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Effect of Entomopathogenic Nematodes and *Trichoderma harzianum* on the Strawberry Black Root Rot Pathogens *Pratylenchus penetrans* and *Rhizoctonia fragariae*

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Abstract: The effects of inundative releases of entomopathogenic *Steinernema carpocapsae* and *S. feltiae* infective juveniles and applications of the biological control fungus *Trichoderma harzianum* T-22 (RootShield) on *Pratylenchus penetrans* and strawberry black root rot caused by *Rhizoctonia fragariae* were determined in field microplots and small plots. Entomopathogenic nematodes were applied as a soil drench at rates of 7.4 or 14.8 billion per ha in May or August for 3 years. RootShield was applied as crown dips at planting or later as a soil drench. There were no differences in *P. penetrans* from plants drenched with water alone or with *S. carpocapsae* or *S. feltiae* nematodes, averaged over rates and timing. The nematode species applied and the rate or timing of application had no effect on lesion nematodes. Our results suggest that *P. penetrans* exposure to living or heat-killed *S. feltiae* and associated bacteria resulted in a temporary lack of motility. A progressively increasing proportion of *P. penetrans* became active again and, after 8 days, had infected tomato roots in similar numbers to unexposed *P. penetrans*. In laboratory assays and field plots or microplots, *S. carpocapsae* and *S. feltiae* did not permanently affect *P. penetrans* in tomato or strawberry.

Key words: Black root rot, entomopathogenic nematodes, *Galleria mellonella,* motility, *Pratylenchus penetrans,* repellence, *Rhizoctonia fragariae,* RootShield; *Steinernema carpocapsae, S. feltiae,* strawberry, *Trichoderma harzianum.*

Black root rot has been the most serious disease of perennial strawberry (*Fragaria* × *ananassa* Duch.) in the Northeast over the last decade (Cooley and Schloemann, 1994; Pritts and Wilcox, 1990). The disease results in a cortical root rot, loss of feeder roots, and reduced plant vigor, productivity, and winter survival (Martin, 1988). Black root rot is a disease of complex etiology involving *Rhizoctonia fragariae* Hussain & McKeen, 1963 anastomosis groups (AG) A, G, or I (Martin, 1988) and the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Shuurmans Stekhoven. *Pratylenchus penetrans* is one of the most important and common soilborne pathogens affecting strawberry (Chen and Rich, 1962; Goheen and Smith, 1956; Klinkenberg, 1955; LaMondia and Martin, 1989). In controlled growth chamber experiments, LaMondia and Martin (1989) demonstrated an interaction between *P. penetrans* and *R. fragariae* resulting in an increase in the severity of disease. Black root rot has been difficult to control despite pre-plant fumigation (where available), various rotations out of strawberries, and avoidance of wet compacted soils (LaMondia, 1999; Pritts and Wilcox 1990). While fumigation has been reported to reduce both nematodes and black root rot (Wolfe et al., 1990; Yuen et al., 1991), in some instances fumigation may increase the disease by allowing low pathogen populations to proliferate in the absence of competitors (Yuen et al., 1991). Similarly, the wide host range of lesion nematodes limits the efficacy of crop rotation, especially if weeds are present in rotation crops (Mai et al., 1977). In fact, many researchers are currently recommending at least a 4 to 5-year rotation out of strawberries for black root rot control (Anonymous, 1991).

Applications of the entomopathogenic nematodes *Steinernema carpocapsae, S. feltiae,* or *S. glaseri* reduce plant-parasitic nematode populations in roots and soil (Bird and Bird, 1986; Gouge et al., 1994; Ishibashi and Choi, 1991; Ishibashi and Kondo, 1986). The nature of the interaction between entomopathogenic and plant-parasitic nematodes is unknown. In addition, *Trichoderma harzianum* has been an effective biological control agent of a number of plant-pathogenic fungi, including *Rhizoctonia* spp. (Haran et al., 1996; Lo et al., 1997; Yuen et al., 1994; Zimand et al., 1996). The fungus has both rhizosphere and phylloplane competence (Lo et al., 1997) and differential expression of chitinases against different target fungi (Haran et al., 1996). *Trichoderma harzianum* is commercially available for use on a number of crops (RootShield, BioWorks, Inc., Geneva, NY) but has not been tested on strawberry.

