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Biological Control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*[1]

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Abstract: The root-knot nematode *Meloidogyne incognita* was controlled more effectively and yields of host plants were greater when *Paecilomyces lilacinus* and *Pasteuria penetrans* were applied together in field microplots than when either was applied alone. Yields of winter vetch from microplots inoculated with the nematode and with both organisms were not statistically different from yields from uninoculated control plots.

Key words: Bacillus penetrans, bacterial spore parasite, biocontrol, fungus parasite, southern root-knot nematode, *Meloidogyne incognita, Paecilomyces lilacinus, Pasteuria penetrans.*

Paecilomyces lilacinus (Thom) Samson and *Pasteuria penetrans* (Thorne) Sayre and Starr have been reported to provide some control of one or more species of *Meloidogyne. Paecilomyces lilacinus,* a common soil hyphomycete with a cosmopolitan distribution, parasitizes eggs of *M. incognita* (Kofoid and White) Chitwood and *Globodera pallida* (Stone) Behrens (2,4–6). *Pasteuria penetrans* is a prokaryotic endoparasite (10) of juveniles of *M. incognita.* Its spores attach to the cuticle of second-stage juveniles in the soil resulting in diseased female nematodes which reproduce little or not at all at maturity (8,9).

Greenhouse and microplot experiments were designed to determine if *P. lilacinus* and *P. penetrans* acting together would reduce population densities of *M. incognita* and hence result in better plant growth than would occur with either organism acting alone.

MATERIALS AND METHODS

Greenhouse experiments: Three experiments were conducted using tomato, *Lycopersicon esculentum* Miller cv. Rutgers; tobacco, *Nicotiana tabacum* L. 'NC 2326'; and pepper, *Capsicum annuum* L. cv. California Wonder, as host plants for *M. incognita.* In each of these experiments we used 15-cm-d clay pots containing 800 cm^3 steam-sterilized Arredondo fine sand (90.6% sand, 3.9% silt, 5.5% clay with 1.9% organic matter). The tomato and tobacco experiments were repeated once, and the pepper experiment was repeated twice. The eight treatments, each replicated six times, were 1) *M. incognita* + *P. lilacinus,* 2) *M. incognita* + *P. penetrans,* 3) *M. incognita* + *P. lilacinus* + *P. penetrans,* 4) *M. incognita* only, 5) *P. lilacinus* only, 6) *P. penetrans* only, 7) *P. lilacinus* + *P. penetrans,* and 8) untreated control. An isolate of the fungus *P. lilacinus,* from the International Potato Center (CIP) in Peru, designated *P. lilacinus* CIP-1, was cultured and distributed on autoclaved wheat seeds. One hundred grams of wheat seed free of any pesticide treatment was placed in each of two 500-ml Erlenmeyer flasks and soaked in water overnight. Then the water was drained off, and each flask was closed with a cotton plug and placed in an autoclave for 15 minutes at 15 psi. After the flasks and contents cooled, *P. lilacinus* as a mycelial mat growing on PDA agar was added aseptically to one flask; the other flask served as an uninoculated control. The flasks were incubated at 25–30 C for 10 days and shaken periodically to better distribute the fungus and to prevent the seeds from sticking together. Four grams of the fungus-infected wheat seed containing 4×10^7 conidia was added to all treatments containing *P. lilacinus* (treatments 1, 3, 5, 7) and incorporated into the soil. One-half gram of dried and finely ground tomato roots which had been grown in soil heavily infested with *M. incognita* and

