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Effect of Entomopathogenic Nematodes on *Mesocriconema xenoplax* Populations in Peach and Pecan

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Abstract: The effect of *Steinernema riobrave* and *Heterorhabditis bacteriophora* on population density of *Mesocriconema xenoplax* in peach was studied in the greenhouse. Twenty-one days after adding 112 *M. xenoplax* adults and juveniles/1,500 cm^3 soil to the soil surface of each pot, 50 infective juveniles/cm^2 soil surface of either *S. riobrave* or *H. bacteriophora* were applied. Another entomopathogenic nematode application of the same density was administered 3 months later. The experiment was repeated once. *Mesocriconema xenoplax* populations were not suppressed ($P \leq 0.05$) in the presence of either *S. riobrave* or *H. bacteriophora* 180 days following ring nematode inoculation. On pecan, 200 *S. riobrave* infective-stage juveniles/cm^2 were applied to the soil surface of 2-year-old established *M. xenoplax* populations in field microplots. Additional applications of *S. riobrave* were administered 2 and 4 months later. This study was terminated 150 days following the initial application of *S. riobrave*. Populations of *M. xenoplax* were not suppressed in the presence of *S. riobrave*.

Key words: biological control, *Carya illinoensis,* entomopathogenic nematodes, *Heterorhabditis bacteriophora, Mesocriconema xenoplax,* nematode, peach, *Prunus persica,* ring nematode, *Steinernema riobrave.*

In the southeastern United States, the productive life span of peach trees does not exceed 6 to 10 years on some sites (Brittain and Miller, 1978). Some tree death has been attributed to a disease complex termed peach tree short life (PTSL) (Savage and Cowart, 1942). The external symptoms are similar to those of any plant deprived of an adequate root system (Taylor et al., 1970). Bacterial canker (*Pseudomonas syringae* pv. *syringae*) (Weaver et al., 1974), cold injury (Prince, 1966), or a combination of both are thought to be the final agents of tree death in the PTSL complex (Brittain and Miller, 1978). The ring nematode, *Mesocriconema xenoplax,* is the only plant-parasitic nematode that has been associated with predisposing trees to PTSL (Nyczepir et al., 1997). Research has shown that management of *M. xenoplax* is essential for minimizing losses in peach to the PTSL syndrome (Sharpe et al., 1989).

Mesocriconema xenoplax also has been found associated with pecan trees in South Africa (Kleynhans, 1986). Recently, *M. xenoplax* was found in association with stressed and stunted pecan trees in Georgia (Nyczepir, unpubl.). The contribution of *M. xenoplax* in stressing pecan trees remains to be determined. The only other ring nematode reported on pecan in Georgia is *M. rusticum* [= *Criconemoides quadricornis* Hendrix, 1973), which was reported to have a synergistic effect in reducing root growth in the presence of either *Pythium irregulare* or *Fusarium solani.*

Preplant chemical treatment with either 1,3-dichloropropene (1,3-D) or methyl bromide (bromomethane) is recommended to control *M. xenoplax* on peach in the southeastern United States. These fumigant nematicides lower nematode population density enough to prevent major root damage during the first years following tree establishment, thus allowing the tree to have a healthy start (Nyczepir, 1991; Ritchie et al., 2003). In recent years nematode management research has focused on alternatives to conventional nematicide applications such as rootstock resistance (Okie et al., 1994), rotation crops (Nyczepir and Bertrand, 2000), biological control (Kluepfel et al., 2002), and ground covers (Nyczepir and Bertrand, 2000). Emphasis on nonchemical control is partly due to apprehension about the environmental problems associated with soil fumigation with methyl bromide. As a result of its role in ozone depletion, a ban on the importation and manufacture of methyl bromide in the United States is scheduled for 1 January 2005 (Clean Air Act, 1990). Therefore, finding a cost-effective and environmetally safe alternative to chemical control of nematodes is warranted.

