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Evaluation of *Paecilomyces lilacinus* as a Biocontrol Agent of *Meloidogyne javanica* on Tobacco[1]

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Abstract: The efficacy of the nematode parasite *Paecilomyces lilacinus,* alone and in combination with phenamiphos and ethoprop, for controlling the root-knot nematode *Meloidogyne javanica* on tobacco and the ability of this fungus to colonize in soil under field conditions were evaluated for 2 years in microplots. Combinations and individual treatments of the fungus grown on autoclaved wheat seed, *M. javanica* eggs (76,000 per plot), and nematicides were applied to specified microplots at the time of transplanting tobacco the first year. Vetch was planted as a winter cover crop, and the fungus and nematicides were applied again the second year to specified plots at transplanting time. The fungus did not control the nematode in either year of these experiments. The average root-gall index (0 = no visible galls and 5 = > 100 galls per root system) ranged from 2.7 to 3.9 the first year and from 4.3 to 5.0 the second in nematode-infested plots treated with nematicides. Plants with *M. javanica* alone or in combination with *P. lilacinus* had galling indices of 5.0 both years; the latter produced lower yields than all other treatments during both years of the study. Nevertheless, the average soil population densities of *P. lilacinus* remained high, ranging from 1.2 to 1.3 × 10^6 propagules/g soil 1 week after the initial inoculation and from 1.6 to 2.3 × 10^4 propagules/g soil at harvest the second year. At harvest the second year the density of fungal propagules was greatest at the depth of inoculation, 15 cm, and rapidly decreased below this level.

Key words: biocontrol, ethoprop, 1,3-dichloropropene, fenamiphos, fungal egg parasite, *Meloidogyne javanica,* nematicide, *Nicotiana tabacum, Paecilomyces lilacinus,* root-knot nematode, tobacco, vetch, *Vicia villosa.*

Several biological control agents of *Meloidogyne* spp. have been reported (7–9,11,12,18,20–22), but until recently none had demonstrated effective suppression of nematode populations under field conditions. The fungus *Paecilomyces lilacinus* (Thom) Samson has been reported to be an effective biological control agent of these nematodes (4,6,11,12). Infestations of soil with the fungus in field and greenhouse experiments have been reported to limit numbers of root-knot nematode galls and increase plant yields (4,5,8,12,19). In these experiments, recovery of the fungus from nematode egg masses, females, and soil indicated that it is capable of establishing itself in soil, but its ability to colonize soil under field conditions has not been studied quantitatively.

The compatibility of *P. lilacinus* with ethylene dibromide was studied, but *P. lilacinus* failed to suppress the residual population of *Meloidogyne incognita* (Kofoid and White) Chitwood following applications of different rates of the fumigant (1). Sublethal doses of ethoprop were evaluated for enhancement of the parasitism of *M. incognita* by *Catenaria anguillulae* Sorokin (20); there was a synergistic effect between the nematicide and the fungus.

Our objectives were to evaluate the efficacy of combinations and individual treatments of *P. lilacinus* and the nematicides phenamiphos and ethoprop for control of *Meloidogyne javanica* (Neal) Chitwood on tobacco and to determine the ability of *P. lilacinus* to colonize soil and reduce nematodes over a 2-year period under field conditions.

MATERIALS AND METHODS

A 2-year study (1984–85) was conducted in 76-cm-d microplots encircled with 60-cm-wide fiberglass inserted 50 cm into the soil (14). The microplots were arranged in rows 1.2–1.5 m apart in an Arredondo fine sand (93% sand, 4% silt, 3% clay, 1% organic matter; pH 5.8) treated with 977 kg methyl bromide/ha (98% a.i.) applied un-

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der 3-mil polyethylene 1 month before planting. No phytoparasitic nematodes were detected when the microplots were sampled before planting. Each treatment was replicated seven times in a randomized complete block design.

Paecilomyces lilacinus obtained from the International Potato Center, Lima, Peru, was grown on PDA at 26 C in test tubes. Inoculum of *P. lilacinus* was produced on an autoclaved mixture of 100 g wheat seed and 100 ml tap water in a 1-liter wide-mouth flask. A conidial suspension, obtained by washing 20-day-old cultures of the fungus on PDA with 10 ml autoclaved water, was added to the autoclaved wheat seed. The flasks were kept at 25 C for 12 days and shaken vigorously daily to insure uniform growth of the fungus. Each fungus-inoculated plot received the contents of one flask.