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P. penetrans (12,13) was added to all treatments containing *P. penetrans* (treatments 2, 3, 6, 7) and mixed with the soil. All treatments not receiving *P. lilacinus* (treatments 2, 4, 6, 8) received 4 g fungus-free sterilized wheat seed. Immediately following the addition of the fungal and bacterial inoculum, 10,000 eggs of *M. incognita* were injected into the soil of treatments 1, 2, 3, and 4. All three experiments were maintained in a greenhouse for 60 days at an average air temperature of 30 C. Plants then were removed from the soil, and the roots were washed and rated for galling using the root gall index scale of 1–5 with 1 = no galls, 2 = 1–25% of roots with galls, 3 = 26–50% with galls, 4 = 51–75% with galls, and 5 = over 75% with galls. Numbers of egg masses per 0.5 g of root system were counted, and the percentage of eggs in the egg masses that hatched was determined and recorded for all three greenhouse experiments by placing 200 freshly extracted eggs (3) into vials containing aerated water and incubating them at 28 C for 24 hours. The above three criteria (i.e., root gall index, number of egg masses, and percentage of egg hatch) were used to indicate how effectively *M. incognita* was controlled by *P. lilacinus* and *P. penetrans*.

The statistical analysis of variance (ANOVA) and Waller-Duncan K-ratio *t*-test at $P = 0.05$ were used to analyze the pooled results of the experiments.

Microplot experiments: Soybean, *Glycine max* (L.) Merrill cv. Hood, and winter vetch, *Vicia villosa* Roth, were used as host plants for *M. incognita*. Twelve 2.4 × 0.9-m concrete-sided rectangular plots containing Arredondo fine sand (90.6% sand, 3.9% silt, 5.5% clay with 1.9% organic matter) to a depth of 60 cm were used. These had been used in a previous test in which four plots were infested with *M. incognita*, four with *M. incognita* and *P. penetrans*, and four (the controls) contained neither *M. incognita* nor *P. penetrans* (1). Each of these 12 plots was divided into two plots 1.2 × 0.9 m (1.08 m^2). This resulted in four replicates of each treatment in which *M. incognita* was present and two replicates of each treatment in which *M. incognita* was absent. The fungus inoculum at 40 g/plot was incorporated into the top 15 cm of the soil of all plots receiving *P. lilacinus* (treatments 1, 3, 5, 7). The same quantity of autoclaved and incubated wheat seed without the fungus was added to all other treatments. The eight treatments were the same as in greenhouse tests; each was replicated four times.

Seeds of soybean were planted on 25 May 1983 in two 1.2-m rows spaced 40 cm apart. The experiment was terminated on 17 October 1983, 146 days after planting. The entire plant tops were weighed fresh, and the beans were shelled, dried, and weighed when the seed moisture content was 9.4%.

The initial, mid-season, and final soil population densities of the nematode were determined from 100 cm^3 soil composed of six subsamples taken randomly from each plot and processed by a centrifugal flotation technique (7).

Winter vetch: Soil samples were taken from the harvested soybean plots (described above) on 1 January 1984 and winter vetch seeds were planted broadcast. No additional fungal or bacterial inocula were added. On 11 April 1984, 102 days after planting, winter vetch tops were cut at ground level and oven-dried at 75 C to constant weight and weights recorded. As in the soybean test, soil samples were taken at mid-season and at harvest to determine population densities of the nematode.

Data were analyzed as in the previous test, and in addition, significant differences in nematode population densities initially, at mid-season, and at harvest were determined by performing tests on the slope of a regression line using a simple regression equation (11).

Results

Greenhouse experiments: In all experiments, root gall indices in treatments containing *M. incognita* and either *P. lilacinus* or *P. penetrans* or both *P. lilacinus* and *P. penetrans* did not differ significantly from each other or from treatments not containing *M. incognita*; however, all of those root gall indices were significantly lower

TABLE 1. Effect of *Paecilomyces lilacinus* and *Pasteuria penetrans* on root gall index, egg mass count, and egg hatch of *Meloidogyne incognita* on tomato, *Lycopersicon esculentum* cv. Rutgers.