The objectives of our research were to determine whether entomopathogenic nematode applications would suppress lesion nematode populations and whether *T. harzianum* alone or in combination with entomopathogenic nematodes would protect strawberry plants from black root rot caused by lesion nematodes and *R. fragariae*. Additionally, we evaluated whether entomopathogenic nematodes affected lesion nematode motility, direction of movement, and infection of host roots.

Materials and Methods

The effects of inundative releases of entomopathogenic *S. carpocapsae* and *S. feltiae* infective juveniles on lesion nematode (*P. penetrans*) populations in strawberry roots were determined in field microplots and small plots. Ninety-six field microplots were filled with

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Þeld soils from one of two commercial strawberry farms with a history of black root rot. Soils were either a Þne sandy loam (56.8% sand, 33.2% silt, 10.0% clay, pH 6.4, organic matter 9.1%) or a silt loam (40.2% sand, 54.8% silt, 5.0% clay, pH 6.3, organic matter 4.9%). Microplots consisted of polyvinyl chloride pipe (15-cm diam., 0.46 m long) buried 0.9 m apart on center to a depth of 35 cm. Additional *P. penetrans* inoculum was applied by incorporating 4 g of strawberry roots containing 2,500 individuals into the soil in each microplot. Microplots were planted with a single 1-year-old strawberry crown (ÔHoneoyeÕ) per plot. Plots were fertilized annually with 10-10-10 at approximately 75 kg N/ha; 50% was applied in April, and the remainder was applied at renovation in July. Herbicides and fungicides were applied as necessary based on commercial practices. Runners were not allowed to root in the microplots in order to maintain plants of the same age during the experiment. Entomopathogenic *S. carpocapsae* and *S. feltiae* nematodes were applied as a soil drench in 250 ml water per plot around strawberry crowns at rates of 7.4 or 14.8 billion/ha. The sources for the nematodes were Buena Biosystems, Ventura, California, in 1998, and Integrated Biocontrol Systems, Aurora, Indiana, in 1999. Different sources were used in different years due to availability. The *S. feltiae* used in 1998 were grown by MicroBio Inc., Cambridge, England, as their trademarked Nemasys product, and the *S. carpocapsae* was an undesignated strain. In 1999, the strains used were designated as *S. carpocapsae,* A11 strain; *S. feltiae,* UmeŒ strain; and *H. bacteriophora,* Lewiston strain. Application dates were 11 June 1998, 19 August 1998, 16 May 1999, 26 August 1999, 10 May 2000, and 21 August 2000. Plots were irrigated with 2.5 cm water after drench application to move nematodes into the root zone. Control plots received water alone.

Crowns were removed from plots each October to evaluate lesion nematode populations. The numbers of runners produced on each crown were counted in 1999 and 2000 to evaluate vigor. Nematodes were extracted from 2 g of root tissue placed in a ßask containing 50 ml water and shaken for 7 days using a wrist-action shaker, and counted. Rye was planted into all microplots as a winter cover. New crowns were planted in microplots on 21 April 1998, 16 April 1999, and 27 April 2000. Statistical differences between treatments were determined by factorial analysis of variance.

An experiment to determine whether the biological control fungus, *T. harzianum,* would protect strawberry plants from black root rot caused by lesion nematodes and *R. fragariae* was established in sandy loam Þeld soil (73.4% sand, 22.3% silt, 4.3% clay, pH 5.9) infested with black root rot pathogens. Application with and without Viterra Agrigel (Nepera Chemical Co., Inc. Harriman, NY) was investigated as a method of RootShield application. Crowns were subjected to Þve treatments: (i) dipped into water alone, (ii) dipped into Agrigel alone (9 g/liter water), (iii) dipped into a RootShield suspension (29 g/liter water), (iv) dipped into a RootShield and Agrigel suspension (29 g RootShield and 9 g Agrigel/liter water), or (v) RootShield was dusted to cover wet roots. Treated crowns were planted in plots of 10 plants each with plants 25 cm apart within rows and 1 m between rows on 16 May 1997. There were four replicate plots of each of the Þve treatments. Vigor determinations were made on 14 October 1997 by counting the number of expanded leaves per crown. Yield was measured on eight occasions between 26 May and 12 June 1998. Six plants per treatment were sampled on 22 July 1998 and assessed individually for lesion nematode counts, root and shoot weights, and percent black root rot. *Rhizoctonia fragariae* was isolated from surface-sterilized roots (exposed to 0.5% NaOCl for 30 seconds) placed on acidiÞed water agar for 48 hours. Hyphal tips were transferred to potato dextrose agar for identiÞcation (Martin, 1988; Vincelli and Beaupre, 1989). A line-intersect method (Tennant, 1975), which we previously modiÞed to photograph roots for a record of disease severity (LaMondia and Martin, 1989), was used to evaluate percent rotted roots. Statistical differences between treatments were determined by StudentÕs *T*-test comparison.

