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Augmenting Entomopathogenic Nematodes in Soil from a Florida Citrus Orchard: Non-Target Effects of a Trophic Cascade

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Abstract: Laboratory experiments were conducted to study non-target effects of augmenting entomopathogenic nematode (EPN) communities in soil. When raw soil from a citrus orchard was augmented with either 2,000 *Steinernema riobrave* or *S. diaprepesi,* fewer EPN ($P \leq 0.05$) survived if the soil had also been treated with 2,000 *S. riobrave* 7 d earlier (i.e., two augmentation events rather than one). EPN survival was unaffected by treatment ($P \leq 0.05$) in soil that was air-dried to disrupt antagonist activity prior to the experiment. When *S. diaprepesi, S. riobrave, Heterorhabditis zealandica* or no EPN were added to raw soil and *S. diaprepesi* was added 5 d later, the survival of both *S. diaprepesi* and of total EPN was greater ($P \leq 0.05$) in soil that received no pretreatment than in soil pretreated with *S. riobrave*. Pretreatment of soil with *H. zealandica* or *S. diaprepesi* had less or no affect on survival of *S. diaprepesi* or total EPN. When nematodes were recovered from soil and placed on water agar, the number of *S. diaprepesi* that were killed by endoparasitic and trapping nematophagous fungi was greater ($P \leq 0.05$) if soil was pretreated with steinernematid species than if the soil was not pretreated or was pretreated with *H. zealandica*. The adverse effects of pretreating soil on EPN survival were density dependent within a range of pretreatment dosages (20–100 IJ/cm^2 soil surface), and the treatment effects required more time to become evident at lower than at higher dosages. These experiments suggest that non-target effects of augmenting the EPN community in soil vary among EPN species and have the potential to temporarily reduce EPN numbers below the natural equilibrium density.

Key words: Antagonism, nematophagous fungi, numerical response, post-application biology, predation, survival.

Entomopathogenic nematodes (EPN) are used in classical and augmentation biological control programs in Florida to manage insect pests of turf, forage crops and citrus. *Steinernema scapterisci* Nguyen and Smart was discovered in Uruguay, near the presumed center of origin of the mole cricket (*Scapteriscus* spp.) and introduced in Florida as a potential biological control agent of several invasive mole cricket species (Nguyen and Smart, 1990, 1991). Research is ongoing to determine whether *S. scapterisci* has established in Florida at levels needed to provide acceptable mole cricket suppression, or whether it is necessary to periodically augment the numbers of the nematode (Adjei et al., 2003). Commercial formulations of four EPN species have also been used at various times since 1991 for augmentation biological control of *Diaprepes abbreviatus* L., a polyphagous pest in the Caribbean Basin that was introduced into Florida more than 40 years ago (Shapiro-Ilan et al., 2005). The use of EPN to manage the subterranean weevil larvae was adopted because available chemical pesticides are ineffective. An exotic species, *Steinernema riobrave* Cabanillas, Poinar and Raulston, and an endemic strain of *Heterorhabditis indica* Poinar, Karunakar and David are currently sold in Florida for control of *D. abbreviatus*. Estimates of the efficacy and profitability of using EPN for weevil control in citrus vary widely and probably reflect variation in factors such as product quality, application rates, suitability of edaphic conditions for EPN and experimental methods (Adair, 1994; Duncan and McCoy, 1996; Duncan et al., 1996b; Stansly et al., 1997; Bullock et al., 1999; McCoy et al., 2000, 2002; Duncan et al., 2002, 2003b).

Despite their widespread use for pest control in Florida, relatively little is known about the post-application biology of EPN or the effects of augmentation on soil food webs (Duncan et al., 2003a, 2003b; El-Borai et al., 2005). Periodic augmentation of the EPN communities in citrus orchards with *S. riobrave* effectively killed sentinel weevil larvae; however, following treatment, the prevalence of EPN and the mortality of sentinel larvae sometimes declined temporarily in treated compared to untreated plots (Duncan et al., 2003b, 2007). Duncan et al. (2003b) speculated that competition between long-lived endemic EPN species (i.e., *S. diaprepesi* Nguyen and Duncan) and the shorter-lived exotic *S. riobrave* could eventually diminish the number of EPN in soil. Equally plausible is the possibility that density-dependent antagonists of nematodes increased following EPN augmentation, thereby reducing EPN numbers below the previous equilibrium density (Ishibashi and Kondo, 1986; Koppenhofer et al., 1996; Kaya, 2002). Numerically, EPN are a minor part of nematode communities in soils; however, they attain numbers high enough in the vicinity of parasitized insect larvae to stimulate a localized numerical response by some species of nematophagous fungi (NF) (Jaffee and Strong, 2005). Entomopathogenic nematodes are typically augmented at rates equivalent to 500 to 2,000 IJ/100 cm^3 in the top 5 cm soil, which are in the range of total nematode abundance reported for many soil habitats. Therefore, it seems possible that augmenting EPN could initiate a trophic cascade in which the NF and other natural enemies proliferate and temporarily reduce the numbers of endemic and exotic EPN enough to suppress the natural control of insect larvae.