Treatment	RGI†	EMC‡	% egg hatch
1. *M. incognita* + *P. lilacinus*	1.5 a	12 a	38 a
2. *M. incognita* + *P. penetrans*	1.5 a	13 a	66 b
3. *M. incognita* + *P. lilacinus* + *P. penetrans*	1.5 a	11 a	40 a
4. *M. incognita* only	4.8 b	32 b	79 c
5. *P. lilacinus* only	1.0 a		
6. *P. penetrans* only	1.0 a		
7. *P. penetrans* + *P. lilacinus*	1.0 a		
8. Untreated control	1.0 a		

Values shown are the means of two experiments.
Means followed by the same letter in each column are not significantly different ($P = 0.05$) according to the Waller-Duncan K-ratio *t*-test.
† Root gall index (1 = no galling, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, 5 = over 75% of roots galled).
‡ Egg mass count per 0.5 g of root system.

than those in treatments containing *M. incognita* only (Tables 1–3). Similarly, the numbers of egg masses in treatments containing *M. incognita* and either *P. lilacinus* or *P. penetrans* or both in the tomato and tobacco experiments were significantly lower than in treatments containing *M. incognita* only. Numbers of egg masses in the pepper experiment followed the trend described for tomato and tobacco, but treatments containing *M. incognita* and either *P. lilacinus* or *P. penetrans* or both were significantly different. Furthermore, treatments containing *M. incognita* and *P. lilacinus* contained the fewest egg masses, followed by treatments containing *M. incognita* and *P. penetrans*; *M. incognita, P. lilacinus,* and *P. penetrans*; and *M. incognita* only.

In all experiments, fewer eggs hatched in treatments containing either *P. lilacinus* or *P. penetrans* or both than in treatments with *M. incognita* only. In the tomato and tobacco experiments, however, the percentages of eggs that hatched were significantly lower in treatments containing *P. lilacinus* with or without *P. penetrans* than in the treatments containing *P. penetrans* only. In the pepper experiment, the percentage of eggs that hatched was higher in treatments containing both *P. lilacinus* and *P. penetrans* than in treatments containing either *P. lilacinus* or *P. penetrans.*

Data were not collected for weights of tomato plants but were collected for the tobacco and pepper experiments. Fresh weights of the tops of tobacco plants were significantly greater in all treatments containing *M. incognita* in the presence of one

TABLE 2. Effect of *Paecilomyces lilacinus* and *Pasteuria penetrans* on root gall index, egg mass count, and egg hatch of *Meloidogyne incognita,* and top weights of tobacco, *Nicotiana tabacum* NC 2326.

Treatment	RGI†	EMC‡	% egg hatch	Fresh top weight (g)
1. *M. incognita* + *P. lilacinus*	1.5 a	12 a	44 a	183 c
2. *M. incognita* + *P. penetrans*	2.0 a	13 a	65 b	131 b
3. *M. incognita* + *P. lilacinus* + *P. penetrans*	1.5 a	10 a	44 a	172 c
4. *M. incognita* only	4.8 b	29 b	73 c	35 a
5. *P. lilacinus* only	1.0 a			187 c
6. *P. penetrans* only	1.0 a			189 c
7. *P. penetrans* + *P. lilacinus*	1.0 a			187 c
8. Untreated control	1.0 a			188 c

Values shown are the means of two experiments.
Means followed by the same letter in each column are not significantly different ($P = 0.05$) according to the Waller-Duncan K-ratio *t*-test.
† Root gall index (1 = no galling, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, 5 = over 75% of roots galled).
‡ Egg mass count per 0.5 g of root system.

TABLE 3. Effect of *Paecilomyces lilacinus* and *Pasteuria penetrans* on reproduction of *Meloidogyne incognita* on pepper, *Capsicum annuum* cv. California Wonder.

Treatment	RGI†	EMC‡	% egg hatch	Fresh top weight (g)
1. *M. incognita* + *P. lilacinus*	1.6 a	7.5 a	30.8 a	261.9 c
2. *M. incognita* + *P. penetrans*	1.6 a	10.0 b	34.8 a	212.8 b
3. *M. incognita* + *P. lilacinus* + *P. penetrans*	2.1 a	14.0 c	54.6 b	219.0 b
4. *M. incognita* only	4.8 b	28.8 d	76.8 c	79.1 a
5. *P. lilacinus* only	1.0 a			286.1 c
6. *P. penetrans* only	1.0 a			283.6 c
7. *P. penetrans* + *P. lilacinus*	1.0 a			285.3 c
8. Untreated control	1.0 a			284.8 c