To determine whether *T. harzianum* would interact with entomopathogenic nematodes in preventing damage from lesion nematodes and *R. fragariae,* an experiment was established in sandy loam Þeld soil (73.4% sand, 22.3% silt, 4.3% clay, pH 5.9) infested with black root rot pathogens at the Valley Laboratory. Strawberry plots (ÔCavendishÕ) were established in a Þeld naturally infested with black root rot pathogens. Crowns were planted on 17 June 1998. There were three rows per plot, 11 plants per row, planted 15 cm apart within rows and 0.75 m between rows. Plots were surrounded by a 0.9-m border. Treatments were arranged in a 2 × 3 × 2 factorial design and consisted of a water control or *T. harzianum* (1.26 g RootShield in 2 liters water per plot) drench around crowns on 18 June 1998 and 26 May 1999 at the rate of 4,700 liters/ha. Applications of entomopathogenic nematodes (*Steinernema carpocapsae* or *S. feltiae* applied at rates of 7.4 billion/ha applied in 4,700 liters/ha water) were applied either in spring (18 June 1998 and 26 May 1999) or late summer (19 August 1998 and 26 August 1999). Nontreated plots were included as controls. Two plants from the outer rows of the plots were destructively sampled on 3 November 1999 to determine pathogen populations. *Rhizoctonia fragariae* was isolated and identiÞed as described above. Ten 0.5-cm-long root segments each from structural and perennial roots were evaluated for each plot. Fruit was harvested and weighed in June 1999 from the center row of each plot. Statistical differences between treatments were determined by factorial analysis of variance.

We conducted additional laboratory and greenhouse

experiments to determine whether living or heat-killed entomopathogenic nematodes directly affected lesion nematode motility, repelled active lesion nematodes, or reduced infection of host roots. The effects of nonsterile entomopathogenic nematodes with associated microorganisms on *P. penetrans* motility were determined in glass tubes. Approximately 5,000 living or heat-killed (heated to 60 ¡C for 5 minutes in a water bath) *S. feltiae* infective juveniles suspended in 0.7 ml water were added to 90 to 120 *P. penetrans* in 0.3 ml water. Two tubes were rinsed into counting dishes after 48, 72, 96, or 120 hours, and the percent of lesion nematodes that were motile or responded to prodding with a pick were determined. The experiment was conducted twice. Statistical differences between treatments were determined by repeated-measures analysis of variance.

Repellency of *P. penetrans* from living or heat-killed *S. feltiae* nematodes in suspension, or from *Galleria mellonella* larvae infected with *S. feltiae,* was determined in 90-mm petri dishes. Approximately 1,500 living or heat-killed *S. feltiae* infective juveniles suspended in 0.5 ml water were placed in a 50-mm-diam. well cut into the water agar 40 mm from the center of a 90-mm petri dish. A control well containing distilled water alone was placed 40 mm from the center in the opposite direction. Freeze-killed *G. mellonella* larvae without entomopathogenic nematodes were similarly placed in dishes as a control for comparison with non-frozen nematode-infected *G. mellonella.* Approximately 30 to 60 freshly extracted *P. penetrans* (24-hour, wrist-action shaker extraction from strawberry roots) were placed on the agar surface in the center of each dish in a single drop that spread to 10 mm diam., and the dishes were held at ambient temperature. Lesion nematode movement was evaluated by determining a nematode-mm value. This value was calculated by multiplying the number of nematodes that moved beyond the center 10-mm section of the dish by the distance each moved either toward or away from the target well or *G. mellonella* larvae. There were Þve replicate dishes of each treatment, and measurements were taken 2, 4, or 6 hours after adding *P. penetrans* to the dish. *Pratylenchus penetrans* repellency from *S. feltiae* infected or freeze-killed *G. mellonella* larvae was determined at 3, 4, or 20 hours after adding *P. penetrans* to each dish. Statistical differences between treatments were determined by StudentÕs *T*-test comparisons within paired treatments.