A local population of *M. javanica* was cultured in the greenhouse on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). The nematode inoculum consisted mainly of eggs (> 98%) extracted from tomato roots washed with 0.5% sodium hypochlorite (9), and plots were inoculated at a rate of 120 eggs/100 cm^3 soil (76,000 per microplot).

Treatments during the first year consisted of inoculations with the fungus alone and in combination with ethoprop applied at the rate of 0.45 g a.i./m^2 (4.5 kg a.i./ha broadcast) and 0.9 g/m^2 (9.0 kg a.i./ha broadcast) and fenamiphos applied at the rate of 0.34 g a.i./m^2 (3.4 kg a.i./ha broadcast) and 0.68 g a.i./m^2 (6.8 kg a.i./ha broadcast) in plots infested with nematodes. Controls included untreated, nematode only, nematode inoculum plus 1,3-dichloropropene applied at the rate of 14 ml a.i./m^2 (140 liters a.i./ha broadcast), ethoprop and fenamiphos alone (rates as with fungus), autoclaved wheat seed alone, and autoclaved fungus-infested wheat seed. Fungal and nematode inoculum and nonfumigant nematicides were incorporated with a rake to a depth of 15 cm into microplots. Two plants of root-knot susceptible tobacco (*Nicotiana tabacum* L. cv. NC 2326) were transplanted 25 cm apart into each microplot in April. Hairy vetch (*Vicia villosa* Roth) was seeded into microplots during October (2.6 g seed/plot) as a cover crop to further enhance the increase of *P. lilacinus* populations for the next tobacco crop. Furthermore, egg masses of *M. javanica* formed overwinter would be vulnerable to infection by *P. lilacinus.*

The second year's treatments which included ethoprop at 9.0 kg a.i./ha and fenamiphos at 6.8 kg a.i./ha were applied at planting into plots that had received ethoprop at 4.5 kg a.i./ha and fenamiphos at 3.4 kg a.i./ha the previous year. Fungus inoculum was applied to plots receiving 1,3-dichloropropene and nematodes the previous year, and the remaining plots were left untreated. Tobacco was planted in April as in the previous year. Fertilizer applications to simulate local agronomic practices were used for tobacco and vetch. The microplots were weeded and irrigated and the plants were sprayed for pest control as needed. Auxiliary and terminal buds of tobacco were removed as necessary.

Soil samples for estimating populations of *P. lilacinus* were taken from treatments indicated (Fig. 1) at 6, 43, and 93 days after planting tobacco the first year, at 2 days preplant and 82 and 142 days after planting vetch, and at planting and 49 and 94 days after planting tobacco the second year. Fungal populations were monitored in the soil by bulking three 15-cm-deep cores (2.5-cm-d) collected from each plot. A 1-g subsample from each bulk sample was serially diluted in deionized water. One milliliter of soil–water suspension was pipetted into an empty, sterile petri dish (10 dishes per sample), and approximately 15 ml of a medium selective for *P. lilacinus* were poured into each of the petri dishes (15). The dishes were swirled gently to distribute the sample within the medium. Petri dishes were incubated at 25–27 C under 12 hours of light for 7–10 days, and the number of fungal colonies per dish was recorded.

Colonization of root segments and of root-knot nematode galls and egg masses by *P. lilacinus* was assessed 142 days after planting vetch and 49 days after planting

tobacco the second year. Presence of the fungus on root segments and on root-knot nematode galls and egg masses was assessed on one tobacco or three vetch plants per plot from treatments indicated in Table 3. Roots were washed in tap water and then soaked in a solution of 50 μg/ml chlortetracycline and 100 μg/ml streptomycin sulfate in sterilized water. One hundred root sections per sample, with or without root-knot nematode galls, were then plated on the selective medium, and presence or absence of the fungus was recorded.

Numbers of *M. javanica* juveniles per 250 cm^3 soil were estimated 43 and 93 days after planting tobacco the first year and at planting and 49 and 94 days after planting tobacco the second year. Soil samples for nematode analyses were taken with a 2.5-cm-d cone-shaped auger; five cores were collected from each microplot and bulked. Soil (250 cm^3) was processed by a modified centrifugation-flotation technique (13). A root-gall index was determined immediately after the final tobacco harvest each year (0 = no visible galls, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 = > 100 galls per root system).

