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Response of *Meloidogyne* spp. to *Pasteuria penetrans*, Fungi, and Cultural Practices in Tobacco[1]

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Abstract: The response of a mixed population of *Meloidogyne incognita* and *M. javanica* to three cultural practices, tobacco cultivars (two cultivars, differing in resistance to *M. incognita*), cover treatments (three treatments), and inorganic nitrogen fertilizer (two treatments), *Pasteuria penetrans*, and soil-borne fungi was investigated in a tobacco field in 1991. On all sampling dates, higher densities of root-knot nematodes were observed on tobacco cv. Coker 371 Gold than on K-326. Initially, forage sorghum decreased the number of *Meloidogyne* spp. on tobacco, compared with weeds and hairy indigo, but had little effect on the density of second-stage juveniles (J2) in soil at mid-season or final harvest. The density of *P. penetrans* endospores also decreased in soil in forage sorghum plots. On one of four sampling dates, high levels of inorganic nitrogen fertilizer increased the number of *P. penetrans* endospores produced per nematode female, and decreased the density of *Meloidogyne* spp. on tobacco. The density of *P. penetrans* endospores increased in these high-N plots after harvest, especially in the plots planted with K-326. The density of J2 in soil was negatively correlated with both the numbers of endospores attached per J2 and the proportion of J2 with attached endospores in midseason soil samples. No correlation was observed between the nematode density and frequency of fungi colonizing the egg masses of *Meloidogyne* spp.

Key words: biological control, fungi, *Meloidogyne incognita, M. javanica,* nematode, *Nicotiana tabacum,* nitrogen fertilizer, *Paecilomyces lilacinus, Pasteuria penetrans,* population, root-knot nematode, tobacco, tobacco cultivar.

Continuing environmental problems associated with the use of nematicides (11) have resulted in a sense of urgency regarding the search for alternative nematode management tactics (6). Biological control of nematodes with microbial agents is an alternative management tactic that is receiving increased interest among nematologists. Biocontrol of plant-parasitic nematodes has been reported from several countries and on different nematodes (2, 3,5,7,8,10). Many soil-borne fungi have been demonstrated to be antagonists of nematodes. These include predaceous fungi, endoparasites of vermiform nematodes, fungi colonizing eggs and females of sedentary endoparasitic nematodes, and fungi that are antibiotic to nematodes (9). Recently, another group of nematode antagonists, endospore-forming bacteria, especially *Pasteuria penetrans,* have been reported throughout the world. *Pasteuria penetrans* is a density-dependent obligate parasite of many species of nematodes, mainly root-knot nematodes, *Meloidogyne* spp. Some reports suggest that *P. penetrans* may suppress nematode population densities below economic damage levels when their densities build up over time (8,9). Additional knowledge about the ecology of these nematode antagonists in agricultural ecosystems is needed before they can be used effectively in nematode management (IPM) programs.

The objective of this study was to determine the effects of nitrogen fertilizer, autumn cover treatments, and tobacco (*Nicotiana tabacum*) cultivars on population dynamics of root-knot nematodes, their fungal antagonists, and *P. penetrans* in a *Meloidogyne*-infested tobacco field in Florida.

MATERIALS AND METHODS

An infestation of root-knot nematodes, a mixed population of *M. incognita* and *M. javanica,* was observed in a tobacco field at the University of Florida Green Acres Agronomy Research Farm in Alachua

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County, Florida. The severity of root-knot of tobacco had decreased over time at this site. *Pasteuria penetrans* was observed attached to second-stage juveniles (J2) extracted from soil collected from the site. Sampling was conducted in 1991 to determine the population densities of *Meloidogyne* spp. and nematode antagonists.

The site was planted to tobacco continuously for 6 years before 1991. The 3 × 2 × 2 factorial treatment design included three autumn cover treatments, hairy indigo, forage sorghum, and weeds; two levels of inorganic nitrogen fertilizers, 89 and 158 kg ammonium nitrate/ha; and two tobacco cultivars, Coker 371 Gold and K-326. Coker 371 Gold is susceptible to both *M. incognita* and *M. javanica*, and K-326 is resistant to *M. incognita* but susceptible to *M. javanica*. Hairy indigo and forage sorghum were planted in August and plowed under in November each year. Rye was planted in early December as a winter cover treatments over the entire field and plowed under in March. Tobacco was transplanted in late March, and the final harvest was taken at the end of July each year. Preplant fertilization included a broadcast application of 1,800 kg of 6-6-18 (N-P-K) and 340 kg of 15-0-14 (sodium-potassium nitrate) per ha. At the final cultivation, in late April, ammonium nitrate was applied to the row middle at the rates described. The plots were arranged in randomized complete blocks replicated four times, and each plot consisted of one row with a row spacing of 1.2 m and length of 12 m.

