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Entomopathogenic Nematodes and Bacteria Applications for Control of the Pecan Root-Knot Nematode, *Meloidogyne partityla,* in the Greenhouse

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Abstract: *Meloidogyne partityla* is a parasite of pecan and walnut. Our objective was to determine interactions between the entomopathogenic nematode-bacterium complex and *M. partityla*. Specifically, we investigated suppressive effects of *Steinernema feltiae* (strain SN) and *S. riobrave* (strain 7–12) applied as infective juveniles and in infected host insects, as well as application of *S. feltiae*'s bacterial symbiont *Xenorhabdus bovienii* on *M. partityla*. In two separate greenhouse trials, the treatments were applied to pecan seedlings that were simultaneously infested with *M. partityla* eggs; controls received only water and *M. partityla* eggs. Additionally, all treatment applications were re-applied (without *M. partityla* eggs) two months later. Four months after initial treatment, plants were assessed for number of galls per root system, number of egg masses per root system, number of eggs per root system, number of eggs per egg mass, number of eggs per gram dry root weight, dry shoot weight, and final population density of *M. partityla* second-stage juveniles (J2). In the first trial, the number of egg masses per plant was lower in the *S. riobrave*-infected host treatment than in the control (by approximately 18%). In the second trial, dry root weight was higher in the *S. feltiae*-infected host treatment than in the control (approximately 80% increase). No other treatment effects were detected. The marginal and inconsistent effects observed in our experiments indicate that the treatments we applied are not sufficient for controlling *M. partityla*.

Key words: Biological control, entomopathogenic nematode, *Meloidogyne partityla*, pecan, *Steinernema, Xenorhabdus*

Pecan (*Carya illinoensis*) is an important nut crop in North America (Wood, 2003). Root-knot nematodes (*Meloidogyne* spp.) are recognized pests of pecan (Hendrix and Powell, 1968; von Broembsen, 2005). The pecan root-knot nematode, *Meloidogyne partityla* (Kleynhans), a species previously only reported in South Africa, has been reported in pecan orchards in the United States over the past 10 years, and the nematode has been associated with tree decline in the orchards or nurseries where it was found (Starr et al., 1996; Thomas et al., 2001; Nyczepir et al., 2002; Crow et al., 2005). *Meloidogyne partityla*'s host range appears to be specific to members of the family Juglandaceae (e.g., hickory [*Carya spp.*] and walnut [*Juglans* spp.]) (Starr et al., 1996). There are currently no curative (e.g., chemical) treatments recommended for the control of root-knot nematodes in pecan; recommended preventative measures consist of destroying infested nursery trees (von Broembsen, 2005). Research toward safe and effective control methods is warranted.

Entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae are biological control agents (Stock, 2005). These nematodes are parasites of insects, killing their hosts with the aid of bacteria carried in their alimentary canals (steinernematids carry *Xenorhabdus* spp., whereas heterorhabditids carry *Photorhabdus* spp.) (Poinar, 1990; Adams and Nguyen, 2002). The infective juvenile nematode (IJ), the only free-living stage, enters its arthropod host via natural openings, i.e., mouth, anus, spiracles (Poinar, 1990), or occasionally through the insect cuticle (Dowds and Peters, 2002). The nematodes then release their symbiotic bacteria, which take a prominent role in killing the host within 24 to 72 hours (Dowds and Peters, 2002; Forst and Clarke, 2002). After the nematodes complete one to three generations within the insect cadaver, IJ exit to find new hosts (Poinar, 1990). Entomopathogenic nematodes are capable of controlling a variety of economically important insect pests (Klein, 1990; Shapiro-Ilan et al., 2002b; Grewal et al., 2005).

Entomopathogenic nematodes can also suppress certain species of plant-parasitic nematodes (Bird and Bird, 1986; Ishibashi and Kondo, 1986; Lewis and Grewal, 2005). Although suppressive effects from entomopathogenic nematodes have been observed on a variety of plant-parasitic nematodes, such as *Belonolaimus longicaudatus*, *Criconemoides* spp. (Grewal et al., 1997), and *Globodera rostochiensis* (Perry et al., 1998), the most consistent suppression has been observed among *Meloidogyne* spp. (Lewis and Grewal, 2005). Our objective was to determine suppressive effects of the entomopathogenic nematode-bacterium complex on *M. partityla*.