Depth samples from five plots with fungus and nematode inoculum were taken 16 July 1985 with a 10-cm-d bucket auger at 15-cm intervals to a depth of 75 cm. Soil cores from each plot at each depth were bulked, and populations of fungi and nematodes were assayed as described. Tobacco leaves were harvested as they matured, and the green weight was recorded.

Data from treatments were subjected to analysis of variance, and treatment means were compared by Duncan's multiple-range test. Data from monitoring *P. lilacinus* populations in the soil were subjected to analysis of variance, and Duncan's multiple-range test. A single degree of freedom contrast was used to test for significance of treatments inoculated with the fungus vs. treatments not inoculated with the fungus.

Results

There were no differences in yield and gall rating between treatments with the nematode alone and those with nematodes plus fungus ($P = 0.05$) (Table 1). At the first sampling date, however, fewer juveniles were recovered from plots receiving nematodes plus fungus than from the control with nematodes alone ($P = 0.05$). Both of these treatments resulted in lower yields and higher gall ratings than did all other treatments ($P = 0.05$). Nonfumigant nematicides limited yield loss and galling caused by *M. javanica* compared with the treatment with nematodes alone. There were no differences in plots infested with nematodes treated with nonfumigant nematicides alone and those treated with the nematicides and the fungus. Except for plots treated with ethoprop at 9.0 kg a.i./ha, the highest numbers of *M. javanica* juveniles were recovered at harvest from treatments that included nonfumigant nematicides. Plots treated with autoclaved wheat seed, with or without fungus and without nematodes, produced yields equal to the untreated control.

At the end of the second (1985) tobacco test, no combination of nematicides, fungus, and nematodes applied in 1984 or 1985 produced yields or gall ratings different from those in soil treated with the nematodes alone. In 1985 the yields of controls with no nematode inoculum were greater than yields from plots with the nematode alone and nematode plus fungus in 1984 or from plots with the fungus in 1985 ($P = 0.05$). Plots treated with the nematode plus the fungus in 1984 produced yields in 1985 that were not different from yields from the treatment with the nematode alone ($P = 0.05$).

There were no differences in densities of fungal propagules in the soil among treatments receiving *P. lilacinus* in samples taken either year (Fig. 1). The initial soil samples had fungus population densities of $1.2–1.3 \times 10^6$ propagules/g soil. The population of *P. lilacinus* that recolonized in treatments not inoculated with the fungus was $2.0–3.8 \times 10^3$ propagules/g soil. Population density in the fungus-inoculated plots decreased over the 2-year period (Fig. 1), and inoculated and uninoculated treat-

TABLE 1. Yield of tobacco, soil populations of *Meloidogyne javanica* juveniles at midseason and harvest, and root-knot gall ratings in microplots treated with *Paecilomyces lilacinus*† and nematicides, 1984.

Treatment	Yield‡ (g)	Nematodes/250 cm³ soil		Gall rating§
		13 June	20 July	
Control, autoclaved wheat only	1,976 a	0 c	0 d	0.9 f
Control, fenamiphos, 6.8 kg a.i./ha	1,973 a	0 c	0 d	0.1 g
Control, autoclaved fungus	1,908 ab	0 c	0 d	0.0 g
Control, fungus	1,875 abc	0 c	0 d	0.5 fg
Control, ethoprop, 4.5 kg a.i./ha	1,865 abc	0 c	0 d	0.0 g
Control, fenamiphos, 3.4 kg a.i./ha	1,843 abcd	0 c	0 d	0.1 g
Nematode + fungus + fenamiphos, 6.8 kg a.i./ha	1,799 abcd	40 b	1,188 b	3.1 de
Control, untreated	1,752 abcd	0 c	0 d	0.1 g
Control, ethoprop, 9.0 kg a.i./ha	1,706 abcde	0 c	0 d	0.0 g
Nematode + fenamiphos, 6.8 kg a.i./ha	1,652 bcde	305 ac	719 bc	3.9 b
Nematode + fenamiphos, 3.4 kg a.i./ha	1,644 bcde	386 a	692 bc	3.5 bcd
Nematode + ethoprop, 4.5 kg a.i./ha	1,635 bcde	47 bc	2,073 b	3.9 b
Nematode + fungus + fenamiphos, 3.4 kg a.i./ha	1,608 bcde	299 a	3,089 a	3.8 bc
Nematode + fungus + ethoprop, 9.0 kg a.i./ha	1,580 cde	44 bc	1,514 b	2.7 e
Nematode + ethoprop, 9.0 kg a.i./ha	1,541 de	28 bc	221 c	3.1 de
Nematode + fungus + ethoprop, 4.5 kg a.i./ha	1,541 de	51 bc	1,603 b	3.2 de
Nematode + 1,3-dichloropropene, 140 liters a.i./ha	1,420 e	0 c	317 c	2.8 e
Nematode + fungus	998 f	114 b	379 c	5.0 a
Control, nematode	932 f	391 a	374 c	5.0 a

Means with the same letter in vertical columns are not significantly different according to Duncan's new multiple-range test ($P = 0.05$).