Soil and root samples were taken with a bucket auger (10-cm-d) from each plot in the root rhizosphere 25 cm deep on 4 June, 3 July, 25 July, and again on 23 August immediately after the soil had been disked. Six cores per plot were taken on 4 June, and 12 cores per plot were taken at all other dates. Soil from each sample, except for the final sample, was screened with a sieve with 4-mm openings to separate tobacco roots from the soil. The soil was mixed, and a 100-cm^3 subsample was taken from each sample to extract nematodes by a centrifugal-flotation technique (4). The numbers of *Meloidogyne* J2/100 cm^3 soil were determined and the numbers of endospores attached to the cuticles of J2 were counted from 20 juveniles per sample with an inverted light microscope. Roots were washed and drained. Lateral roots were removed and chopped with a food blender for 20 seconds, put on tissue paper supported with a screen in a jar, and incubated at 23–24 C for 48 hours. The J2 were collected and counted.

On 2 August, six tobacco plants were removed from each plot and the galling indices were recorded according to the following scale: 0 = no galls, 1 = 1–10, 2 = 11–20, 3 = 21–55, 4 = 56–80, and 5 = 81–100% roots galled (1). Twenty females were extracted from each root sample for estimation of the average number of endospores of *P. penetrans* per female. The females were ruptured and blended with a glass tissue grinder in 2 ml water and the *P. penetrans* endospores were transferred to a hemacytometer and counted. Ten egg masses were taken from each sample to determine frequency of colonization by fungi. The egg masses were washed three times with sterile water, treated with 0.5% NaOCl for 1 minute, washed with sterile water three times again, and treated with a solution of chlortetracycline (50 ppm) + streptomycin (100 ppm) for 10 minutes. The treated egg masses were transferred onto 1.5% water agar in petri dishes, five egg masses per dish. Following an incubation of 5 days at 23–24 C, the egg masses with and without fungi were recorded.

The density of nematodes and the numbers of *P. penetrans* endospores produced per female were transformed to $\log_{10}(x + 1)$ values. The numbers of *P. penetrans* endospores attached per J2 were transformed to $\log_{10}(100x + 1)$ values. Data that were calculated as percentages were arcsin (x) transformed. All data were subjected to analysis of variance (ANOVA). The means were compared with Duncan's multiple-range test or *t*-test when only one factor and two treatments were included. Linear regressions were performed to deter-

TABLE 1. Population densities of second-stage juveniles (J2) of *Meloidogyne* spp. in soil samples and the numbers of J2 hatched within 2 days from roots of two tobacco cultivars in a tobacco field treated with two inorganic nitrogen fertilizer rates and three autumn cover treatments.

Treatment	Level of treatment	J2/100 cm^3 soil				J2/g root		
		4 June	3 July	25 July	23 August	4 June	3 July	25 July
Nitrogen	89 kg N	169	866	1,584	190	161	234	134
	158 kg N	114	832	1,691	127	47	168	88
Cultivars	Coker 371 Gold	162	1,263	2,425	210	141	283	112
	K-326	121	434	850	107	66	120	110
Cover treatments	Weeds	135	961	1,689	234	82	239	123
	Hairy indigo	204	810	1,494	120	177	170	149
	Forage sorghum	85	775	1,730	122	52	195	63
ANOVA	Block	NS	NS	NS	NS	NS	NS	**
	Nitrogen (N)	NS	NS	NS	NS	*	NS	NS
	Cultivar (C)	*	***	***	**	**	**	NS
	Cover treatments (Ct)	NS	NS	NS	NS	NS	NS	**
	N × C	NS	NS	NS	NS	NS	NS	NS
	N × Ct	NS	NS	NS	NS	*	NS	NS
	C × Ct	NS	NS	NS	NS	NS	NS	NS
	N × C × Ct	NS	NS	NS	NS	NS	NS	*

Data are the means of main effects. The values were transformed to $\log_{10}(x + 1)$ values before being subjected to ANOVA. *, **, *** represent $P \leq 0.05$, 0.01, 0.001, respectively. NS = not significant at $P \leq 0.05$.

mine the relationship between nematode density (transformed) or galling index (untransformed) with the number of nematode antagonists (transformed).