Based on prior research, our treatments focused on the nematode-bacterium complexes of *Steinernema feltiae* (Filipjev) and *Steinernema riobrave* Cabanillas, Poinar, & Raulston. Among the entomopathogenic nematodes tested for control of plant-parasitic nematodes, *S. feltiae* has been the most consistent in providing at least some level of control (Lewis and Grewal, 2005). In several studies, negative impacts on *Meloidogyne* spp. have been observed following *S. riobrave* applications (Grewal et al., 1997; Perez and Lewis, 2002, 2004). In addition to suppressing plant-parasitic nematodes through direct application of *S. feltiae* and *S. riobrave* IJ (in aqueous suspension), exposure of steinernematid-infected insect host cadavers to *M. incognita* caused repellency in the plant-parasitic nematode (Grewal et al., 1999). Fur-

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thermore, application of the entomopathogenic nematode's bacteria and associated metabolites (without the nematodes themselves) has resulted in suppression of *Meloidogyne* spp. (Grewal et al., 1999; Fallon et al., 2004). Thus, we investigated suppressive effects of *S. feltiae* and *S. riobrave* applied as IJ and in infected host insects, as well as application of *S. feltiae*'s symbiont *Xenorhabdus bovienii* (Akhurst) on *M. partityla*.

Materials and Methods

Nematode and bacterial cultures: Entomopathogenic nematodes *S. feltiae* (SN strain) and *S. riobrave* (7–12 strain) were cultured in the laboratory at 25°C based on procedures described by Kaya and Stock (1997). The cultures had been passed through *Galleria mellonella* (L.) fewer than five times prior to experimentation. For nematodes used in aqueous applied treatments, IJ were passed an additional time through *G. mellonella* and stored at 13°C until experiments were initiated. For nematodes used in infected host applications, *Tenebrio molitor* L. were infected on filter paper in 60-mm-diam. plastic petri dishes with either *S. feltiae* or *S. riobrave* at a rate of 500 IJ/insect and stored at 25°C until application. The same batch of nematodes was used to infect *G. mellonella* for the aqueous treatments and *T. molitor* for the infected host applications. The different hosts were used to simulate a comparison of current commercial products, i.e., aqueous applied-nematodes cultured in *G. mellonella* and infected host-applied nematodes reared in *T. molitor*.

A monoxenic culture of *X. bovienii* was established from *S. feltiae*-infected *G. mellonella* according to procedures described by Lunau et al. (1993). Bacteria used in experiments were cultured in 250-ml Erlenmeyer flasks containing 50 ml TSY (per liter: 40 g tryptic soy broth + 5 g yeast extract [Sigma-Aldrich, Inc., St. Louis, MO]); the flasks were shaken at 25°C and 200 rpm for approximately 24 hr. Primary phase of the bacteria was confirmed on selective T7 agar (Oxoid Ltd., Hampshire, England), which is similar to NBTA (see Kaya and Stock, 1997).

A population of *M. partityla* isolated from pecan in Georgia was maintained on pecan in the greenhouse. Root-knot nematode egg inoculum was extracted from pecan roots using NaOCl solution (Hussey and Barker, 1973).

Experimental parameters: Experiments to determine effects of entomopathogenic nematodes and their bacteria on *M. partityla* were conducted under greenhouse conditions. Experimental units consisted of plastic pots (15-cm-diam. x 14-cm-deep) containing steam pasteurized loamy sand (86% sand, 10% silt, 4% clay; 0.54% organic matter; pH 6.1) and one pecan seedling each (cv. 'Elliott,' approximately 60-d-old, 15–20 cm height). The pots were watered daily as needed.