The inßuence of living or heat-killed *S. feltiae* infective juveniles on *P. penetrans* infection of tomato roots was determined by inoculating approximately 20,000 entomopathogenic nematodes to four holes (1-cm-diam., 1-cm-deep) around 4-week-old ÔRutgersÕ tomato seedlings. Control pots were treated with water alone. The seedlings were grown in 50 cm^3 pasteurized sandy soil in a 4-cm-diam. container. Approximately 700 *P. penetrans* also were inoculated into the same holes within 5 minutes after inoculating the entomopathogenic nematodes. Plant roots were washed free of soil 2, 4, 6, or 8 days after inoculation, and *P. penetrans* was extracted from all roots using a wrist-action shaker. There were four replicate plants for each treatment, and the experiment was conducted twice. Statistical differences between treatments were determined by factorial analysis of variance.

Results

There were no differences in numbers of *P. penetrans* extracted from roots of plants drenched with water alone or with *S. carpocapsae* or *S. feltiae* (Table 1). The nematode species applied, the rate of application, and the timing of application had no effect on lesion nematode populations or on the number of runners per plant in 1999 or 2000. The only effects on *P. penetrans* involved soil type and year (P = 0.001). Lesion nematode numbers were higher in the silt loam soil initially and throughout the experiment. These data are not shown because they are averaged in Table 1. *Pratylenchus penetrans* populations increased each year, averaging 198 per 2 g root in 1998, 305 per 2 g root in 1999, and 600 per 2 g root in 2000.

Trichoderma harzianum (RootShield) treatment in water or Agrigel suspension or dust applied to roots did not affect plant vigor as determined by shoot or root weight the subsequent year, yield, nematode population in roots, or percent root rot (Table 2). *Rhizoctonia fragariae* was consistently isolated from symptomatic roots.

The experiments in small plots with *S. carpocapsae and S. feltiae* in factorial combination with an annual Rootshield drench resulted in no differences in lesion nematodes extracted in November of the second year (Table

Table 1. Effect of entomopathogenic nematodes on strawberry vigor and *Pratylenchus penetrans* populations in strawberry roots in Þeld microplots over 3 years.

Nematode treatment[a]	Year	*Pratylenchus* per 2 g root	Number of runners per plant
None	1998	208	Ð[b]
	1999	343	1.6
	2000	887	0.5
Steinernema carpocapsae	1998	166	Ð
	1999	373	1.5
	2000	484	1.5
Steinernema feltiae	1998	220	Ð
	1999	209	1.5
	2000	427	0.5
SigniÞcance		*P*	*P*
Nematode		ns	ns
Year		0.001	0.05
Nematode × Year		ns	ns

[a] Nematodes applied as a soil drench of 7.4 or 14.8 billion/ha in June and August of each year.
[b] Runners not counted in 1998.

TABLE 2. Effect of *Trichoderma harzianum* (RootShield) drench on strawberry vigor, yield, *Pratylenchus penetrans* populations, and percent black root rot in Þeld soils.

Preplant treatment[a]	Leaf no. 1997	Shoot wt. 1998	Root wt. 1998	*P. penetrans* per 2 g root	Berry wt. (kg)	Percent root rot
Water	15.1	58.6	9.6	72.5	3.88	47.9
Agrigel	13.3	61.3	9.3	100.0	2.01	33.4
RootShield in water	12.8	47.6	7.4	102.5	3.62	43.7
RootShield in Agrigel	15.0	59.8	10.5	80.0	3.27	35.8
RootShield dust	14.3	39.1	7.0	42.5	3.01	27.6
SigniÞcance	ns	ns	ns	ns	ns	ns

[a] RootShield applied as a root dip (29 g/l product in water with or without Agrigel) or as formulated product dusted onto damp roots.

3). In addition, the percent recovery of *R. fragariae* from root segments was higher ($P = 0.001$) from secondary perennial roots than for structural roots, but there were no differences between treatments within root types. There were no differences between treatments in percent rotted roots (data not shown). The application of *Steinernema* again had no effects on the number of *P. penetrans* extracted from strawberry roots.

Exposure to living or heat-killed *S. feltiae* in water in tubes reduced ($P = 0.001$) *P. penetrans* motility (Table 4). *Pratylenchus penetrans* in some tubes had no motility or response to prodding with a pick after 48 or 72 hoursÕexposure to living or dead *S. feltiae.* However, motility was restored after 2 or more hours in the counting dish and after an additional 48 hours exposure in tubes.