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To explore this possibility, we conducted laboratory experiments to compare the survival of several EPN species and the prevalence of nematophagous fungi in soil with and without prior EPN augmentation. We tested the hypotheses that augmentation of EPN in soil at commercial application rates can (i) increase the prevalence of nematode antagonists, thereby (ii) increasing mortality rates in EPN communities in soil and that (iii) these effects will be less pronounced for heterorhabitid than steinernematid EPN because heterorhabditid IJ more often remain ensheathed in the protective second-stage cuticle (Timper and Kaya, 1989, 1992).

Materials and Methods

Nematode inoculum: Steinernema diaprepesi (Sd), *S. riobrave* (Sr) and *Heterorhabditis zealandica* Poinar (Hz) were isolated from *Diaprepes abbreviatus* sentinel larvae buried in a commercial citrus orchard near Bartow, FL. *Steinernema riobrave* were descendents of commercially formulated EPN that were applied periodically for more than 4 yr to manage the insect. The other two species are endemic. Nematodes were maintained in culture by periodically infecting *D. abbreviatus* larvae in moist (10%) autoclaved sand. Nematodes recovered from insect cadavers in White traps were stored in shallow water in transfer flasks (15°C) for up to 15 d before re-transfer. Nematodes used in these experiments were stored in this manner for between 1 and 5 d before use.

Assay for survival of IJ in soil: A bioassay was developed to determine whether augmenting EPN in soil can result in soil that is temporarily suppressive to EPN. Surface litter was removed, and 500 cm^3 of soil from 0 to 30 cm depth was obtained from the undercanopy of each of eight mature citrus trees in a commercial orchard near Bartow, FL. Soil texture in the orchard is a fine sand (97% sand, 2%, silt, 1% clay; pH 6.7; organic matter <1.0%). To reduce the prevalence of nematode antagonists, the soil was mixed, spread in a thin layer, air-dried in the laboratory for 2 wk and stored in a plastic container. Forty-eight hours prior to initiating an experiment, additional soil (hereafter referred to as raw soil) was collected from beneath the same trees, mixed and sampled for moisture determination (24 hr at 70°C). In all experiments, the moisture content of the air-dried and raw soil was adjusted to 8% (wt water: wt dry soil) with tap water. Experimental units consisted of petri dishes (53-mm-diam. × 12-mm-deep) filled with either type of soil. Predetermined numbers of EPN IJ in 600 µl tap water or tap water without nematodes were pipetted onto the soil surface, and the dishes were sealed with parafilm and stored in the dark at 25°C to allow population growth of natural enemies. After 7 d (unless otherwise indicated), 2,000 EPN IJ of the same or different species were added in the same manner to all of the dishes. The dishes were then resealed and stored for either 3 or 7 additional d to determine the treatment effects on the survival of EPN and the prevalence of natural enemies. The assay was terminated by recovering nematodes from soil with sugar centrifugation (Jenkins, 1964; nematodes recovered on 500 µm sieve) or Baermann funnels (48 hr). IJ on the dish inner surfaces were rinsed either directly into glass tubes (experiment 1) or back into the soil prior to extraction. IJ in aliquots (5 cm^3 of 25 cm^3) of the recovered nematodes were identified and counted using an inverted compound microscope (×20–×400). In some experiments, nematodes in the remaining 20 cm^3 water in the tubes were processed to recover nematophagous fungi (NF) as described in the following section.

Assay for prevalence of nematophagous fungi: The IJ and endemic nematodes extracted from soil in the IJ survival assay were further purified by magnesium sulfate centrifugation (Kaplan and Davis, 1990), concentrated in 500 µl tap water and spread across 2% water agar containing 2 ppm streptomycin sulfate in 5-cm-diam. petri dishes. Excess water was allowed to evaporate, and dishes were then sealed with parafilm and incubated for 6 d at 25°C. At this point, sporulation by trapping fungi frequently was not initiated, even though most or all IJ were killed. Therefore, 6 to 8 d after placing the nematodes on agar, 1,000 additional, healthy *S. riobrave* or *S. diaprepesi* from the stock cultures in 500 µl tap water were added to the dishes, and excess water allowed to evaporate. After 48 hr, sporulation of some species was prevalent, and dishes were examined to determine the numbers of live and dead IJ and to classify NF associated with nematode cadavers as either trappers or endoparasites, based on the morphology of spores or specialized hyphae. The cause of death for cadavers without evidence of NF was denoted as unknown.