Treatments and *M. partityla* eggs were added to pots simultaneously. Prior to addition of nematode eggs and treatments, the soil in each pot was tilled approximately 2 cm deep with a metal spatula. Aqueous and infected host treatments of nematodes were applied on the same day along with the control. For the aqueous entomopathogenic nematode treatment, a 40 ml tap water suspension of approximately 2,000 *M. partityla* eggs and 32,250 IJ (approximately 200 IJ/cm^2) of *S. riobrave* or *S. feltiae* was poured (from a beaker) evenly over the soil. Entomopathogenic nematodes applied in aqueous suspension had been stored for less than 2 wk prior to use. For the cadaver treatment, two *T. molitor* infected with *S. riobrave* or *S. feltiae* were buried 1 cm below the soil surface approximately 2 cm on either side of the seedling's stem; a 40 ml suspension containing 2,000 *M. partityla* eggs was then poured onto the soil surface. The cadavers were 1-wk-old when they were applied. The control pots received only water containing 2,000 *M. partityla* eggs in 40 ml. After application, approximately 1 cm of water was applied to all treatment pots as a means to wash these nematodes into the soil. Approximately 5 to 10 ml of *X. bovienii* in TSY suspensions was diluted to 40 ml in a mixture that included 2,000 *M. partityla* eggs and poured onto pots 1 wk after the other treatments. Each pot in the bacteria treatment received approximately 1.45 x 10^9 cells (as estimated through hemocytometer counts). All treatment applications were re-applied (without *M. partityla* eggs) 2 mon after the initial treatments (at which time control pots received only water).

The experiment contained 10 replicates (pots) for each treatment, arranged in a randomized block design (blocked by row on the greenhouse bench). The entire experiment (including two applications) was repeated once, i.e., there were two trials of the same experiment. Temperature was monitored throughout the experimental periods and averaged 30.1 ± 2.2°C and 31.6 ± 1.2°C in the first and second trial, respectively. Each trial was evaluated 4 mon after initial treatments were applied (bacteria applications were evaluated at 4 mon minus 1 wk). For each plant (replicate), variables that were assessed included number of galls, total number of egg masses, total number of eggs, number of *M. partityla* J2, dry root weight, dry shoot weight, eggs per egg mass, and eggs per gram of dry root weight. Treatment effects among these variables were analyzed through analysis of variance, and if a significant F-test was detected ($P \leq 0.05$) treatment differences were elucidated through the Student-Newman-Keuls' (S-N-K) test (SAS Software, version 9.1, 2001, SAS Institute, Cary, NC).

Results

In trial 1, the average number of egg masses per plant was lower in the *S. riobrave*-infected host treatment than in the control (by approximately 18%) and all other

treatments (F = 3.34; df = 5,45; P = 0.01) (Fig. 1). No other treatment differences were detected in other variables (P > 0.05; Fig. 1).

In trial 2, dry root weight was higher in the *S. feltiae*-infected host treatment than in the control (approximately 80% increase) as well as the aqueous *S. riobrave* and *X. bovienii* treatments; no other treatments differed from the control in dry root weight (F = 4.40; df = 5,43; P = 0.003) (Fig. 2). No other treatment differences were detected in other variables (P > 0.05; Fig. 2).