Values shown are the means of three experiments.
Means followed by the same letter in each column are not significantly different ($P = 0.05$) according to the Waller-Duncan K-ratio *t*-test.
† Root gall index (1 = no galling, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, 5 = over 75% of roots galled).
‡ Egg mass count per 0.5 g of root system.

or both biological control organisms than when *M. incognita* was alone. Additionally, weights of plants from pots containing *M. incognita* and *P. lilacinus* or *M. incognita* and *P. lilacinus* plus *P. penetrans* did not differ from each other, or from those treatments not containing *M. incognita*, but were significantly greater than weights of plants from pots containing *M. incognita* and *P. penetrans*. Weights of the tops of pepper plants were significantly greater when one or both organisms were present with the nematode than when the nematode was alone. Weights of plants from pots containing *M. incognita* and *P. lilacinus* were similar to those from plots without the nematode but greater than those from pots containing the nematode and *P. penetrans* or the nematode and both *P. lilacinus* and *P. penetrans*.

Microplot experiments: Total top weights and seed yields of soybean in treatments containing *M. incognita* and both *P. lilacinus* and *P. penetrans* were significantly greater than those in treatments containing *M. incognita* and either organism alone but not as great as from the untreated controls (Table 4). Further, plots containing *M. incognita* and *P. penetrans* yielded more than did plots containing *M. incognita* and *P. lilacinus*. Yields from treatments containing *M. incognita* and either *P. lilacinus* or *P. penetrans* or both were 172%, 212%, and 260%, respectively, of yields from plots

TABLE 4. Effect of *Paecilomyces lilacinus* and *Pasteuria penetrans* on soil population densities of *Meloidogyne incognita* and the top weights and seed yield of soybean, *Glycine max* cv. Hood, in microplots.

Treatment	Soil nematode populations/ 100 cm³ soil			Soybean	
	Pi†	Pm†	Pf†	Top dry weight (g)‡	Seed yield (g)
1. *M. incognita* + *P. lilacinus*	464	264 c	172 d	325 b	260 b
2. *M. incognita* + *P. penetrans*	244	192 b	144 c	419 c	321 c
3. *M. incognita* + *P. lilacinus* + *P. penetrans*	244	96 a	92 a	432 d	393 d
4. *M. incognita* only	464	808 d	1,064 e	189 a	151 a
5. *P. lilacinus* only	0	0	0	570 e	518 e
6. *P. penetrans* only	0	0	4	573 e	516 e
7. *P. lilacinus* + *P. penetrans*	0	0	4	572 e	518 e
8. Untreated control	0	1.2	5.2	572 e	518 e

Values shown are the means of four replicates.
Means followed by the same letter in each column are not significantly different ($P = 0.05$) according to the Waller-Duncan K-ratio *t*-test.
† Initial, mid-season (72 days), and final (146 days) population densities.
‡ Top weights include seed.

TABLE 5. Effect of *Paecilomyces lilacinus* and *Pasteuria penetrans* on soil population densities of *Meloidogyne incognita* and top dry weight of winter vetch, *Vicia villosa*, in microplots.

Treatment	Soil nematode population densities/ 100 cm³ soil			Top dry weight (g)
	Pi†	Pm†	Pf†	
1. *M. incognita* + *P. lilacinus*	152	84 a	68 c	373 b
2. *M. incognita* + *P. penetrans*	136	108 b	64 a	375 b
3. *M. incognita* + *P. lilacinus* + *P. penetrans*	120	72 a	40 b	418 c
4. *M. incognita* only	552	636 c	692 d	171 a
5. *P. lilacinus* only	0	2	4	413 c
6. *P. penetrans* only	0	0	0	418 c
7. *P. lilacinus* + *P. penetrans*	0	0	0	387 c
8. Untreated control	2	2	4	396 c

Values shown are the means of four replicates.

Means followed by the same letter in each column are not significantly different ($P = 0.05$) according to the Waller-Duncan K-ratio *t*-test.

† Initial, mid-season (50 days), and final (102 days) population densities.

containing *M. incognita* only, but were only 50%, 62%, and 76%, respectively, as much as yields from untreated control plots. Soil nematode populations showed significant downward trends as the season progressed in treatments containing *M. incognita* and either one or both biocontrol organisms but a significant upward trend in treatments containing *M. incognita* only.