RESULTS AND DISCUSSION

The number of J2 in Coker 371 Gold plots was higher ($P \leq 0.05$) than that in plots planted with K-326 at all sampling dates. No effect of inorganic nitrogen fertilizer or autumn cover treatments was observed on the population density of J2 in soil. All three cultural practices affected ($P \leq 0.05$) the number of J2 hatched from roots (Table 1). There was an interaction between the inorganic nitrogen fertilizer and autumn cover treatments affecting the numbers of J2 hatched from tobacco roots (Table 2). The high rate of ammonium nitrate suppressed J2 hatched from roots in plots of hairy indigo sampled on 4 June. In plots treated with the low rate of ammonium nitrate, a higher number of J2 hatched from tobacco roots from weed and hairy indigo plots than from forage sorghum plots. No similar effect was found in plots treated with the high rate of ammonium nitrate. In the weed plots, there was an interaction between cultivar and inorganic nitrogen fertilizer affecting the number of J2 hatched from roots sampled on 25 July. High levels of inorganic nitrogen decreased the number of J2 hatched from K-326 tobacco roots (Table 3).

The weed and hairy indigo plots yielded higher initial numbers of *P. penetrans* endospores attached to J2 and resulted in a higher percentage of J2 encumbered with *P. penetrans* endospores in samples collected on 4 June (Table 4). A lower percentage of J2 from Coker 371 Gold compared with K-326 was observed to have *P. penetrans* endospores attached in samples from 3 July. The high rate of ammonium

TABLE 2. Interaction between nitrogen fertilizer levels and autumn cover treatments and their effects on the number of *Meloidogyne* spp. second-stage juveniles (J2) hatched over 2 days per gram tobacco root collected on 4 June.

Cover plant	J2/gram root	
	89 kg N	158 kg N
Weeds	131 aA	33 aA
Hairy indigo	314 aA	40 aB
Forage sorghum	38 bA	67 aA

The values were transformed to $\log_{10}(x + 1)$ values before being subjected to ANOVA and Duncan's multiple-range test. The same lowercase letter in columns or uppercase letter in rows indicate no significant differences at $P \leq 0.05$ according to Duncan's multiple-range test.

TABLE 3. Interaction between nitrogen fertilizer levels and tobacco cultivars and their effects on the number of *Meloidogyne* spp. second-stage juveniles hatched over 2 days per gram tobacco root collected from weed plots on 25 July.

	J2/gram root	
Cultivar	89 kg N	158 kg N
Coker 371 Gold	92 aA	138 aA
K-326	193 aA	67 aB

The values were transformed to $\log_{10}(x + 1)$ values before being subjected to a *t*-test. The same lowercase letter in columns or uppercase letter in rows indicate no significant differences at $P \leq 0.05$ according to the *t*-test.

TABLE 5. Interaction between inorganic nitrogen fertilizer levels and tobacco cultivars and their effects on numbers of *Pasteuria penetrans* endospores adhering to *Meloidogyne* spp. second-stage juveniles (J2) in soil 1 month after harvest of tobacco.

	Endospores/J2	
Cultivars	89 kg N	158 kg N
Coker 371 Gold	2.0 aA	2.7 aA
K-326	0.9 bB	3.8 aA

The values were transformed to $\log_{10}(100x + 1)$ values before being subjected to ANOVA. The same lowercase letter in columns or uppercase letter in rows indicate no significant differences at $P \leq 0.05$.

nitrate decreased the percentage of J2 that had *P. penetrans* endospores attached in samples from 4 June (Table 4). There was an interaction between inorganic nitrogen fertilizer and cultivar affecting the number of *P. penetrans* endospores attached to J2 in soil 1 month after harvest (Table 5). *Meloidogyne* spp. females collected from tobacco grown in plots treated with the high rate of ammonium nitrate yielded higher numbers of *P. penetrans* endospores than did females collected from plots treated with the low rate (Table 6).

Pasteuria penetrans was suppressive to the root-knot nematodes. The population density of J2 in soil on 3 July was negatively correlated with both percentage of J2 with *P. penetrans* endospores attached ($r = -0.51$, $P \leq 0.001$) and average number of *P. penetrans* endospores attached to the J2 ($r = -0.33$, $P \leq 0.05$). A negative correlation ($r = -0.46$, $P \leq 0.01$) was also observed between the population density of J2 in soil on 25 July and the percentage of J2 in soil with *P. penetrans* endospores attached in the 3 July sample. The number

TABLE 4. Percentages of second-stage juveniles (J2) of *Meloidogyne* spp. attached with *Pasteuria penetrans* endospores, and average numbers of *P. penetrans* endospores attached per J2 in soil collected from two tobacco cultivars in a field treated with two nitrogen fertilizer rates and three autumn cover treatments.