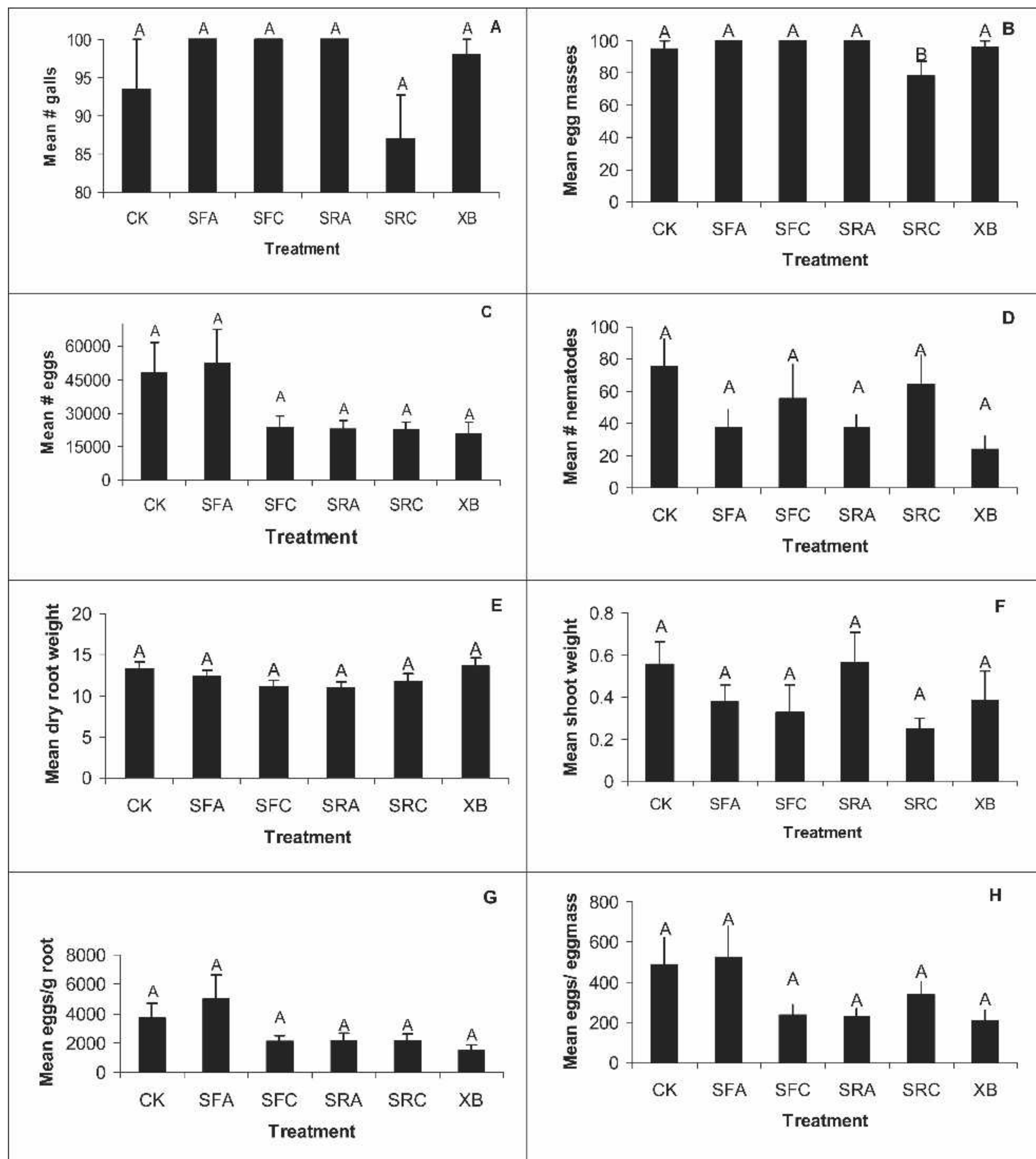

FIG. 1. Assessment of *Meloidogyne partityla* suppression (trial 1) following treatments of *Steinernema feltiae* (SF) or *S. riobrave* (SR) in aqueous suspension (A) or infected host cadavers (C), *Xenorhabdus bovienii* (XB), or an untreated check (CK). Variables assessed in each pot were average (± SE) number of galls per plant (A), number of egg masses per plant (B), number of eggs per plant (C), *M. partityla* J2 (D), dry root weight in grams (E), dry shoot weight in grams (F), number of eggs per gram root weight (G), number of eggs per egg mass (H). All numbers are per replicate (pecan seedling). Different letters above bars indicate statistical differences ($P \leq 0.05$, based on S-N-K test).