Applications of entomopathogenic nematodes *Steinernema carpocapsae, S. feltiae, S. glaseri, S. riobrave,* or *Heterorhabditis bacteriophora* have been reported to reduce plant-parasitic nematode populations in roots and soil (Bird and Bird, 1986; Gouge et al., 1994; Grewal et al., 1997; Ishibashi and Kondo, 1986; Smitley et al., 1992). Of particular interest is the investigation by Grewal et al. (1997), who reported the suppression of *Mesocriconema* sp. by *S. riobrave* on turf. The effectiveness of other entomopathogenic nematodes on *M. xenoplax* population density has not been tested.

The objective of our research was to determine whether entomopathogenic nematode applications would suppress *M. xenoplax* populations on peach and pecan.

Materials and Methods

Greenhouse experiment: The effects of inundative releases of *S. riobrave* (355 strain) and *H. bacteriophora* (Hb strain) infective juveniles (IJ) on *M. xenoplax* popula-

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tions and plant growth in peach were determined. Approximately 2-week-old Nemaguard peach (*Prunus persica* L. Batsch) seedlings were planted singly in 15-cm-diam. plastic pots containing 1,500 cm^3 steam-pasteurized loamy sand (86% sand, 10% silt, 4% clay; pH 6.1; 0.54% organic matter) in February 2000. The susceptible peach cultivar, Nemaguard (Beckman et al., 1993), was used to verify nematode infectivity. Six days later (29 February 2000), the soil in each of 30 pots was infested with 112 *M. xenoplax* adults and juveniles in 40 ml water (Nyczepir and Bertrand, 1990). Ten additional pots received no *M. xenoplax* and served as the uninoculated control. The nematode isolate was obtained from an orchard previously diagnosed as a PTSL site in Byron, Georgia, and cultured on Nemaguard peach in the greenhouse. Nematodes were extracted from the soil by centrifugal-flotation (Jenkins, 1964). Twenty-one days later (21 March 2000), 50 IJ/cm^2 soil of *S. riobrave* or *H. bacteriophora* in approximately 1 ml water were applied in late afternoon (5:00 p.m.) to the soil surface of 10 *M. xenoplax*-infested pots that had been previously tilled to a depth of 2 cm. Applications were made by evenly distributing droplets in a circle around each peach seedling using a 2000-µl micropipet. After application, approximately 1 cm of water was applied to all treatment pots as a means to wash these nematodes into the soil. The amount of irrigation water used to wash the *S. riobrave* or *H. bacteriophora* into the soil is consistent with what has been recommended for other *Steinernema* and *Heterorhabditis* spp. under field turf conditions (Georgis, 1990; Shetlar et al., 1988). Additional *S. riobrave* and *H. bacteriophora* were applied 78 days (7 June 2000) later as described above. Generally, a minimum of 25 IJ/cm^2 soil is required for effective insect suppression; however, application rates of up to 250 IJ/cm^2 soil are commonly used in field research (Shapiro-Ilan et al., 2002). We selected rates on the mid-to-upper range (i.e., 50–200 IJ/cm^2 soil) because if there was a treatment effect, we did not want to miss detecting it due to a low application rate. *Steinernema riobrave* and *H. bacteriophora* were cultured in the last instar of *Galleria mellonella* (L.) as described by Kaya and Stock (1997). The *S. riobrave* and *H. bacteriophora* isolates were provided by Certis USA (Columbia, MD) and E. Lewis' culture collection (Blacksburg, VA), respectively. All seedlings were pruned to a height of 18 cm about 3 months (31 May 2000) after soil was infested with *M. xenoplax*. Upon bud break following pruning, only a single bud was allowed to break dormancy and grow for the remainder of the study. Treatments [i.e., (i) *M. xenoplax;* (ii) *M. xenoplax + S. riobrave;* (iii) *M. xenoplax + H. bacteriophora;* and (iv) untreated control] were replicated 10 times and arranged in a randomized complete block design on a bench in an air-conditioned greenhouse (25 ± 5°C). Plants were watered daily and fertilized with Osmocote (14-14-14, N-P-K) (Scotts-Sierra Horticultural Products Co., Marysville, OH) as needed.

