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Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in Field Soil[1]

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Abstract: The role of *Pasteuria penetrans* in suppressing numbers of root-knot nematodes was investigated in a 7-year monoculture of tobacco in a field naturally infested with a mixed population of *Meloidogyne incognita* race 1 and *M. javanica*. The suppressiveness of the soil was tested using four treatments: autoclaving (AC), microwaving (MW), air drying (DR), and untreated. The treated soil bioassays consisted of tobacco cv. Northrup King 326 (resistant to *M. incognita* but susceptible to *M. javanica*) and cv. Coker 371 Gold (susceptible to *M. incognita* and *M. javanica*) in pots inoculated with 0 or 2,000 second-stage juveniles of *M. incognita* race 1. Endospores of *P. penetrans* were killed by AC but were only slightly affected by MW, whereas most fungal propagules were destroyed or inhibited in both treatments. Root galls, egg masses, and numbers of eggs were fewer on Coker 371 Gold in MW, DR, and untreated soil than in AC-treated soil. There were fewer egg masses than root galls on both tobacco cultivars in MW, DR, and untreated soil than in the AC treatment. Because both *Meloidogyne* spp. were suppressed in MW soil (with few fungi present) as well as in DR and untreated soil, the reduction in root galling, as well as numbers of egg masses and eggs appeared to have resulted from infection of both nematode species by *P. penetrans*.

Key words: bacterium, biological control, *Meloidogyne incognita, M. javanica,* nematode, *Nicotiana tabacum, Pasteuria penetrans,* root-knot nematode, suppressive soil, tobacco.

Pathogen-suppressive soils are defined as soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease (conducive soil), but thereafter the disease is reduced even though the pathogens may persist in the soil (1). Soils suppressive to plant pathogens may occur naturally as an inherent characteristic of their physical, chemical, and(or) biological structure. Suppressiveness may also be induced by some agronomic practice, such as planting a crop or adding organic or nutritional amendments, which changes the microflora (1). Induced suppressiveness apparently develops as a result of the buildup of antagonists in response to a high pathogen population (1), especially in cases where susceptible crops are grown in succession.

The endospore-forming bacterial parasite, *Pasteuria penetrans* (Thorne) Sayre & Starr, is widely distributed in agricultural soil throughout the world (10,23) and contributes to natural and induced nematode control, especially of *Meloidogyne* spp. (3–5,7,17–19,21). In most observations, the suppression of *Meloidogyne* spp. by *P. penetrans* has resulted after several years of monoculture with susceptible hosts (3,6, 18,19).

In 1994, a site near Gainesville, Florida, was identified as a suppressive soil to *Meloidogyne* spp. (6). The field had been planted continuously for 7 years to flue-cured tobacco (*Nicotiana tabacum* L.). No nematicides or other nematode management practices had been applied to the site during this time. Root-knot damage was initially severe and was caused by a mixed population of *M. incognita* race 1 (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood. Yield reductions decreased over the years to a point were there was little difference in the growth of the susceptible tobacco cv. Coker 371 Gold and the resistant cv. Northrup King 326 (Whitty, pers. comm.). Endospores of *P. penetrans* were found in mature females and attached to second-stage juveniles (J2) of both nematode species from samples collected throughout the field, and several

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TABLE 1. Survival of soil fungi and *Pasteuria penetrans* in soil autoclaved (AC) twice for 1.5 hours at 0.5 kg/cm^2, microwaved (MW) for 3 minutes/kg soil, air dried (DR) for 2 weeks in the greenhouse, and untreated.

