percentage of insect mortality in treatments with *S. diaprepesi* and *S. diaprepesi* + *Pellioditis* sp. did not differ (Fig. 3A).

Steinernema riobrave did not significantly suppress the number of *Pellioditis* sp. that developed in insect cadavers ($P = 0.12$; df = 1, error df = 69; F = 2.36) (Fig. 3B), but the presence of the EPN increased the number of *Pellioditis* sp. produced in the available insect prey by nine-fold ($P = 0.02$; df = 1, error df 116; F = 5.28) (Fig. 3C). Compared to *S. riobrave* alone, the presence of *Pellioditis* sp. suppressed the number of *S. riobrave* per cadaver by 84% ($P = 0.001$; df = 1, error df = 69; F = 39.29) (Fig. 3B), and the number of *S. riobrave* per available insect by 82% ($P = 0.001$; df = 1, error df = 116; F = 12.44) (Fig. 3C). *Pellioditis* sp. did not significantly reduce the numbers of *S. diaprepesi* produced per cadaver or per insect (Fig. 3B,C).

EPN IJ production: Steinernema diaprepesi emerged from insect cadavers faster than *S. riobrave* (Fig. 4A). Twelve days after exposure of insects to nematodes, 42% of the insects treated with *S. diaprepesi* had ruptured, compared to 10% of those treated with *S. riobrave* ($t = 5.73$; $P = 0.02$). The difference in emergence rate disappeared between days 12 and 16. *Steinernema riobrave* produced 49% more IJ3 during 28 days than *S. diaprepesi,* but the difference was not significant (Fig. 4B). Faster emergence by *S. diaprepesi* was not due to a faster rate of infecting and killing insects. *Steinernema riobrave* killed nearly 80% of insect larvae by 8 days post-infection, whereas *S. diaprepesi* required nearly twice as long to attain the same level of insect mortality (Fig. 4C).

DISCUSSION

Free-living bactivorous nematodes are opportunists that rapidly colonize ephemeral habitats (Ferris et al., 2001). Results of this study demonstrate that this trait makes some FLBN species formidable competitors of