Living or heat-killed *S. feltiae* either repelled *P. penetrans* or reduced nematode movement from 2 to 6 hours as they moved in the direction of wells containing *S. feltiae* when compared to movement toward control

TABLE 3. Effect of entomopathogenic nematodes and *Trichoderma harzianum* (RootShield) drench on *Rhizoctonia fragariae* and *Pratylenchus penetrans* populations in Þeld plots.

			Percent *R. fragariae* infection	
Drench treatment[a]	Entomopathogenic nematode treatment[b]	*Pratylenchus* per 2 g root	Structural root	Perennial root
Water control	None	48.6	3	53
Water control	*S. carpocapsae*	46.7	1	58
Water control	*S feltiae*	35.6	3	54
RootShield	None	24.4	0	56
RootShield	*S. carpocapsae*	37.5	3	58
RootShield	*S. feltiae*	56.3	1	44
SigniÞcance		*P*	*P*	*P*
Nematode		ns	ns	ns
RootShield		ns	ns	ns
Nematode × RootShield		ns	ns	ns

[a] 2.9 kg/ha RootShield in 4,675 liters/ha.
[b] 74 billion nematodes/ha applied in spring or late summer.

TABLE 4. Effects of 48 to 120-hour exposure to living or heat-killed *Steinernema feltiae* on *Pratylenchus penetrans* motility.

	Percent responsive after hours exposure[a]			
Treatment[b]	48	72	96	120
Water control	82.6	92.8	82.9	94.6
Steinernema feltiae living	20.0	40.1	60.8	70.2
Steinernema feltiae heat-killed	23.6	40.1	37.2	79.1
SigniÞcance	*P*			
Nematode	0.001			
Time	0.003			

[a] *Pratylenchus penetrans* responsiveness determined by motility or to probing with a pick.
[b] Approximately 5,000 living or heat-killed (heated to 60 ¡C for 5 minutes in a water bath) *S. feltiae* infective juveniles suspended in 0.7 ml water were added to 90Ð120 *P. penetrans* in 0.3 ml water.

wells (Table 5). Living *S. feltiae* dispersed over much of the dish surface after 6 hours. *Steinernema feltiae*-infected *G. mellonella* were not as effective in altering *P. penetrans* movement as nematodes added directly to dishes, despite the fact that the numbers of *S. feltiae* in infected *G. mellonella* were approximately 30 × higher than those added to the dish.

Pratylenchus penetrans infection of tomato roots after exposure to *S. feltiae* in soil for 2 to 8 days was reduced ($P = 0.02$) in comparison to the water controls (Table 6), regardless of whether the *S. feltiae* were viable or had been heat-killed. *Pratylenchus penetrans* infection of roots increased with time ($P = 0.04$). The differences between numbers of *P. penetrans* extracted from roots of plants inoculated with *S. feltiae* or water alone appeared to disappear by 8 days, although there appeared to be no interaction between treatment and time.

DISCUSSION

A number of biological controls may have potential for management of difficult-to-control insects and diseases of perennial strawberries. For example, entomopathogenic nematodes have been shown to be effective

TABLE 5. *Pratylenchus penetrans* movement toward or away from living or heat-killed *Steinernema feltiae* or *S. feltiae*-infected *Galleria mellonella* over time.

	Nematode-mm[a]			
Treatment	2-hour	4-hour	6-hour	20-hour
S. feltiae living	14	30	20	Ñ
Control	104	72	124	Ñ
SigniÞcance, $P =$	0.001	0.05	0.001	
S. feltiae heat-killed	42	14	40	Ñ
Control	90	108	98	Ñ
SigniÞcance, $P =$	0.05	0.05	ns	
S. feltiae-Galleria	25	86	Ñ	96
Control	88	144	Ñ	136
SigniÞcance, $P =$	ns	ns		ns

[a] Number of nematodes × distance from the center 1 cm inoculation point toward either the *S. feltiae*-treated or control side of the dish.

TABLE 6. Effect of co-inoculation with *Steinernema feltiae* and *Pratylenchus penetrans* on *P. penetrans* extraction from tomato roots after 2 to 8 days.