Effect of augmenting with Sr on survival of Sr, Sd and Hz: Fifteen replications of the following treatments were established in both air-dried and raw soil: 0-Sr; 0-Sd; 0-Hz; Sr-Sr; Sr-Sd; and Sr-Hz, in which the first term indicates the initial treatment (±2,000 IJ Sr) and the second term indicates the species added to the soil 7 d later. Seven days after the second augmentation, nematodes were extracted from soil using Baermann funnels. Nematodes on the dish surfaces were rinsed into tubes and counted separately. The experimental design was a 2 × 2 × 3 factorial (air-dried vs. raw soil *x* pretreatment or no pretreatment with Sr *x* Sr or Sd or Hz, 7 d after adding Sr).

Effect of augmenting with Sr, Sd and Hz on survival of Sd: The following treatments were established in raw soil in a completely randomized design: 0-Sd; Sd-0; Sd-Sd; Sr-Sd; Hz-Sd. Either 600 or no IJ were added initially. Fifteen replicates were prepared for three treatments and 30 replicates for treatments 0-Sd and Sr-Sd. Seven days after the second augmentation, nematodes in soil

from 15 replicates of all treatments were extracted for 72 hr on Baermann funnels. The IJ in the remaining 15 replicate dishes of treatments 0-Sd and Sr-Sd were extracted by sugar centrifugation and assayed for nematophagous fungi. *Steinernema diaprepesi* were the sentinel IJ added to the fungus bioassays after 8 d.

In a second experiment, 15 replicates of the treatments Sd-Sd, Sr-Sd or Hz-Sd (600 IJ) were established in both air-dried and raw soil. The experimental design was a 2 × 3 factorial (soil type × nematode species). Nematodes were recovered from soil by sugar centrifugation 7 d after the second augmentation. IJ that were not enumerated after extraction from soil were bioassayed for nematophagous fungi. *Steinernema riobrave* were used as bait in the second part of the NF bioassay.

Temporal responses to EPN augmentation: One hundred-twenty IJ survival assay dishes were prepared with raw soil. Sixty plates were augmented with 600 IJ Sr and the remaining plates received only 600 µl tap water. After 7, 14 and 28 d, 2,000 additional Sr were added to 20 dishes each with or without prior augmentation. Baermann funnels were used to recover nematodes 7 d after the second augmentation when numbers of IJ Sr were counted. We also counted endemic, second-stage juvenile (J2) *Tylenchulus semipenetrans* Cobb, which were especially numerous at this time of year, to determine whether augmenting EPN affected this plant-parasitic nematode. The experiment was repeated, but with an additional augmentation time interval (21 d). Each experiment was arranged in a completely randomized 3 × 2 or 4 × 2 factorial design (augmentation time interval × number of augmentations).

Effects of EPN augmentation density: Eighty IJ survival assay dishes were prepared using raw soil, and 20 replicates of four treatments (0, 500, 1,000 and 2,000 IJ *S. riobrave*) were arranged completely randomly. Two thousand *S. diaprepesi* were added to the dishes after 14 d. Three days later, nematodes were recovered by sugar centrifugation. An aliquot was counted, and the remaining IJ were used in the NF assay. *Steinernema diaprepesi* were used in the second part of the NF assay.

Statistical tests: Data from factorial experiments were subjected to ANOVA with the General Linear Model Procedure (Minitab Inc., Release 13). Tukey's Honestly Significant Difference Test and Fisher's LSD test were used to compare treatment means. Pairwise comparisons were by Student's *t*-test. Proportions and counts were transformed (arcsin-square root and log (X + 1), respectively) prior to analysis, but untransformed means are reported.

Results

Effect of augmenting with Sr on survival of Sr, Sd and Hz: All two-way interactions between the main factors were at or near a significant level ($P \leq 0.07$; Table 1). Accordingly, each factor was analyzed separately. For the steinernematid species, recovery of live IJ from soil was 138% higher from air-dried than from raw soil for Sd ($F = 43.31$; df = 1, 56; $P = 0.001$) and 73% higher for Sr ($F = 10.12$; df = 1, 56; $P = 0.002$) (Fig. 1A,C). The same trends occurred for the steinernematid nematodes recovered from dish surfaces (Fig. 1B,D). However, IJ were recovered in greater numbers from soil than from dish surfaces ($P = 0.001$) in treatments containing Sd, whereas fewer IJ were recovered from soil than from

Table 1. Analysis of variance for recovery of three EPN species from soil that was raw or air-dried and either pretreated or not pretreated with *Steinernema riobrave*.

Source	DF	Adjusted mean square	F	*P*
Soil treatment	1	76,594	46.89	0.000
EPN species	2	126,252	77.30	0.000
Pretreatment with Sr	1	3,085	1.89	0.172
Soil × species	2	45,609	27.92	0.000
Soil × pretreatment	1	5,563	3.41	0.067
Species × pretreatment	2	12,473	7.64	0.001
Soil × species × pretreatment	2	2,598	1.59	0.208
Error	128	1,633		
Total	139			