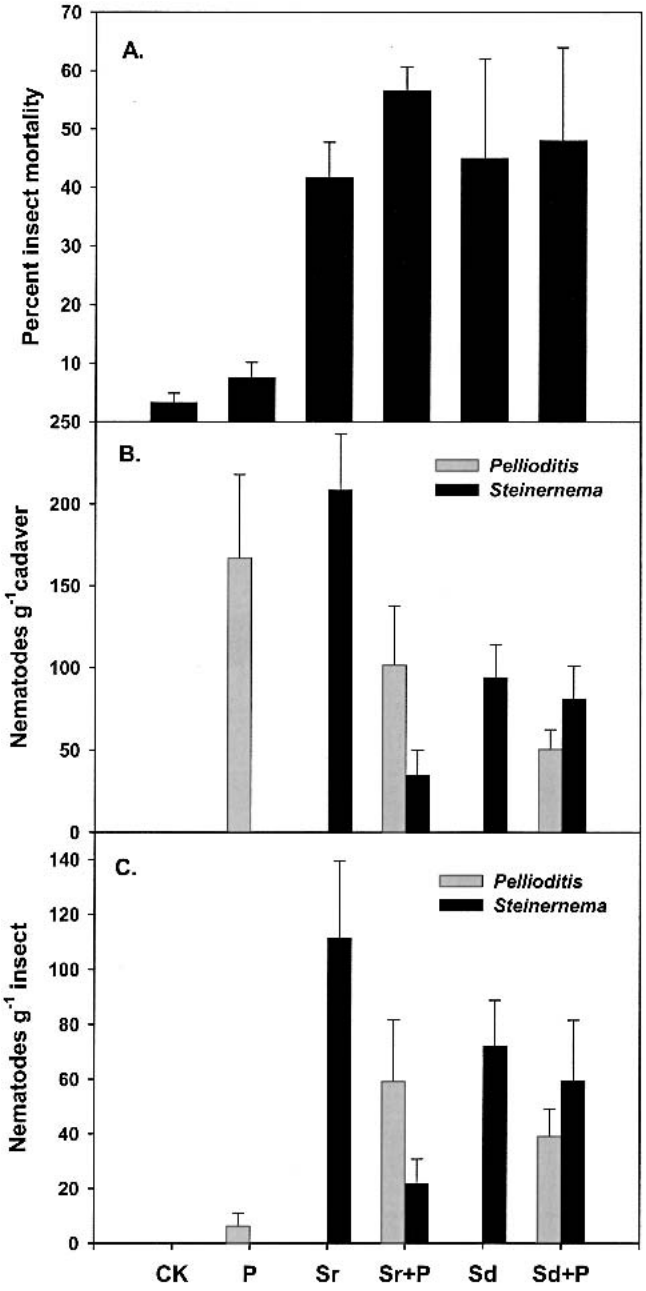

FIG. 3. The effects of *Steinernema riobrave* (Sr), *S. diaprepesi* (Sd), and *Pellioditis* sp. (P), alone or combined, on (A) the percentage mortality of larvae of *Diaprepes abbreviatus;* (B) the number of nematodes recovered per insect cadaver; (C) the number of nematodes recovered per available insect. Data for panel A are means and standard errors pooled from seven experiments ($n = 7$) for *S. riobrave* and two experiments for *S. diaprepesi* ($n = 2$). Data for panels B and C are pooled from two experiments ($n = 2$). Numbers of nematodes are expressed per milligram of insect body weight to adjust for variation in size of insect prey.