Treatment[a]	*P. penetrans* extraction from roots after exposure in soil for: 2 days	4 days	6 days	8 days
Water control	30.2	54.6	60.0	40.6
Steinernema feltiae living	18.7	22.5	25.1	43.4
Steinernema feltiae heat-killed	21.4	32.4	34.8	49.5
SigniÞcance	*P*			
Nematode	0.02			
Days	0.04			
Nematode × Days	ns			

[a] 20,000 *Steinernema feltiae* and 700 *P. penetrans* were inoculated into four 1-cm-diam., 1 cm-deep holes around 4-week-old Rutgers tomato seedlings. The seedlings were grown in 50 cm^3 pasteurized sandy soil in a 4-cm-diam. container.

controls of black vine weevils in commercial strawberry production (Cowles, 1997; Curran, 1992; Sampson, 1994; Wilson et al., 1999). Suppression of plant-parasitic nematode populations also has been demonstrated in a number of greenhouse (Ishibashi and Choi, 1991; Ishibashi and Kondo, 1987) and Þeld studies (Grewal et al., 1997; Smitley et al., 1992) in other cropping systems. Root lesion nematodes have been associated with reduced strawberry vigor and yield and increased severity of black root rot, caused by *R. fragariae* (LaMondia, 1999). *Trichoderma harzianum* has been an effective biological control agent of a number of plant-pathogenic fungi, including *Rhizoctonia* spp. (Haran et al., 1996; Harman, 2000; Lo et al., 1997; Yuen et al., 1994; Zimand et al., 1996). The fungus has both rhizosphere and phylloplane competence (Lo et al., 1997) and is commercially available for use on a number of crops.

The integration of successful biocontrol agents that could be used to control an array of pests and pathogens would seem a promising approach to sustainable production. The primary objective of this study was to evaluate whether entomopathogenic nematode applications would suppress lesion nematode populations. In addition to this, the effect of *T. harzianum* alone or in combination with entomopathogenic nematodes to protect strawberry plants from black root rot caused by lesion nematodes and *R. fragariae* was also evaluated. As a secondary objective, we evaluated the potential toxicity and repellency of entomopathogenic nematodes to lesion nematodes as well as suppression of root infection.

Our results indicate that the inundative application of *S. feltiae* or *S. carpocapsae* did not reduce *P. penetrans* populations over 2 or 3 years in Þeld microplots or in small Þeld plots. Previous research demonstrating suppression of plant-parasitic nematodes after the application of entomopathogenic nematodes was primarily conducted with root-knot nematodes (Bird and Bird, 1986; Ishibashi and Choi, 1991). Suppression of other nematodes has yielded inconsistent results. Ishibashi and Kondo (1987) reported a reduction in recovery of total numbers of tylenchid nematodes after *S. carpocapsae* application in 1 of 2 years. Smitley et. al. (1992) demonstrated that applications of *Heterorhabditis bacteriophora* resulted in reduced *Tylenchorhynchus* populations in irrigated plots in 1 of 2 years. Alternatively, *P. penetrans* numbers were not affected by entomopathogenic nematode application in the Þrst year, but were reduced 1 week after *H. bacteriophora* application, but not at 3, 5, or 8 weeks in the second year. They concluded that populations of *P. penetrans* might be temporarily suppressed following inoculation with entomopathogenic nematodes.

Similarly, *T. harzianum* (RootShield) treatment alone or in combination with *S. feltiae* or *S. carpocapsae* did not affect strawberry vigor, yield, nematode population in roots, or percent root rot. The method of application of *T. harzianum* did not increase or affect biocontrol activity, consistent with previous observations (Harman, 2000). *Rhizoctonia fragariae* was consistently isolated from symptomatic roots. *Trichoderma harzianum* strain T-22 has been shown to be rhizosphere competent and to control *R. solani* on crops such as turfgrass (Lo et al., 1996). *Trichoderma harzianum* does not produce diffusible factors but coils around and lyses *R. solani* hyphae (Benhamou and Chet, 1993).