		Percentage of J2 with *P. penetrans* attached				Number of *P. penetrans* endospores attached per J2			
Treatment	Level of treatment	4 June	3 July	25 July	23 August	4 June	3 July	25 July	23 August
Nitrogen	89 kg N	55.6	51.3	71.5	39.6	3.5	3.2	10.1	1.4
	158 kg N	46.3	46.9	73.8	36.9	3.2	2.7	8.9	3.2
Cultivars	Coker 371 Gold	50.6	42.7	70.2	37.9	3.2	2.8	9.7	2.4
	K-326	51.2	55.4	75.1	38.6	3.6	3.1	9.2	2.3
Cover treatments	Weeds	57.5 a	47.8	75.3	41.6	3.8 a	1.7	10.6	2.7
	Hairy indigo	53.1 a	51.3	77.0	41.0	4.1 a	3.9	11.0	3.1
	Forage sorghum	42.1 b	48.2	65.6	32.2	2.2 b	3.2	6.8	1.2
ANOVA	Block	**	NS	*	NS	NS	NS	*	NS
	Nitrogen (N)	*	NS	NS	NS	NS	NS	NS	NS
	Cultivar (C)	NS	*	NS	NS	NS	NS	NS	NS
	Cover treatment (Ct)	**	NS	NS	NS	*	NS	NS	NS
	N × C	NS	NS	NS	NS	NS	NS	NS	*
	N × Ct	NS	NS	NS	NS	NS	NS	NS	NS
	C × Ct	NS	NS	NS	NS	NS	NS	NS	NS
	N × C × Ct	NS	NS	NS	NS	NS	NS	NS	NS

Data are means of main effects. The percentages were arcsin (x) transformed and the average number of endospores per juvenile were transformed to $\log_{10}(100x + 1)$ values before being subjected to ANOVA. *, **, *** represent $P \leq 0.05$, 0.01, 0.001, respectively. NS = not significant at $P \leq 0.05$. Means followed by different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple-range test.

TABLE 6. Average numbers of *Pasteuria penetrans* endospores produced per *Meloidogyne* spp. female taken from roots of two tobacco cultivars, percentages of nematode egg masses colonized by fungi, and galling indices of tobacco roots grown in a field treated with two nitrogen fertilizer rates and three autumn cover treatments.

Treatment	Level of treatment	Number of endospores (10^5/female)	Percentage of egg masses colonized by fungi	Galling index
Nitrogen	89 kg N	1.15	46.4	3.4
	158 kg N	1.71	57.5	3.2
Cultivars	Coker 371 Gold	1.48	59.3	4.1
	K-326	1.38	44.6	2.5
Cover treatments	Weeds	1.15	52.8	3.4
	Hairy indigo	2.00	49.0	3.4
	Forage sorghum	1.14	54.1	3.0
ANOVA	Block	NS	NS	NS
	Nitrogen	*	NS	NS
	Cultivar	NS	*	***
	Cover treatment	NS	NS	NS

Data are the means of main effects. The average numbers of endospores per females were transformed to $\log_{10}(x + 1)$ values and the percentage of egg masses colonized by fungi were arcsin (x) transformed before being subjected to ANOVA. *, **, *** represent $P \leq 0.05, 0.01, 0.001$, respectively. NS = not significant at $P \leq 0.05$. No significant interaction among the factors was observed.

The galling index scale: 0 = no galls, 1 = 1–10, 2 = 11–20, 3 = 21–55, 4 = 56–80, and 5 = 81–100% roots galled.

of J2 hatched from roots of the 25 July sample was negatively correlated ($r = -0.57$, $P \leq 0.05$) with the number of *P. penetrans* endospores produced per female in K-326 plots previously covered with weeds or hairy indigo.

The frequency of fungal colonization of egg masses was higher on Coker 371 Gold than that on K-326, but no significant difference was observed between the two inorganic fertilizer levels or among the autumn cover treatments (Table 5). *Paecilomyces lilacinus* was the predominant fungal species encountered in egg masses of *Meloidogyne* spp. (data not shown); however, no significant correlation was observed between the density of nematodes and the frequency of fungal colonization of the egg masses.

Although significant changes in density of *Meloidogyne* spp. were observed at times with all three cultural practices, a difference ($P \leq 0.001$) in galling indices was observed only between the two cultivars (Table 6). A negative correlation ($r = -0.42$, $P \leq 0.01$) was observed between the galling index of tobacco roots and percentage of J2 with *P. penetrans* endospores attached in soil 3 July. However, the overall density of *Meloidogyne* spp. in soil was relatively high in 1991. The above-ground symptoms associated with root-knot disease and root galling of tobacco were obvious in some plots, and a few plants died.

The density of *P. penetrans* endospores is important for suppression of its host nematode population. Our results show some indications that a higher nitrogen level may increase the density of *P. penetrans* on *Meloidogyne* infecting a resistant tobacco cultivar. More studies are needed, however, before we can conclude whether these tactics could be used in IPM programs.

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