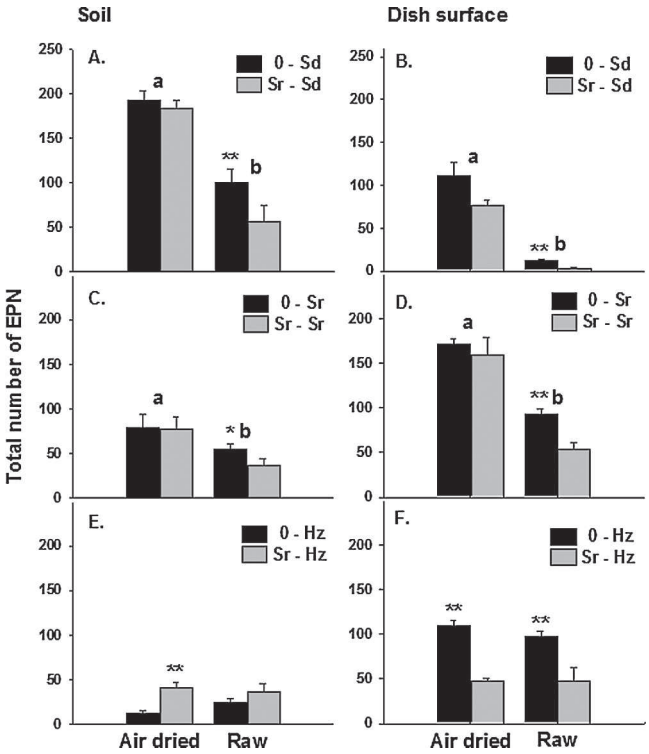

Fig. 1. Effects of augmenting the numbers of *Steinernema riobrave* (Sr) in raw or air-dried soil on the numbers of EPN recovered from Baermann funnels 2 wk later. A second EPN cohort (Sr, *S. diaprepesi* [Sd] or *Heterorhabditis zealandica* [Hz]) was added to all experimental units 7 d after Sr were added to half of the units. Error bars are standard errors of means. Significant differences between means within a soil type (air dried or raw) are denoted by * ($P \leq 0.05$) and ** ($P \leq 0.01$) according to Tukey's Honestly Significant Difference Test. Treatment differences between the soil types ($P \leq 0.05$) are indicated by different letters.

dish surfaces ($P = 0.001$) in treatments with only Sr. Pretreatment of raw soil with Sr caused fewer total IJ to be recovered from soil (Sr-Sd, $P = 0.01$; Sr-Sr, $P = 0.03$) and dish surfaces (Sr-Sd, $P = 0.01$; Sr-Sr; $P = 0.01$), but IJ recovery was unaffected by pretreatment with Sr ($P > 0.10$) when dishes contained air-dried soil.

Unlike the treatments containing steinernematid species, in treatments containing Hz the recovery of IJ from soil or container surfaces was unaffected by the type of soil (raw vs. air-dried; Fig. 1E,F). More IJ were recovered from dish surfaces than from soil ($P = 0.001$) in treatments containing Hz, and there was an interaction between the location of the nematodes (soil vs. dish surface) and pretreatment with Sr. On dish surfaces, fewer IJ were recovered using both types of soil (air-dried vs. raw) if they were pre-inoculated with Sr ($P = 0.01$). However, pretreatment of air-dried soil with Sr resulted in higher recovery of Sr+Hz from soil ($P = 0.01$), and a similar non-significant trend was observed in raw soil.

Effect of augmenting with Sr, Sd and Hz on survival of Sd: In the first experiment, significantly fewer Sd were recovered from soil pretreated with Sr compared to non-pretreated soil (Fig. 2). Pretreatment with Hz did not significantly affect recovery of Sd compared to recovery from non-pretreated soil, and pretreatment with Sd increased recovery of Sd to levels numerically higher (ns) than the combined recovery from treatments Sd-0 and 0-Sd. The same trend occurred for total EPN recovered from these treatments. Fewer total live EPN were recovered from soil pretreated with Sr than from soil that received no pretreatment with EPN (Fig. 2). The proportion of dead Sd among the Sd recovered from Baermann funnels was higher ($P < 0.001$, Tukey's HSD) in soil pretreated with Sr (39%) than in the other treatments (range 9.8–19.1%) which did not differ from one another (data not shown).

Pretreatment of soil with Sr resulted in a 10-fold increase in Sd cadavers in the NF assay (Fig. 3). More Sd were killed by endoparasitic fungi, trapping fungi and unidentified sources if soil was pretreated with Sr than if no pretreatment occurred.

In the second experiment, pretreatment of raw soil with Sd or Hz resulted in recovery of more than three times as many EPN than did pretreatment with Sr ($P = 0.01$) (Fig. 4A). In air-dried soil, pretreatment with Sr and Hz had similar effects on recovery of EPN which were approximately half as numerous as in soil pretreated with Sd ($P = 0.01$). *Heterorhabditis zealandica* appeared to be less affected by antagonists than was either steinernematid species because fewer EPN ($P = 0.01$) were recovered from raw soil than from air-dried soil pretreated with steinernematids, whereas pretreatment with Hz had the same effect in both soils.