† The fungus on wheat was added to microplots.

‡ Green weight yield from two plants per plot.

§ Gall rating averaged from two plants per plot based on the following index: 0 = no visible galls, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 = > 100 galls per root system.

ments had population densities of 1.6–2.3 × 10^4 and 1.9–4.9 × 10^2 propagules/g soil, respectively, at harvest the second year.

The highest fungus populations in plots treated with the fungus plus nematodes were at the 0–15-cm depth at the end of the 1985 tobacco test (Table 2). The fungal population density decreased rapidly with depth, and the fungus was not isolated below 30 cm deep in two of four replicates sampled. Root-knot nematode juveniles were found at all of the depths sampled.

The fungus successfully colonized galls and egg masses of *M. javanica* on vetch roots in the treatment with fungus plus nematodes (Table 3). In one plot 90 of 100 galls or egg masses were colonized by the fungus. Galls and root segments from tobacco transplanted into the same treatment had lower rates of infestation at midseason. Only 20 of 100 galls colonized the highest proportion recorded. A recolonized fungus population was found in a low percentage of both vetch and tobacco root galls in the treatment inoculated with nematodes alone. A very low percentage of vetch root segments and no tobacco root segments treated with the fungus alone were colonized by the recolonizing fungus population.

DISCUSSION

Applications of *P. lilacinus*-infested wheat seed resulted in fungus soil population densities more than 400 times greater than the recolonized population of *P. lilacinus* in uninoculated fumigated soil. Neither ethoprop or fenamiphos affected the densities of the fungus in soil. The population densities of *P. lilacinus* in the inoculated plots did not control *M. javanica*, and no additive or synergistic activity was observed in any of the fungus plus nematicide treatments. *Paecilomyces lilacinus* also failed to suppress populations of *M. incognita* and improve yields of okra or cotton grown in microplots treated with different rates of ethylene dibromide (1).