Yields of winter vetch were significantly greater in treatments containing *M. incognita* and either or both organisms than in treatments containing *M. incognita* only (Table 5). Further, yields from plots containing *M. incognita* and both organisms were not statistically different from those treatments (untreated control) not containing *M. incognita*. Yields of treatments containing *M. incognita* and either *P. lilacinus* or *P. penetrans* or both were 218%, 219%, and 243%, respectively, of yields from plots with *M. incognita* only, and were 94%, 95%, and 100%, respectively, of yields from untreated control plots. Nematode population densities showed the same trend as observed in the soybean test.

DISCUSSION

P. lilacinus suppressed root galls, number of egg masses, and egg hatch in greenhouse tests. The extent to which *P. lilacinus* reduced egg hatch is particularly striking, but not surprising because *P. lilacinus* is an egg parasite (4,5). In microplots where *P. lilacinus* was applied, yields of soybean and winter vetch were increased by 172% and 218% over the yields in plots containing *M. incognita* only. These yield increases represent 50% of the soybean and 94% of the winter vetch yields in the untreated control plots. Jatala et al. (4) reported increased yields of potatoes when *P. lilacinus* was applied to control *M. incognita* and *Globodera pallida*. In our tests, the initial introduction of *P. lilacinus* to plots infested with *M. incognita* increased yields of soybeans by 172% and of winter vetch by 218% in the subsequent test in the same plots without reapplication of the fungus. The greater increase of vetch probably was the direct result of the progressive reduction of soil populations of *M. incognita* following the application of *P. lilacinus*. According to Jatala et al. (6), *P. lilacinus* has the ability to reduce population densities of *M. incognita* progressively with succeeding generations and without reapplication of the fungus.

These results confirm that *P. penetrans* suppressed root galling and egg mass production by *M. incognita* and resulted in greater yields in both greenhouse and microplot experiments. To a lesser extent, *P. penetrans* also reduced the percentage hatch of eggs of *M. incognita*. The reduction of root galling confirms earlier reports (8,9) in which greenhouse tomatoes inoculated with *M. incognita* had fewer galls on roots

grown in soil containing *P. penetrans* than in soil without *P. penetrans*. In microplot tests, the application of *P. penetrans* substantially increased yields of soybean and winter vetch, confirming the report of Stirling (12) who observed that *P. penetrans* significantly reduced populations of *M. javanica*. Soybean yields were 212% greater in plots containing *P. penetrans* and *M. incognita* than in plots containing *M. incognita* only. This was 62% of the yield from the untreated control plots. Similarly, in the winter vetch test, yields from plots containing *P. penetrans* and *M. incognita* were 219% greater than the yields from plots containing *M. incognita* only. This yield increase, representing 96% of the yield from untreated control plots, is comparable to that often achieved with nematicides.

Nematode population densities in the microplots after the harvest of soybeans (Pf, Table 4) were greater than the initial densities (Pi, Table 5) when winter vetch was planted. There was a period of 2.5 months between the harvest of soybean and the seeding of vetch. Also, we experienced an unusually long period of cold weather, with low temperatures of −9, −10, and −3 C on 25, 26, and 27 December, and −1, −4, −2, +3, −2, +2, −4, and 0 C on 30 December through 7 January. The decrease in population densities probably was due to both lack of host plants for 2.5 months and the unusual low temperatures.

In microplot experiments, crop yields were greater and nematode population densities were less when both biocontrol organisms were used together than when either was used alone. This was expected since each one attacks different life stages of the nematode. *Pasteuria penetrans* attacks second-stage juveniles, killing some of them; those that survive and become adult females produce few or no eggs, but instead their bodies become filled with spores of *P. penetrans*. *Paecilomyces lilacinus* attacks eggs and sometimes adult females and therefore should reduce nematode population densities and plant damage to a greater extent than would either organism alone. Our report appears to be the first on the combined use of two biocontrol organisms to control a nematode.

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