The survival of Sr in the nematophagous fungi assay was unaffected by pretreatment of air-dried soil with any species, but pretreatment of raw soil with Sd resulted in fewer surviving Sr than did pretreatment with Hz (Fig. 4B). The prevalence of trapping or endoparasitic fungi was not significantly different among treat-

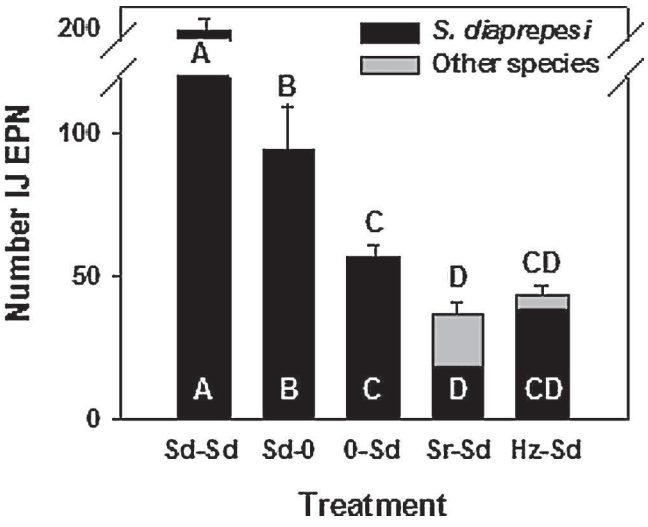

FIG. 2. Effect of augmenting the numbers of *Steinernema diaprepesi* (Sd), *S. riobrave* (Sr) or *Heterorhabditis zealandica* (Hz) in raw soil, followed by augmentation of *S. diaprepesi* 7 d later on numbers of entomopathogenic nematodes recovered from Baermann funnels. Error bars are standard errors of means. Bars with the same letter do not differ from each other according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$). White letters represent differences between numbers of *S. diaprepesi* and black letters represent differences between total numbers of EPN.

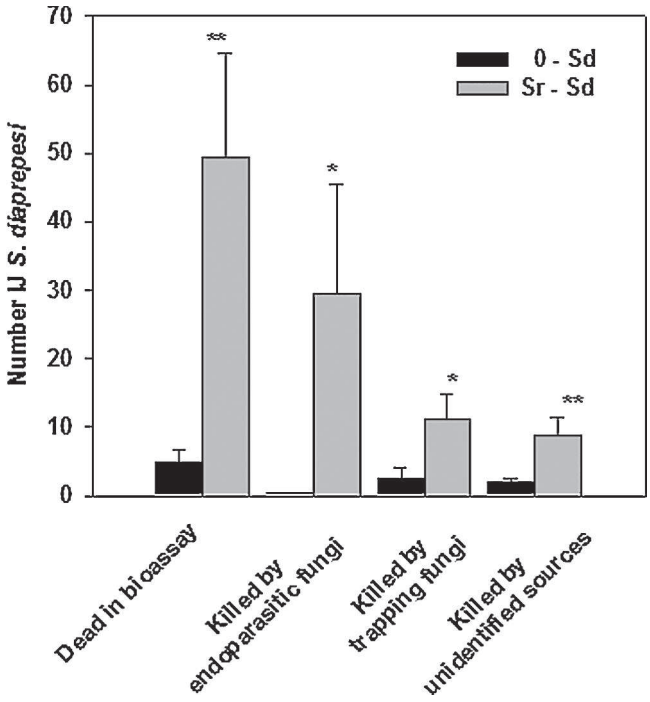

FIG. 3. Effect of augmenting the numbers of *Steinernema riobrave* (Sr) in raw soil on the survival of sentinel *S. diaprepesi* in the NF assay and on the relative prevalence of nematophagous fungi recovered from soil and transferred to water agar (NF assay). Error bars are standard errors of means. Significant differences between means within a category are denoted by * ($P \leq 0.05$) and ** ($P \leq 0.01$) according to Tukey's Honestly Significant Difference Test.