Soil treatments	Cfu per g of soil	Mean number of *P. penetrans* endospores attached per 20 J2
AC	0 c	0 b
MW	204 b	9.9 a
DR	27 × 10^3 a	12.7 a
Untreated	24.4 × 10^4 a	7.6 a

Values are means of six replicates. Means within columns followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple-range test. The number of fungal propagules was determined by dilution plating and is presented as colony-forming units (cfu). Attachment of *P. penetrans* endospores were counted on second-stage juveniles (J2) of *Meloidogyne incognita* race 1.

species of fungi were isolated from J2 and egg masses (6). The objective of this study was to determine the role of *P. penetrans* in suppressing *M. incognita* and *M. javanica* in soil from this tobacco field.

MATERIALS AND METHODS

Field site: The field site was located at the University of Florida, Green Acres Agronomy Farm, Alachua County, Florida. Approximately 90 liters of soil, a loamy, siliceous, hypothermic Grossarenic Paleudults with 90% sand, 4% silt, 6% clay, and 2.5% organic matter, was collected from the tobacco field with a bucket auger (10-cm-d) in March 1993 (greenhouse experiment 1) and in July 1993 (greenhouse experiment 2). The soil was sieved through a screen with 5-mm × 5-mm openings, mixed, and divided into four subsamples of 22 liters each. The subsamples were either autoclaved (AC) twice for 1.5 hours at 0.5 kg/cm2, microwaved (MW) for 3 minutes/kg soil (9) at full power (625 W; 2,450 MHz), air dried (DR) for 2 weeks in the greenhouse to eliminate or reduce the natural nematode population, or left untreated.

Nematode origin: The *Meloidogyne incognita* race 1 isolate used in this study originated from tobacco grown on the University of Florida, Green Acres Agronomy Farm, Gainesville, Florida. The nematode was cultured in a greenhouse on tomato (*Lycopersicum esculentum* Mill. cv. Rutgers) from a single egg mass. Eggs of *M. incognita* were extracted from infected tomato roots (13), hatched on a Baermann funnel

TABLE 2. Effects of tobacco cultivars, soil treatments (autoclaving, microwaving, air drying, and untreated), and *Meloidogyne incognita* race 1 inoculum levels (0 or 2,000 second-stage juveniles) on nematode reproduction and plant performance.

ANOVA	Number of galls	Number of egg masses	Number of eggs	Total plant fresh weight	Shoot fresh weight	Root fresh weight	Shoot height
				Experiment 1			
Cultivar (C)	***	***	***	**	**	**	**
Soil treatment (ST)	***	***	***	***	***	**	***
Inoculation level (I)	***	***	***	NS	NS	NS	NS
C × ST	***	***	***	***	**	***	**
C × I	***	***	***	NS	NS	NS	NS
I × ST	***	***	***	*	**	NS	**
I × ST × C	***	***	***	NS	*	NS	**
				Experiment 2			
Cultivar (C)	***	***	***	**	*	**	NS
Soil treatment (ST)	***	***	***	***	***	***	***
Inoculation level (I)	***	***	***	NS	NS	NS	NS
C × ST	**	***	***	***	**	***	**
C × I	***	***	***	NS	NS	NS	NS
I × ST	***	***	***	NS	**	NS	**
I × ST × C	***	***	***	NS	NS	NS	**

*, **, *** represent $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively, and NS = no significant differences at $P \leq 0.05$ according to Duncan's multiple-range test.

TABLE 3. Effect of autoclaving (AC), microwaving (MW), and air drying (DR) on soil suppressiveness to *Meloidogyne* spp. and the expression of root-knot on tobacco cultivar Coker 371 Gold (susceptible to both *Meloidogyne incognita* and *M. javanica*) following inoculation with 0 or 2,000 second-stage juveniles of *M. incognita* race 1.