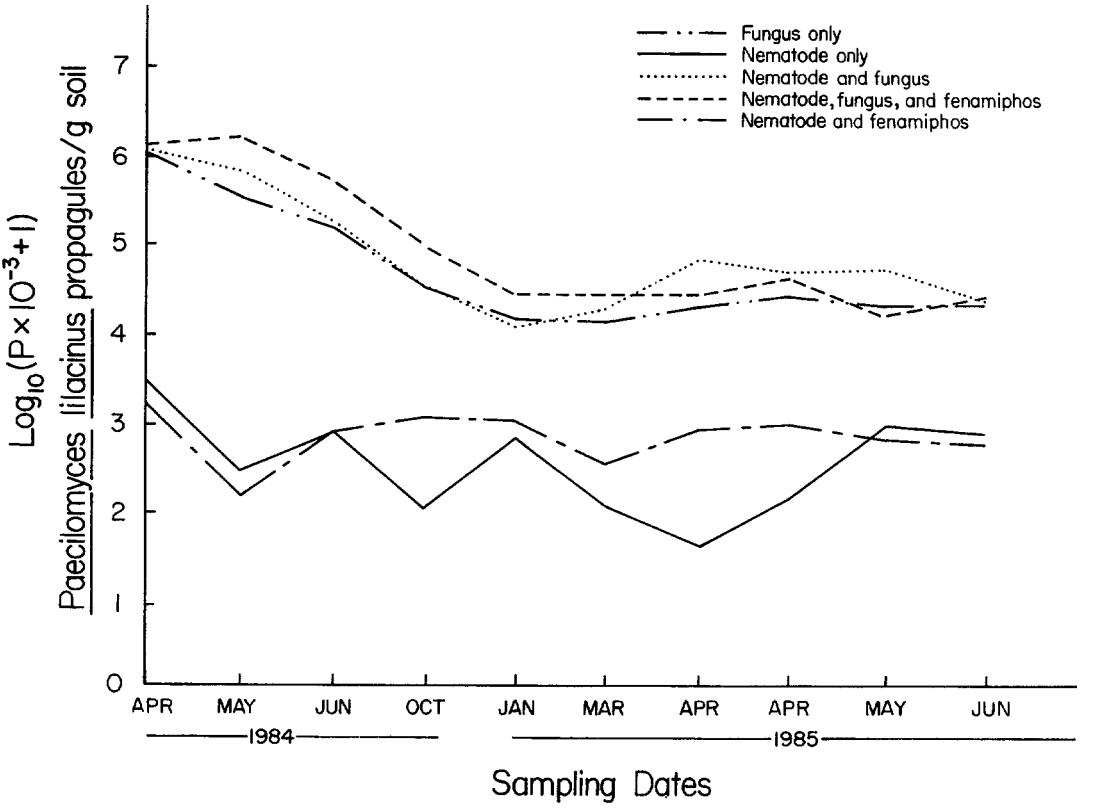

FIG. 1. Population dynamics of *Paecilomyces lilacinus* in the soil in treatments with and without *P. lilacinus* inoculum added (100 g infested wheat seed) 16 April 1984. Contrasts between treatments with and without fungus inoculum were different at all sampling times ($P = 0.05$).

The difference in the ability of *P. lilacinus* to colonize root galls of vetch and tobacco may be due to differences in the type of root system. The tobacco root systems at midseason extended well beyond the 15-cm level of fungus inoculation. The low number of fungal propagules detected on root segments and at soil levels below 15 cm deep indicate that *P. lilacinus* was unable to colonize the rhizosphere of tobacco roots and did not move down through the soil with the roots. Galls and egg masses collected from tobacco roots below the 15-cm level, therefore, were not exposed to a high population of the fungus. This may have caused the lower degree of colonization detected, compared with shallow-rooted vetch plants. The type of root system and depth of soil inoculation may play roles in the ability of this fungus, as well as other biological control organisms, to give adequate control of root-knot nematodes.

There have been a few assertions of *P. lilacinus* controlling *M. incognita* and *Tylen-*

TABLE 2. Numbers of *Paecilomyces lilacinus* propagules and *Meloidogyne javanica* juveniles recovered from soil samples taken at harvest in 1985 from five depths in plots infested with the fungus and nematodes in 1984.

Depth of sample (cm)	Propagules/g soil†	Nematodes/ 250 cm³ soil‡
0–15	55,600 (± 11,623)§	352
16–30	9,600 (± 1,758)	180
31–45	1,000 (± 600)	88
46–60	400 (± 231)	112
61–75	600 (± 383)	460

† Average number of propagules from four replicates.
‡ Soil sample from four replicates bulked to make one composite sample at each depth.
§ Standard error of the mean.

TABLE 3. Infestation (percentages) by *Paecilomyces lilacinus* from 100 nematode galls or root segments with egg masses of vetch at harvest and of tobacco at midseason, 1985.

Treatment	Vetch†		Tobacco‡	
	Average	Range	Average	Range
Nematode (galls)	4.0	0–23.0	0.3	0–1.0
Fungus (root segments)	0.1	0–4.0	0.0§	
Fungus + nematode (galls)	53.0	8.0–90.0	7.1	0–20.0

† Samples from three plants taken from each of five replicates of each treatment.
‡ Samples from one plant taken from each of three replicates of each treatment.
§ Average from 50 root segments.

chulus semipenetrans Cobb in on-farm trials (12). Other studies under greenhouse or microplot conditions have shown a suppression of root galling or reduced yield losses in *P. lilacinus* treatments (1,2,5). The lack of control of *M. javanica* by *P. lilacinus* in our test even after 2 years of exposure may be due to differences in species of *Meloidogyne*, hosts, or isolates of *P. lilacinus*, or to the amount and type of carrier of the fungus. In *M. javanica*, we chose the most aggressive root-knot nematode species among those that infect tobacco (3). More work under field conditions will be necessary to determine whether *P. lilacinus* is effective in the biological control of different species of root-knot nematodes. In this study, lack of control of *M. javanica* was not attributed to the inability of the fungus to invade soil. Future research should include the establishment of delivered antagonists in soil as well as their abilities to colonize roots, nematodes, and galls. The future of *P. lilacinus* as a potential commercial control agent is compromised, however, by its ability to act as a pathogen of humans and other animals with injuries or immune deficiencies (2,16,17).

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