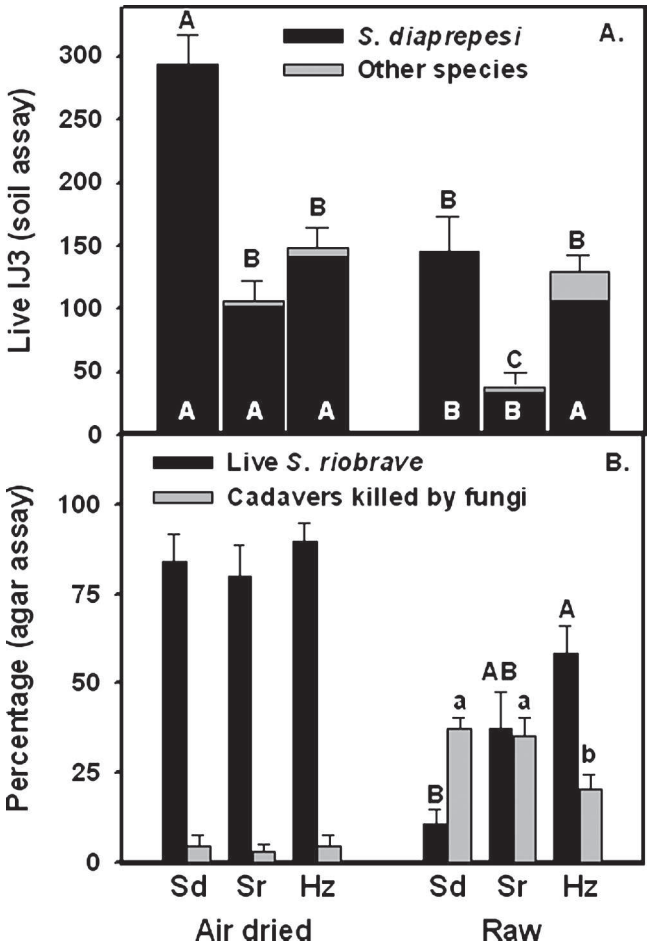

FIG. 4. Effects of sequentially augmenting air-dried or raw soil, first with *Steinernema diaprepesi, S. riobrave* or *Heterorhabditis zealandica,* followed 7 d later by *S. diaprepesi* on A) the survival of entomopathogenic nematodes (EPN) and B) the prevalence of nematophagous fungi (NF). Error bars are the standard error of the mean. Different letters above bars denote significant treatment differences ($P \geq 0.05$; Tukey's Honestly Significant Difference) for both *S. diaprepesi* and total EPN within a soil type. Different letters at the base of bars denote significant differences in recovery of both *S. diaprepesi* and total EPN for a specific pretreatment across soil types.

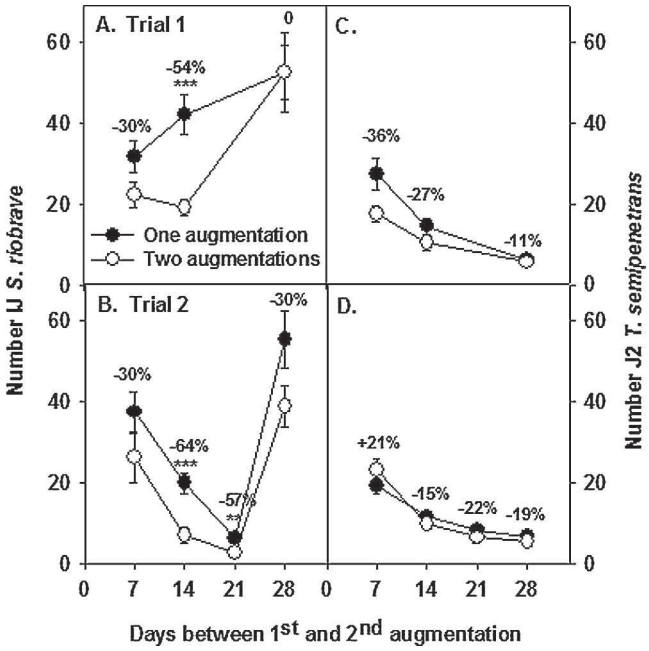

FIG. 5. Effects of interval between the first and second augmentation of soil with *Steinernema riobrave* on numbers of *S. riobrave* (A–B) or *Tylenchulus semipenetrans* (C–D) recovered from soil that was augmented once or twice with the nematode. Numbers above symbols represent the percentage reduction in numbers of nematodes from soil augmented twice compared to once. Significant treatment differences according to Student's *t*-test are denoted by ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

ments, but when considered together these fungi were less prevalent ($P = 0.02$) when raw soil was pretreated with Hz than with Sr or Sd.

Temporal response to EPN augmentation: The numbers of EPN recovered from soil treated once or twice with 600 Sr differed ($P < 0.01$) when the interval between treatments was 2 to 3 wk (Fig. 5A,B). The effect of augmenting EPN in soil on EPN population density was ephemeral; no differences were detected when 28 d separated the first and second application of Sr.

Although there was a tendency to recover fewer *T. semipenetrans* from soil that was augmented twice compared to once, the trend was not significant in either experiment (Fig. 5C,D).

Effects of EPN augmentation density: Fewer EPN ($P = 0.05$) were recovered from soil in all treatments that received two EPN augmentations compared to that receiving a single augmentation (Fig. 6A). There was no significant treatment effect on numbers of live Sd at the end of the NF assay (Fig. 6B). The numbers of Sd killed by trapping fungi increased with increasing dosage of IJ Sr used in the initial augmentation, and a similar non-significant trend occurred for IJ killed by endoparasitic fungi (Fig. 6C,D).