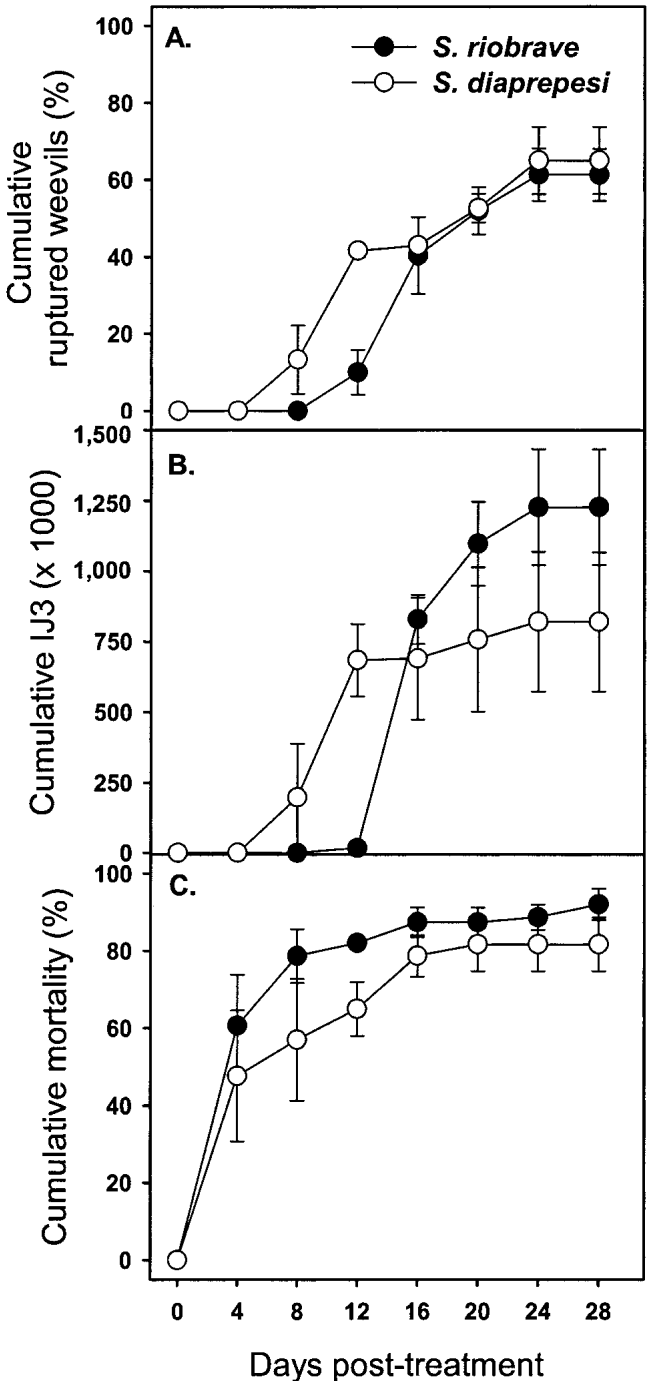

FIG. 4. Rate of emergence of (A) *Steinernema riobrave* and *S. diaprepesi* from cadavers of *Diaprepes abbreviatus,* (B) cumulative numbers of infective juveniles (IJ3) recovered from insect larvae ($n = 25$ in each experiment), and (C) rate of mortality of the insects. Data are means and standard errors pooled from three experiments ($n = 3$).

some EPN. A correlation between the prevalence of *S. riobrave* and FLBN revealed the possibility that application of *S. riobrave* induced population growth of FLBN at the Bartow site. This hypothesis is supported by other evidence. Concomitant infections by FLBN and EPN, detected in up to 11% of the cadavers at Bartow each month, revealed competition between these nematodes. The introduction of *S. riobrave* greatly increased the prevalence of insect cadavers yielding FLBN and the numbers of FLBN in the field experiment at Lake Alfred, Florida. Sympatry of *Pellioditis* sp. and *S. riobrave* in microcosms increased insect mortality compared to that from *S. riobrave* alone and favored reproduction of FLBN at the expense of the EPN. Thus, although the causes of mortality for cadavers yielding only FLBN at

the Bartow site are unknown, it is likely that FLBN displaced EPN some of the time. Between 21% and 29% of the cadavers in control and nematode-treated plots, respectively, yielded only FLBN. If EPN killed most of the insects that yielded only FLBN, then FLBN could be major regulators of EPN at the Bartow site. The suppression of *S. riobrave* recycling by *Pellioditis* in the laboratory experiments supports this possibility. These data also reveal the possibility that FLBN can cause an underestimation of EPN parasitism of insects in the field.

FLBN parasitism of insects and other soilborne animals such as earthworms and diplopods is one adaptation that permits the nematode immediate access to nutrients released on the death of the host (Gerber and Giblin-Davis, 1990; Poinar and Thomas, 1975; Sudhaus and Schulte, 1989). Nematodes that employ such a life strategy have been termed "necromenic" (Sudhaus and Schulte, 1989). We dissected a number of insects that survived in the *Pellioditis* sp. treatments but found no evidence that this nematode is necromenic. Rather, it appears to respond opportunistically to cues involved in the infection of *D. abbreviatus* by EPN in order to invade and appropriate resources in the cadaver. Peters (1996) noted that other soilborne pathogens, predators, and omnivores may prevent reproduction of EPN in prey they have killed and speculated that the presence of necromenic nematodes was responsible for inferior development of *H. bacteriophora* and *S. feltiae* in experimental insects. Competitive displacement of *Heterorhabditis bacteriophora* by the fungus *Beauveria bassiana* from cadavers of *Spodoptera exigua* was seen in laboratory experiments similar in design to our study (Barbercheck and Kaya, 1990).

Steinernema riobrave and *S. diaprepesi* differed in their relative ability to compete with *Pellioditis* sp. Several determinants of competitive ability in EPN have been proposed in previous reports. The rate of colonization may have differed for the two EPN species (Alatorre-Rosas and Kaya, 1990). The *Xenorhabdus* strains carried by the two EPN may differ in nutritional value to *Pellioditis* sp. or in their ability to suppress bacteria carried on and in the FLBN (Akhurst, 1982). Development rate is an important trait in competition between these opportunistic nematodes because it affects the speed of resource appropriation (Kaya and Koppenhöfer, 1996). Infective juveniles of *S. diaprepesi* depleted the host resources at a faster rate than *S. riobrave*. The IJ3 of *S. diaprepesi* are longer than *S. riobrave* (1,002 vs. 622 µm), with approximately twice the volume (Nguyen and Duncan, 2002). Because fewer IJ3 of *S. diaprepesi* ruptured the host sooner than *S. riobrave,* the larger *S. diaprepesi* is either less fecund or depletes the insect resources in fewer generations than *S. riobrave*. Our findings are similar to those of Koppenhöfer et al. (1995), who demonstrated an ability of the larger *S. glaseri* (length = 1,300 µm) to complete development faster and displace *S. carpocapsae* (630 µm) in concomitant infections of *G. mellonella*. It would be interesting to test whether, given similar generation times, large bactivorous nematodes are generally more fit than smaller species to compete in concomitant infections between species.