Six months (28 August 2000) following soil infestation with ring nematode, the study was terminated and the following data were collected: dry root weight, dry shoot weight, shoot length increase of new terminal growth following bud break, and *M. xenoplax* population density. Nematodes were extracted from a 100-cm^3 soil subsample with a semi-automatic elutriator (Byrd et al., 1976) and centrifugal-flotation (Jenkins, 1964). The experiment was repeated once with the following modifications: (i) the initial population density (Pi) of ring nematode was 2,000 *M. xenoplax* adults and juveniles in 40-ml water; (ii) 15 days later (9 November 2000), 75 IJ/cm^2 soil of *S. riobrave* or *H. bacteriophora* in approximately 1 ml water were applied to the soil surface; and (iii) a second application of *S. riobrave* and *H. bacteriophora* occurred 84 days (1 February 2001) later.

Microplot experiment: Approximately 7-week-old 'Schley' pecan seedlings were planted singly in 14 bucket microplots (25-cm diam. × 31 cm deep) (Barker, 1985) containing 15,000 cm^3 of untreated field soil collected from a pecan orchard in Sumter County, Georgia, on 24 July 2000. The soil was a sand (96% sand, 0% silt, 4% clay; pH 7.0; 1.27% organic matter) obtained from under pecan trees exhibiting stressed symptoms and(or) stunted in growth. Microplots were established in a shaded area (30% shade) in the field. The soil was naturally infested with the ring nematode, *M. xenoplax* (Pi of 30 *M. xenoplax* adults and juveniles per 100 cm^3 soil). Ring nematode species identification was confirmed based on morphological examination of the nematodes by Z. Handoo (USDA. Nematode Collection, Beltsville, MD). Treatments were applied approximately 22 months (21 May 2002) after planting seedlings in microplots. Two hundred *S. riobrave* IJ/cm^2 soil in approximately 10 ml water were applied in the morning (10:30 a.m.) to the soil surface of 7 out of 14 microplots that had been previously tilled to a depth of 2 cm. The ambient temperature was approximately 28 °C at time of application. Applications were made by evenly distributing droplets in a circle around each pecan seedling using a 10-ml pipet. After application, approximately 1 cm of water was applied to all treatment pots as a means to wash the *S. riobrave* into the soil. Two additional applications of *S. riobrave* occurred 59 and 121 days (i.e., 19 July 2002 and 19 September 2002, respectively) later. The experimental design was a randomized complete block with seven single tree replications per treatment. Plants were watered as needed and fertilized with Osmocote (14-14-14, N-P-K).

The initial (pretreatment) soil population density of *M. xenoplax* was determined on 21 May 2002 from four soil cores (2.5 cm dia. × 30 cm deep) collected around each tree of each experimental unit. The four soil cores were composited and nematodes were extracted from a 100-cm^3 subsample as described for the greenhouse experiment. Nematode population densities were also determined on 19 August and 21 October 2002.

Statistical analysis: Nematode data were transformed to $\log_{10}(x+1)$ values and subjected to analysis of variance with the general linear models procedure of SAS (SAS Institute, Cary, NC). Analysis of variance was also performed to determined treatment effect on dry root and shoot weights, shoot length, and nematodes per gram dry root. Means were compared according to Fisher's protected least significant difference test following a significant F test. Nontransformed data are presented in tables.

Results and Discussion

Greenhouse experiment: Mesocriconema xenoplax soil populations and number of nematodes per gram dry root in peach did not differ among treatments following inundative applications of *S. riobrave* or *H. bacteriophora* (Table 1). These data indicate that *S. riobrave* or *H. bacteriophora* did not reduce *M. xenoplax* populations over 6 months in the greenhouse.