DISCUSSION

Augmenting the EPN in these soil microcosms caused species-specific, non-target effects. Within two weeks following the first of two applications of steinernematid nematodes to raw but not air-dried soil, the prevalence of some nematophagous fungi increased, whereas numbers of EPN declined compared to treatments that only received a single augmentation treatment. Jaffee and Strong (2005) found that population densities of some species of trapping fungi increased as much as 100-fold in localized responses to the large numbers of IJ steinernematids that egress from insect cadavers. The substantial NF numerical responses reported by Jaffee and Strong (2005) resulted in approximately one-third higher EPN mortality, which is of the same order of magnitude that we observed. These results are consistent with the hypothesis that EPN antagonists can respond to EPN augmentation in the field with population growth high enough to temporarily reduce numbers of EPN and rates of sentinel insect mortality compared to those in non-augmented soil (Duncan et al., 2003b, 2007).

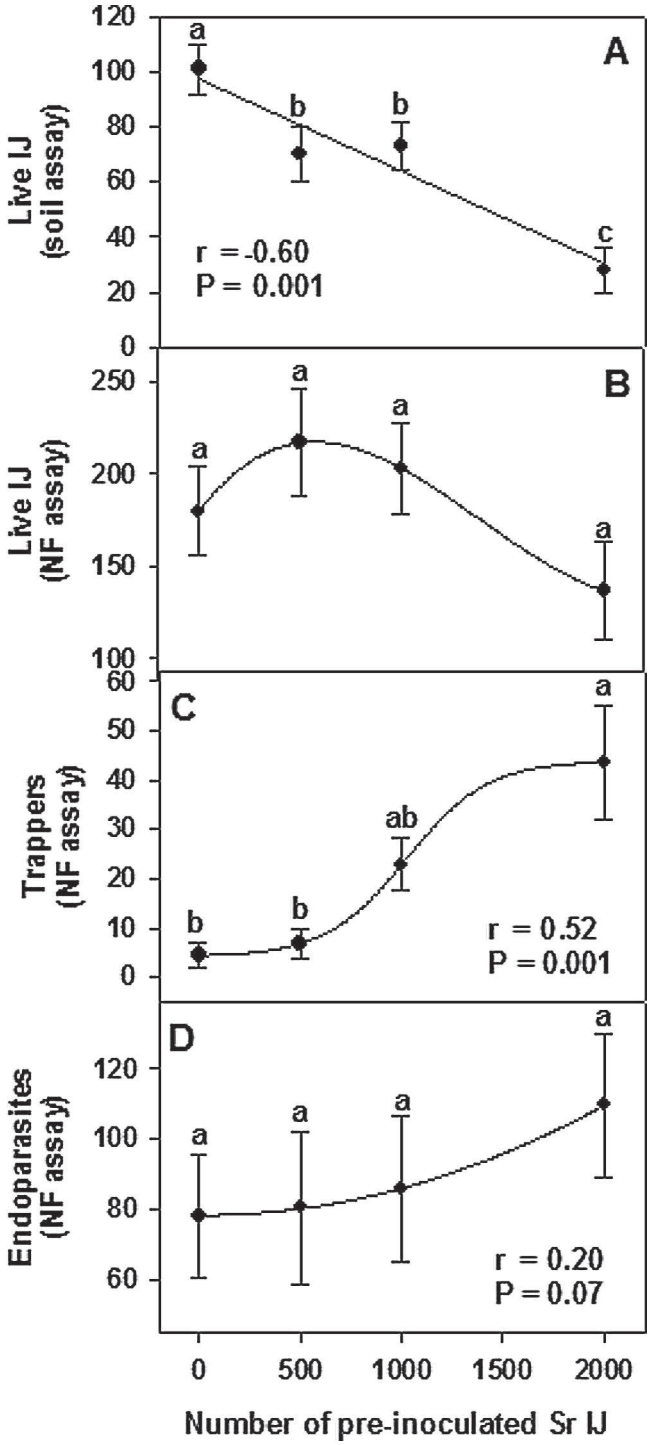

FIG. 6. Effect of augmenting raw soil with various numbers of *Steinernema riobrave* and then adding 2,000 *S. diaprepesi* 2 wk later on A) numbers of live entomopathogenic nematodes extracted from the soil 1 wk after the second augmentation, B) numbers of *S. diaprepesi* that survived the NF bioassay, C) numbers of *S. diaprepesi* killed by trapping fungi in the NF bioassay and D) numbers of *S. diaprepesi* killed by endoparasitic fungi in the NF bioassay. Error bars represent the standard errors of means (n = 20). Linear correlation coefficients (r) were determined for data in A, C and D. Data in A were fitted by linear regression, whereas arbitrary curves were fitted to data in the other panels. Data points with the same letters within a panel do not differ from each other according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).