There may be several reasons for the lack of biocontrol using RootShield in these experiments. The interaction between *T. harzianum* and binucleate *R. fragariae* may be different than with the multinucleate *R. solani.* The extent or persistence of *T. harzianum* root colonization in the Þeld was not determined. Previously, *T. harzianum* was shown to be most effective as a mycoparasite of *R. solani* at acid pH, and was more suppressive at pH 4.0 than 6.0 (Chet and Baker, 1980; Huang and Kuhlman, 1991). Subsequent research indicated that T-22 may be effective in both acid and alkaline soils (Lo et al., 1996). The pH of our Þeld plot was 6.0, within the range of 5.8 to 6.2 recommended for strawberry production (Cooley and Schloemann, 1994). The use of any single chemical or biocontrol such as RootShield as a methyl bromide replacement on strawberry to simultaneously control multiple pathogens may be asking too much (Harman, 2000). The black root rot in these experiments was the result of at least two pathogens, *P. penetrans* and *R. fragariae.*

The inconsistent and temporal effects of entomopathogenic nematodes on plant-parasitic nematodes may be partially explained in context of the mechanism(s) proposed for plant-parasitic nematode suppression. First, competition at the root surface may affect plant-parasitic nematode behavior and subsequent population densities (Bird and Bird, 1986). Ishibashi and Choi (1991) determined that nearly half of inoculated *S. carpocapsae* moved to the area of the root tips

and remained there for some time. Infective juveniles of *Meloidogyne incognita* were repelled by *S. feltiae, S. carpocapsae,* or *H. bacteriophora* treatments that included symbiotic bacteria (Grewal et al., 1999). The repellence of plant-parasitic nematodes from root tips may explain signiÞcant delays in root penetration by plant-parasitic nematodes associated with entomopathogenic nematode treatments.

The second proposed mechanism concerns the build-up of natural nematode antagonists in soil as a result of massive increases in numbers of nematodes resulting from entomopathogenic nematode application (Ishibashi and Choi, 1991; Ishibashi and Kondo, 1987). While this may occur, effects on plant-parasitic nematodes were observed in sterile sand (Grewal et al., 1999).

The third proposed mechanism was that allelochemicals produced by the entomopathogenic nematodes or their associated symbiotic bacteria (*Xenorhabdus* and *Photorhabdus* spp.) may be directly toxic or have detrimental behavioral effects on plant-parasitic nematodes (Grewal et al., 1999; Hu et al., 1999) Cell-free culture Þltrates of symbiotic bacteria were toxic to *M. incognita* (Hu et al., 1999). Relatively high concentrations of these Þltrates paralyzed *M. incognita* juveniles. Paralyzed nematodes did not recover after transfer to aerated water for 24 hours. Grewal et al. (1999) also determined that heat-killed *S. feltiae* temporarily reduced *M. incognita* infection of tomato roots and concluded that allelochemicals produced by *Xenorhabdus* spp. were at least partially responsible. In addition to a delay in root infection, bacterial allelochemicals also were associated with reduced *M. incognita* egg hatch, mortality, and repellence.

Our results in these experiments indicated that exposure to living or heat-killed *S. feltiae* and associated microbes in water reduced *P. penetrans* motility. All individuals of *P. penetrans* in some tubes had no motility or response to prodding with a pick after 48 or 72 hoursÑexposure to living or dead *S. feltiae.* However, motility was restored after several hours in the counting dish and after additional hours exposure in tubes. Grewal et al. (1999) determined *M. incognita* mortality based upon lack of motility at 72 hours and did not report whether motility was restored after additional time or transfer to water. We also determined that living or heat-killed *S. feltiae* either repelled *P. penetrans* or reduced nematode movement from 2 to 6 hours as they moved in the direction of wells containing *S. feltiae.* Living *S. feltiae* dispersed over much of the dish surface after 6 hours. In contrast, *S. feltiae*-infected *G. mellonella* were not as effective as nematodes added directly to dishes, despite the fact that the numbers of *S. feltiae* in infected *G. mellonella* were approximately 30-fold higher than those added directly to the dish. Indole, produced by *Photorhabdus* in culture, was associated with *M. incognita* paralysis, but was not produced in *G. mellonella* cadavers (Hu et al., 1999). A similar mechanism may explain our results with *P. penetrans* and *S. feltiae.* Our data also indicated a trend for a temporary suppression of *P. penetrans* root infection by application of living or heat-killed *S. feltiae,* similar to results previously reported for *M. incognita* (Grewal et al., 1999). Our data demonstrate that lack of responsiveness to tactile stimulation may not be adequate for determination of viability, as many non-responsive nematodes later recovered motility.

Overall, while intriguing, the temporary effects of entomopathogenic nematodes on plant-parasitic nematodes may not be sufficient to result in meaningful management of nematode diseases under Þeld conditions. However, additional research to determine the mechanism of suppression may result in the development of new biological or biorational pathogen-speciÞc management tactics against plant-parasitic nematodes.

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