Tree dry root weight, dry shoot weight, and shoot length did not differ among the treatments in test 1. However, differences in tree growth were detected in test 2 (Table 1). The presence of *M. xenoplax* alone or in combination with *S. riobrave* or *H. bacteriophora* reduced tree growth as compared with the uninoculated control. One explanation for the different results obtained between the two tests may be the difference in initial population density (Pi) of *M. xenoplax* used. In test 2, the Pi of *M. xenoplax* was almost 18 times greater than that in test 1, thus resulting in greater root and shoot damage 180 days after *M. xenoplax* infestation. These findings substantiate an earlier report describing the effect of Pi density of *M. xenoplax* on peach growth in the greenhouse (Nyczepir et al., 1987). No differences in plant growth were detected among the three *M. xenoplax* treatments. These data further indicate that *S. riobrave or H. bacteriophora* (i) did not suppress *M. xenoplax* populations over 6 months and (ii) did not compromise the pathogenicity of *M. xenoplax* in reducing peach tree growth.

Microplot experiment: The populations of *M. xenoplax* for the seven replications in the untreated and *S. riobrave* treatment microplots were 529 and 321/100 cm^3 soil, respectively, 22 months after planting (Fig. 1; 0 days after first application of *S. riobrave*). Differences were not detected between treatments despite the fact that *M. xenoplax* populations were well established in all microplots before initiating the inundative applications of *S. riobrave.* No difference in *M. xenoplax* populations were evident between the two treatments at 90 and 153 days later following two more inundative applications of *S. riobrave.*

Our results indicate that repeated inundative applications of *H. bacteriophora or S. riobrave* do not reduce populations of *M. xenoplax* on peach and pecan. Previous reports of suppression of plant-parasitic nematodes following applications of entomopathogenic nematodes were mostly conducted with *Meloidogyne* spp. (Bird and Bird, 1986; Grewal et al., 1997; Ishibashi and Choi, 1991). Results with other plant-parasitic genera (i.e., *Mesocriconema*) has been inconsistent. Grewal et al. (1997) reported a reduction in recovery of a *Mesocriconema* sp. from turfgrass after *S. riobrave* application in Georgia. We believe that the ring nematode species detected on turfgrass was probably *M. ornatum* or another grass-feeding ring nematode and not *M. xenoplax,* which prefers woody perennials (Nyczepir et al., 1988; Ratanaworabhan and Smart, 1970). In contrast, Smitley et al. (1992) demonstrated that applications of *H. bacteriophora* did not reduce populations of *M. rusticum* in turf.

The parasitic behavior of *M. xenoplax* and *Meloidogyne* spp. may partially explain the lack of biocontrol. *Meloidogyne* spp. are sedentary endoparasites with the J2 stage hatching from the egg and invading the new roots above the root cap. Ishibashi and Choi (1991) reported that *S. carpocapsae* were attracted to the tomato root tips and remained there for some time, thus repelling *M. incognita* J2 and suppressing root gall formation. Similar

Table 1. Effect of entomopathogenic nematodes on populations of *Mesocriconema xenoplax* and growth of Nemaguard peach in the greenhouse over 180 days in Byron, Georgia.

	M. xenoplax per				Dry weight (g)					
	100 cm^3 soil		g dry root		Root		Shoot		Shoot Length (cm)	
Treatment	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Control	—[a]	—	—	—	2.82 a	3.72 a	0.59 a	0.91 a	30.5 a	11.2 a
M. xenoplax (Mx)[b]	138 a	606 a	31 a	298 a	2.95 a	2.12 b	1.05 a	0.13 b	34.8 a	2.6 b
Mx + *S. riobrave*[c]	48 a	390 a	23 a	189 a	3.01 a	2.13 b	1.08 a	0.24 b	31.6 a	3.8 b
Mx + *H. bacteriophora*[c]	248 a	388 a	61 a	187 a	2.99 a	2.06 b	1.09 a	0.07 b	36.5 a	1.3 b

Data are means of 10 replicates. Means within a column followed by the same letter are not different according to Fisher's least significant difference ($P \leq 0.05$).

[a] = not determined.