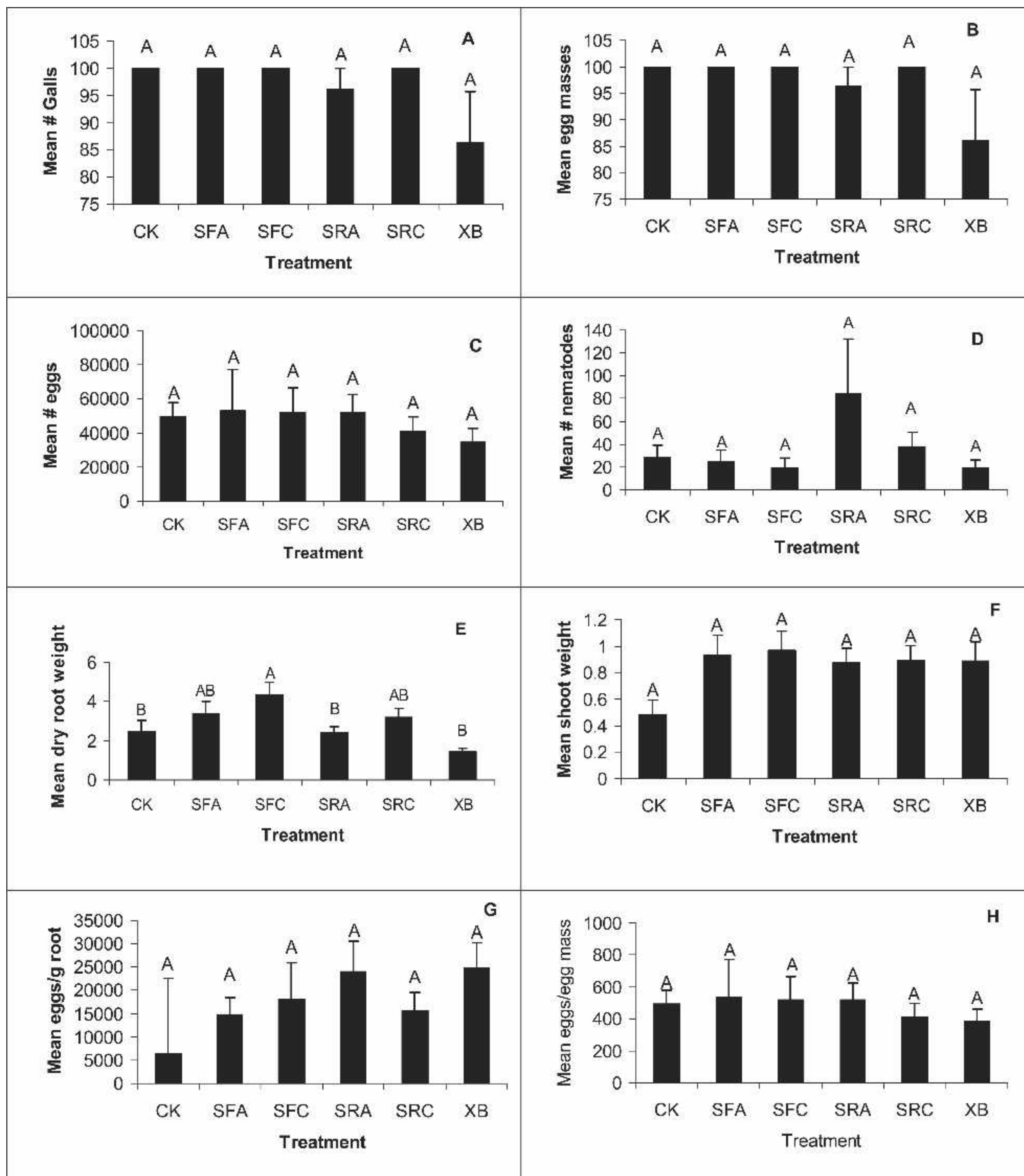

Fig. 2. Assessment of *Meloidogyne partityla* suppression (trial 2) following treatments of *Steinernema feltiae* (SF) or *S. riobrave* (SR) in aqueous suspension (A) or infected host cadavers (C), *Xenorhabdus bovienii* (XB), or an untreated check (CK). Variables assessed in each pot were average (± SE) number of galls per plant (A), number of egg masses per plant (B), number of eggs per plant (C), *M. partityla* J2 (D), dry root weight in grams (E), dry shoot weight in grams (F), number of eggs per gram root weight (G), number of eggs per egg mass (H). All numbers are per replicate (pecan seedling). Different letters above bars indicate statistical differences ($P \leq 0.05$, based on S-N-K test).