The ability of *S. riobrave* to compete with FLBN may not differ from that of other EPN as much as suggested by the current laboratory studies. The prevalence of mixed infections of FLBN and EPN at the Bartow site was not higher for *S. riobrave* than for the other EPN species, and the prevalence of FLBN in plots that received dry fertilizer at the Bartow site was not affected by treatment with *S. riobrave*. The increased prevalence of FLBN in plots treated with liquid fertilizer and *S. riobrave* may have been primarily a response of bacteria and FLBN to the greater frequency of fertilizer applications in much the same way that rhabditid and other bacterial-feeding nematodes have been shown to increase in soil amended with organic mulches (Ferris et al., 1996; Porazinska et al., 1999). The numbers of FLBN and EPN used in the laboratory microcosms were arbitrary, although similar densities of FLBN from the citrus rhizosphere have been reported (Porazinska et al., 1999). Nevertheless, different initial population densities may produce different results. Study of additional species of FLBN in combination with *S. riobrave* and *S. diaprepesi* is also needed to understand if the results of these experiments can be generalized. Further studies are warranted because competition with FLBN may contribute to the lower persistence of *S. riobrave* compared to *S. diaprepesi* in Florida citrus orchards (Duncan et al., 2003).

An interesting question is whether competition by FLBN contributes in a density-dependent manner to the regulation of endemic EPN. Seasonal patterns in the prevalence of FLBN and EPN in the citrus rhizosphere were found in studies in Spain and Florida, respectively (Duncan et al., 2003; Galeano, 2002). Although we found no relationship between prevalence of endemic EPN and FLBN, the correlation between *S. riobrave* and FLBN suggests that FLBN may increase in response to EPN density. This possibility is supported by Somasekhar et al. (2002), who showed that inundative augmentation of EPN in the field increased the prevalence of free-living bactivorous nematodes on the order of 50% to 80% for at least 60 days following application. Density-independent factors may also cause increased numbers of FLBN coincidentally with an increase in EPN. The seasonal recruitment of neonate *D. abbreviatus* larvae into the soil favors population growth of EPN, and may initiate a series of events that increase numbers of FLBN. Increased insect waste, root damage and decomposition, and mortality of these insects due to many factors would all be favorable for population growth of bacteria and FLBN. Therefore, in addition to antagonists such as nematophagous fungi, bacterial pathogens, and microarthropod predators,

competitors such as FLBN should be studied as potentially significant regulators of EPN.

The mechanism by which sympatric populations of *Pellioditis* sp. and *S. riobrave* can increase mortality of *D. abbreviatus* above that caused by *S. riobrave* alone is unknown. The significant effect of *Pellioditis* sp. and an absence of a significant interaction between the two nematodes suggests that *Pellioditis* sp. or microorganisms associated with the nematode are mildly pathogenic to *D. abbreviatus,* even in the absence of EPN. However, the very low mortality in both the untreated control and the *Pellioditis* sp. treatment and a lack of evidence of parasitism by *Pellioditis* sp. suggest that the ANOVA results should be viewed with caution. On the other hand, insect mortality was greater when both nematodes were combined than that for *S. riobrave* alone in all but one of the seven experiments. Those data suggest that insects are more susceptible to infection by *S. riobrave* in the presence of FLBN, or that infection by both nematodes is more likely to result in the death of the insect. Additional research is needed to reveal the mechanism(s) by which FLBN affect insect mortality.

In conclusion, FLBN are ubiquitous soil inhabitants that may contribute to the regulation of EPN species through resource competition. The question of whether some EPN species are better adapted than others to compete with FLBN warrants additional research because this trait may influence the persistence and efficacy of EPN selected for use in insect management.

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