Soil treatment	Nematode reproduction						Plant performance							
	Number of root galls per root system		Number of egg masses per root system		Number of eggs per root system		Total fresh weight (g)		Shoot weight (g)		Root weight (g)		Shoot height gained (cm)	
	Experiment 1													
	+[a]	−	+	−	+	−	+	−	+	−	+	−	+	−
AC	223 a	0 b	191 a	0 b	13,086 a	0 c	89 a	98 a	77 a	82 a	13 a	17 a	27 a	26 a
MW	129 b	0 b	25 b	0 b	1,312 b	0 c	79 a	72 b	61 a	56 c	17 a	15 a	18 a	16 b
DR	118 b	15 b	12 b	3 b	524 c	279 b	75 a	88 ab	60 a	72 ab	15 a	16 a	23 a	20 b
Untreated	107 b	102 a	35 b	21 a	1,290 b	828 a	74 a	75 b	62 a	58 b	12 a	17 a	22 a	19 b
	Experiment 2													
AC	470 a	0 c	437 a	0 b	49,219 a	0 c	52 a	74 a	37 a	61 a	14 a	14 a	4 a	7 a
MW	105 b	0 c	74 b	0 b	1,858 b	0 c	54 a	54 b	43 a	41 b	11 a	12 a	5 a	3 b
DR	155 b	135 b	94 b	92 a	9,169 b	3,992 b	46 a	36 c	32 a	25 c	13 a	11 a	3 a	2 b
Untreated	283 b	229 a	99 b	117 a	6,051 b	13,582 a	51 a	58 b	36 a	44 b	13 a	14 a	3 a	4 a

Values are means of five replicates. Means within columns and within experiments followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple-range test.

[a] + = Inoculated with 2,000 J2 of *M. incognita* race 1; − = No nematodes added, resident population following air drying for 2 weeks in the greenhouse.

TABLE 4. Effect of autoclaving (AC), microwaving (MW), and air drying (DR) on a soil suppressive to *Meloidogyne* spp. and the expression of root-knot on tobacco cultivar Northrup King 326 (resistant to *Meloidogyne incognita* but susceptible to *M. javanica*) following inoculation with 0 or 2,000 second-stage juveniles of *M. incognita* race 1.

Soil treatment	Nematode reproduction						Plant performance							
	Number of root galls per root system		Number of egg masses per root system		Number of eggs per root system		Total fresh weight (g)		Shoot weight (g)		Root weight (g)		Shoot height gained (cm)	
	Experiment 1													
	+[a]	−	+	−	+	−	+	−	+	−	+	−	+	−
AC	1.2 b	0 b	1 b	0 b	118 b	0 b	127 a	102 a	104 a	81 a	23 a	21 a	26 a	17 a
MW	0.3 b	0 b	0 b	0 b	0 b	0 b	92 b	74 b	71 b	57 b	21 a	18 a	14 b	13 b
DR	1.7 b	1 b	2 b	1 b	113 b	159 b	88 b	62 b	69 b	50 b	19 a	11 b	13 b	8 c
Untreated	15 a	14 a	6 a	10 a	385 a	395 a	74 b	94 a	59 b	73 a	15 a	21 a	13 b	16 ab
	Experiment 2													
AC	0 c	0 b	0 a	0 b	36 b	0 b	70 b	69 a	52 bc	55 a	18 a	15 b	4 a	4 a
MW	0 c	0 b	0 a	0 b	0 b	0 b	74 a	76 a	54 a	53 a	20 a	23 a	4 a	4 a
DR	60 cb	24 b	44 a	10 b	2,288 a	681 b	51 b	48 b	37 bc	36 b	15 a	12 b	3 a	3 a
Untreated	73 a	103 a	48 a	75 a	2,470 a	2,522 a	44 c	43 b	33 c	32 b	11 a	11 b	3 a	3 a

Values are means of five replicates. Means within columns and within experiments followed by the same letter do not differ at $P \leq 0.05$ according to Duncan's multiple-range test.

[a] + = Inoculated with 2,000 J2 of *M. incognita* race 1; − = No nematodes added, resident population following air drying for 2 weeks in the greenhouse.

(22), and used as 1- to 4-day-old J2 in bioassays and suppressive soil experiments.