As predicted, the survival of Hz was less affected than that of either steinernematid when soil was pretreated with EPN. Moreover, pretreatment of soil with Hz or Sd had less effect on the survival of Sd than if soil was pretreated with Sr, and the prevalence of NF was significantly lower in soil pretreated with Hz than with either steinernematid. These results and a similar pattern of responses by NF to *H. marelatus* and *S. glaseri* (Jaffee and Strong, 2005) support the observation of Timper and Kaya (1989, 1992) that, in contrast to steinernematids, the tendency of IJ heterorhabditids to retain the second-stage cuticle as a sheath affords them increased protection against some natural enemies. The reasons for greater survival of EPN in soil pretreated with Sd compared to Sr are unknown. The innate longevity of Sd in autoclaved soil (>2 yr) is much greater than that of Sr (<3 mon) (Duncan, unpublished); however, the relatively short duration of these experiments would seem to preclude longevity as a reason that augmentation using Sr reduced EPN survival more than did use of Sd. Other possible causes such as differences in nematode cuticular chemistry and adhesion of NF infection structures (Jansson, 1993; Den Belder et al., 1996; Yang et al., 2005) and differences in nematode behavior (Jaffee and Strong, 2005) remain to be investigated. The fact that Sd was less likely than the other species to move out of soil and onto the container surfaces may have contributed to relative differences between species in predation by NF. Nevertheless, the strongest effect of pretreatment on NF and EPN survival occurred with Sr, which had a high propensity in this and other studies to aggregate on the dish surfaces (Duncan et al., 1996a).

Tylenchulus semipenetrans juveniles were occasionally plentiful in the raw soil, but EPN augmentation did not measurably affect their prevalence. Duncan et al. (2007) reported that augmentation of Sr in field plots did not affect numbers of *T. semipenetrans* measurably for several months until a period of major population growth occurred in untreated, but not Sr-amended plots.

Additional research is needed to evaluate the spatial patterns of antagonist responses to EPN augmentation. The microcosms were amended with EPN within a range of rates recommended for pest control on a surface area basis. Because the EPN were restricted to just one centimeter of downward migration in the assay dishes, the EPN concentration per volume of soil was constrained at a higher level than occurs over time in the field. Therefore, it is likely that our assays amplified the numerical responses of NF and their effects on EPN mortality. For example, an interval of two weeks between EPN augmentations was required to measure significant differences in IJ survival in soil when dishes were treated with 20 IJ/cm^2, compared to just one week when treated with 100 IJ/cm^2. Differences in numerical responses by NF to one vs. two EPN augmentations

were always detected at higher EPN treatment rates, but not at lower rates. These trends suggest that detecting food web cascades at the lower EPN densities typically found at greater depths in the field will require substantially greater replication or more sensitive detection methods. Use of real-time PCR to better quantify low population densities of NF offers promise in this regard (Arora et al., 1996; Atkins et al., 2003).

From a practical standpoint, the frequency with which NF respond strongly enough to EPN augmentation to interfere with biological control of insects may be as important as determining threshold EPN densities needed to elicit a measurable increase in NF. Duncan et al. (2007) reported increased NF prevalence following two of three EPN augmentation events in the field; however, only once did EPN prevalence decline enough to reduce the mortality of buried sentinel insects below that in untreated plots. Perhaps NF responses to EPN augmentation have the greatest potential to affect EPN prevalence during times of high insect recruitment in soil, because the response to increased numbers of exotic EPN could intensify the localized responses to EPN emerging from insect cadavers (Jaffee and Strong, 2005). An additional reason to consider temporal patterns of insect recruitment is that high recruitment soon after EPN augmentation may permit large numbers of insects to escape EPN predation. Ideally, EPN augmentation would be timed to occur immediately following peak insect recruitment and prior to a period of low recruitment to avoid the possibility that non-target effects may reduce the normal mortality rate of young weevils entering the soil (Duncan et al., 2007).

There is some evidence that biological control of *D. abbreviatus* larvae by endemic EPN is greater on the deep sandy soils of Florida's Central Ridge than in other parts of the state with soils of finer texture and shallower water tables (Duncan et al., 2003b). During a two-year survey in an orchard on the Central Ridge, the mortality of buried sentinel weevil larvae averaged 52% per week, with 81% of cadavers containing endemic EPN (Duncan et al., 2007). Non-target effects caused by EPN augmentation could be important in orchards with vigorous EPN communities, whereas they should be of little concern in habitats less suitable to endemic EPN. Nevertheless, it has been speculated that the Central Ridge may be uniquely suited for use of EPN augmentation because reduced prevalence of *D. abbreviatus* there, compared to other regions, makes it more feasible to suppress insect numbers below a damaging level (Futch et al., 2005; Shapiro-Ilan et al., 2005).

Our results are consistent with the basic paradigm that population size is maintained in equilibrium with density dependent forces such as food, competitors and antagonists (Jaffee et al., 1993). Augmentation biological control is a tactic employed to temporarily increase the numbers of a biocontrol agent beyond its equilibrium density in order to increase the mortality of a pest. Implicit in this tactic is the expectation that numbers of the biocontrol agent will eventually decline to the equilibrium state, either gradually or in a series of dampening oscillations above and below the equilibrium. The significant bottom-up and top-down cascades that developed from successive EPN augmentations of our microcosms suggest that augmenting EPN in the field may sometimes result in the latter condition and thereby influence biological control.

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