[b] Initial pretreatment population density of *M. xenoplax* in test 1 (February 2000) and test 2 (November 2000) was 112 and 2,000 *M. xenoplax* adults and juveniles nematodes per 1,500 cm^3 soil, respectively.

[c] Entomopathogenic nematodes applied as a drench of 50 infective juveniles/cm^2 soil in test 1 (March and June 2000) and 75 infective juveniles/cm^2 soil in test 2 (November 2000 and February 2001).

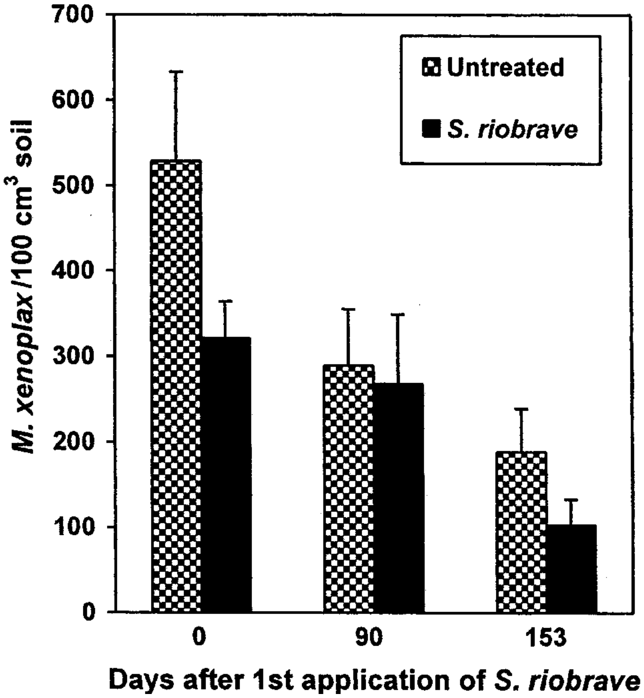

FIG. 1. Effect of *Steinernema riobrave* on populations of *Mesocriconema xenoplax* on Schley pecan in microplots over 5 months in Byron, Georgia. Sampling dates for *M. xenoplax* included: 21 May 2002 (0 days after first application of *S. riobrave*), 19 August 2002 (90 days after first application of *S. riobrave*), and 21 October 2002 (153 days after first application of *S. riobrave*). Initial population density of *M. xenoplax* was 30 *M. xenoplax* adults and juveniles/100 cm^3 soil in July 2000. *Steinernema riobrave* applied as a drench of 200 infective juveniles/cm^2 soil in May, July, and September 2002. Bars indicate standard error of the mean. No significant differences were found between treatments on a given sampling date according to analysis of variance ($P \leq 0.05$; $n = 7$).

attraction of *S. glaseri* to tomato root tips and suppression in reproduction of *M. javanica* was also reported by Bird and Bird (1986). Recently, Grewal et al. (1999) reported that heat-killed nematodes of *S. feltiae* and *S. riobrave* temporarily suppressed *M. incognita* penetration into tomato roots. Repellence of *M. incognita* J2 was thought to be the result of ammonium toxicity. In contrast, *M. xenoplax* is an ectoparasite and exhibits no apparent partiality toward feeding at any specific root region, since it was observed feeding along roots as well as on root tips. Thomas (1959) observed some specimens to feed continuously for 18 hours. Hussey et al. (1991) observed feeding from a single cell in the outer root cortex of clover, carnation, and tomato for up to 8 days. Preferred feeding cells by *M. xenoplax* were located in the first to second layer of cortex tissue. Thus, compared to *M. xenoplax, Meloidogyne* spp. appear to have a greater spatial overlap with entomopathogenic nematodes, which may explain the disparity in effects.

Based on our studies, live inundative applications of *S. riobrave* or *H. bacteriophora* did not suppress *M. xenoplax.* However, additional research to determine the effect (s) of entomopathogenic nematodes and their associated symbiotic bacteria on *Meloidogyne* spp. in pecan and peach is warranted.

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