Laboratory experiments: One day after all soil treatments were completed, a dilution plating technique was used to assay for selected fungi (15). Six replicates were plated for each dilution of each soil treatment. After 3 days the fungal colonies were counted.

A soil bioassay (20) used *M. incognita* race 1 as the host to determine the presence of *P. penetrans*. Dry soil (50 g) from each treatment was placed in a petri dish and replicated six times. The soil water content was adjusted to 100% field capacity to increase the rate of endospore attachment, and the dishes were left uncovered at room temperature (4). After 3 days, 500 J2 of *M. incognita* were added and the moisture level was adjusted to 50% of field capacity. The J2 were extracted by centrifugal flotation 2 days later (14), and the number of endospores attached per J2 was determined with the aid of an inverted microscope for 20 J2 per replicate.

Greenhouse experiments: Treatments were arranged in a 4 × 2 × 2 factorial design that included four soil treatments (listed previously under *Field site*), inoculum levels of 0 and 2,000 J2 of *M. incognita* race 1 per pot, and tobacco cultivars Coker 371 Gold (susceptible to both *M. incognita* and *M. javanica*) and Northrup King 326 (resistant to *M. incognita* but susceptible to *M. javanica*). The soil from each treatment was placed into sterilized 15-cm-d clay pots (ca. 1 liter/pot). The moisture level was adjusted to approximately 50% of field capacity. Pots were covered with aluminum foil and held for 3 days in a growth room at 28–30 C and 14 hours of light. *Meloidogyne incognita* race 1 J2 were suspended in 10 ml of water and dispensed equally to each of five holes, 5 cm deep, in soil of each pot to receive nematodes. Water only was added to the pots that had no nematode inoculum added. One 5–10-cm high tobacco seedling was transplanted into each pot 5 days later. A 20-20-20 (N-P-K) fertilizer was applied once per week, and insects were controlled as needed using recommended insecticides.

Two experiments were conducted, one with soil collected March 1993 and one with soil collected July 1993. Treatments were replicated six times in the first experiment and five times in the second experiment. The two experiments were run for 45 days and 46 days, respectively. Plant heights and fresh shoot weights were recorded. The root systems were washed free of soil, excess water removed, weighed, and then stored in plastic bags at 4 C. The following day, roots were stained with Phloxine B (8), and the number of root galls and egg masses were counted. Eggs were extracted with 1.05% sodium hypochlorite (13) and the number of eggs counted.

Statistical analysis: All data were subjected to factorial analysis of variance (ANOVA). Significantly different means were separated and compared with Duncan's multiple-range test or *t*-test.

Results and Discussion

Laboratory experiments: Survival of the fungal populations and *P. penetrans* in the field soil varied by soil treatments (Table 1). Counts of colony-forming units in AC, MW, and DR soil were different ($P \leq 0.05$); however, quantitative survival of fungi in the DR soil did not differ from the untreated soil ($P \leq 0.05$). The number of *P. penetrans* endospores attached to J2 was not different among the MW, DR, and untreated soil ($P \leq 0.001$), but all endospores were killed in the AC soil (Table 1). In the MW soil, fungal populations were eliminated or reduced, whereas the bacterial endospores were not affected ($P \leq 0.05$). Thus, MW treatment allowed us to separate the possible antagonistic effects of two biological components, namely, nematophagous fungi and *P. penetrans*.

Greenhouse experiments: Suppressive soil treatment results were similar in both experiment 1 and 2. An interaction was detected for cultivars, soil treatments, and in-

oculation levels ($P \leq 0.05$) (Table 2); thus, data for the different cultivars and inoculation levels are presented separately (Tables, 3,4).