Discussion

The entomopathogenic nematode and associated bacteria treatments applied to suppress *M. partityla* either exhibited variable results or lacked a detectable impact altogether. Marginally effective or mixed results in suppression of plant-parasitic nematodes with entomopathogenic nematode-bacterium complexes have been reported in a number of other studies (Gouge et al., 1994; Perry et al., 1998; Fallon et al., 2002; LaMon-

dia and Cowles, 2002; Fallon et al., 2004), and no effect of entomopathogenic nematode applications was reported in others (e.g., Smitley et al., 1992; Riegel et al., 1998; Nyczepir et al., 2004). LaMondia and Cowles (2002) observed short-term (approximately within a week) repellency and reduced infection in tomatoes when exposing *S. feltiae* to *Pratylenchus penetrans* in laboratory or greenhouse experiments, but long-term effects on *P. penetrans* populations under field applications were not detected. Possibly, our treatments also produced short-term effects that were not detected (not looked for) in our experiments.

Overall, more positive reports of suppression with entomopathogenic nematodes have been reported for *Meloidogyne* spp. than for other plant-parasitic nematode species (Lewis and Grewal, 2005). Conceivably, *M. partityla* is less susceptible to entomopathogenic nematodes than other root-knot nematodes such as *M. incognita* or *M. javanica*. Additionally, it is conceivable that pecan is less conducive to control of plant-parasitic nematodes with entomopathogenic nematodes than some other crops; other studies have indicated differences in efficacy among crops (Fallon et al., 2004).

Previously, entomopathogenic nematode-infected hosts were reported to repel *M. incognita* (Grewal et al., 1999). Chemicals that are repellant or toxic to other plant-parasitic nematodes or other organisms, e.g., nitrogen compounds, are emitted from entomopathogenic nematode-infected hosts (Grewal et al., 1999; Shapiro et al., 2000). Recently, Kunkel et al. (2006) reported that infected host exudates may also be repellant to conspecific entomopathogenic nematodes (possibly an adaptation to avoid infecting a depleted host). In contrast, LaMondia and Cowles (2002) did not detect any repellant effects of *S. feltiae*-infected hosts on *P. penetrans*. In this study, the only differences detected between treatments and the control were in the infected host treatments (as indicated by reduced egg masses or increased dry weight), yet even these effects were not consistent among nematode species and the variables that were impacted in each trial.

We applied IJ cultured in *G. mellonella* and used *T. molitor* in the infected host treatments. Thus, in addition to, or instead of, allelochemical effects, one might argue that the observed differences between aqueous IJ treatments and infected host treatments were due to having different insect hosts. Host species can affect the quality and fitness of entomopathogenic nematodes (Abu Hatab et al., 1998; Shapiro-Ilan et al., 2005). Therefore, it is conceivable that the ability to suppress plant-parasitic nematodes could also be affected by host species. However, it must be noted that *S. feltiae* and *S. riobrave* IJ cultured in *G. mellonella* have previously been reported to suppress *Meloidogyne* spp. in other studies (Lewis et al., 2001; Perez and Lewis, 2002, 2004). Furthermore, the quality (virulence to insects) and fitness (reproductive capacity per gram host) of nematodes produced in *G. mellonella* and *T. molitor* were found to be similar (Blinova and Ivanova, 1987; Shapiro-Ilan et al., 2002a; unpublished data). Therefore, we hypothesize that it was the application method (infected host vs. IJ) and not the host species that caused the observed differences in treatment effects. The goal of our comparison, however, was not to differentiate host species vs. application method effects, but rather to determine effects of one type of product vs. another. We used the two different hosts to reflect current commercial products stemming from in vivo production. Thus, further research is required to verify the underlying causes for differences among the treatments.

Infestation of *M. partityla* and application of the *X. bovienii* treatment were initiated one week after the other treatments. Perhaps one might argue that the timing difference may have been partially responsible for the observed treatment effects. However, given that the entire experiment lasted more than 15 weeks, we feel it is unlikely that one week's difference in the duration of *X. bovienii*-treated pots affected the outcome relative to the control and other treatments.

The marginal and inconsistent effects observed in our experiments indicate that the treatments we applied are not viable strategies for controlling *M. partityla*. However, due to a lack of alternatives and the fact that at least some suppression was observed, additional studies may be warranted toward enhancing the suppressive effects. Entomopathogenic nematodes are currently being investigated as alternative control strategies for the pecan weevil, *Curculio caryae* (Horn) (Shapiro-Ilan, 2003). Thus, if the control strategies were deemed economically feasible, it is possible that *C. caryae* and *M. partityla* could be targeted simultaneously.

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