In inoculated treatments of the *M. incognita*-susceptible cultivar, root galling, number of egg masses, and individual eggs were less in MW, DR, and in untreated soil than in AC soil ($P \leq 0.05$) (Fig. 1; Table 3). This reduction most likely resulted from the infection of *M. incognita* by *P. penetrans*, since the number of egg masses and eggs was reduced more than galling (Table 3). This supports earlier observations that infection by *P. penetrans* causes sterility or reduces fecundity in females (18,19). Although nematode suppression by soilborne fungi and *P. penetrans* could not be evaluated separately, an additive nematode suppression would be expected to cause differences in nematode reproduction between the MW and DR treated soils. This effect was observed in experiment 1, where the number of eggs was less in the DR soil compared with MW and AC treated soil ($P \leq 0.05$) (Table 3). However, this effect was not observed in experiment 2 (Table 3). In this case, the MW treated soil (with few fungi present, Table 1) was just as suppressive as the DR and untreated soil. These results support our hypothesis that *P. penetrans* is the main contributor to the suppressiveness of the soil.

Meloidogyne incognita race 1 reproduced slightly on the resistant tobacco cultivar (Table 4). Several studies have shown reproduction of *Meloidogyne* spp. in resistant cultivars (11,24,25). Reproduction on the resistant cultivar in the untreated soil likely resulted from infection by the resident population of *M. javanica* and was not increased by the addition of *M. incognita* race 1 (Table 4). When no nematode inoculum was added, root galls, egg masses, and individual eggs were formed on both cultivars in untreated soil ($P \leq 0.05$) (Tables 3,4). Some nematodes survived the air-drying treatment.

Plant performance (total weight, shoot weight, and shoot height) in the AC treated soil was generally greater than that in the other treatments ($P \leq 0.05$) (Tables 3,4). Increased release of nutrients into the soil solution from autoclaving may have had some effect (9); however, plant performance did not differ in the case of the susceptible tobacco cultivar (Table 3). In this case, water and nutrient absorption may have been limited by the heavy nematode infection (12). Plant performance of the resistant cultivar was improved in the untreated soil (Table 4) but declined when nematode inoculum was added ($P \leq 0.05$) (Table 4). Severe stunting and yield reductions caused by *M. incognita* on resistant cultivars has been described as being caused by the hypersensitive response of the resistant roots to nematode infection (2).

Nematode-suppressive soils containing *P. penetrans* have been reported (3,7,18). Nematode antagonists can be increased by continuous planting of nematode-susceptible crops (18). Bird and Brisbane (3) demonstrated qualitatively the biological origin of nematode suppression caused by *P. penetrans* in field soil in Australia. In our experiments the MW treatment was helpful because it reduced nematophagous fungi that might also be a factor in suppressive soils. Formalin has been used in the past to reduce the incidence of nematophagous fungi (7,16); however, it performs poorly and leaves a nematicidal residue in the soil. The MW treatment

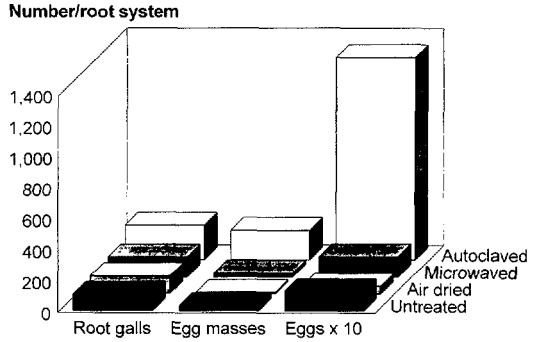

FIG. 1. Effect of soil treatments on a soil suppressive to *Meloidogyne* spp. and the expression of root-knot on tobacco cultivar Coker 371 Gold following inoculation with 2,000 second-stage juveniles of *M. incognita* race 1.

does not leave a nematicidal residual effect. The lower number of developing *Meloidogyne* spp. in the MW soil with viable *P. penetrans* endospores suggests that *P. penetrans* was important in nematode suppression and the reduction of plant damage. Further improvement on the bioassay for nematophagous fungi is required to make conclusions about their contribution to the suppressiveness of a soil to plant-parasitic nematodes.

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