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Impact of *Paecilomyces lilacinus* Inoculum Level and Application Time on Control of *Meloidogyne incognita* on Tomato[1]

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Abstract: Microplot experiments were conducted to evaluate the effects of inoculum level and time of application of *Paecilomyces lilacinus* on the protection of tomato against *Meloidogyne incognita*. The best protection against *M. incognita* was attained with 10 and 20 g of fungus-infested wheat kernels per microplot which resulted in a threefold and fourfold increase in tomato yield, respectively, compared with tomato plants treated with this nematode alone. Greatest protection against this pathogen was attained when *P. lilacinus* was delivered into soil 10 days before planting and again at planting. Yield was increased twofold compared with yield in nematode-alone plots and plots with *M. incognita* plus the fungus. Percentages of *P. lilacinus*-infected egg masses were greatest in plots treated at midseason or at midseason plus an early application, compared with plots treated with the fungus 10 days before planting and (or) at planting time.

Key words: biological control, *Lycopersicon esculentum, Meloidogyne incognita, Paecilomyces lilacinus,* root-knot nematode, tomato.

Crops attacked by *Meloidogyne incognita* (Kofoid and White) Chitwood have been protected to varying levels by the fungus *Paecilomyces lilacinus* (Thom) Samson (1,5,10). The degree of protection against this nematode with *P. lilacinus* has been positively correlated with the amount of fungus applied (1). The greatest nematode suppression on tomato, 42 and 70%, was obtained with 20 or 80 g of fungus-infested rice kernels per 15-cm-d pot, respectively, in the greenhouse; however, *P. lilacinus* failed to suppress residual populations of *M. incognita* in microplots (1). Small plots of tomato, okra, pepper, and eggplant treated with this fungus yielded more than nematode-control plots, but the fungus suppressed *M. incognita* populations only up to midseason (J. P. Noe, unpubl.)

Paecilomyces lilacinus possesses attributes of a successful biological control agent against nematodes (1,10). It parasitizes eggs of *Meloidogyne* spp. (7,9), a characteristic suitable for long-term biological control (10). The fungus also may parasitize young root-knot and cyst nematode females (8,10). Unfortunately, *P. lilacinus* has been reported as a human pathogen (15). The fungus seems to be opportunistic and generally does not exhibit severe pathogenicity. Although undue precautions may not be necessary (13), this potential hazard must be considered in working with this organism.

Although *P. lilacinus* is associated with *Meloidogyne* spp. and can penetrate their eggs and females, its successful deployment as a biological agent against nematodes may depend on a thorough understanding of this fungus. Factors that may account for the observed variability concern the antagonist directly (age, virulence, longevity, inoculum level, strains, method of establishment), the environment (soil type, fertility, amendments, organic matter, temperature, moisture, pH), and host susceptibility (genotype, age) (6,10,12,14). There is a need for more ecological information concerning *P. lilacinus.*

The objectives of this study were to determine the effects of inoculum density and time of application of *P. lilacinus* on its activity against *M. incognita* on tomato.

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Materials and Methods

Nematodes, fungus, and crop cultivar used: Two experiments were conducted in 0.76-m-d microplots (3) established in a Varina sandy loam soil (89% sand, 9.5% silt, 1.5% clay, 0.8% organic matter) at Central Crops Research Station near Clayton, North Carolina. They were initiated on 30 June 1984 and terminated on 25 October 1984. The microplots were tilled and fumigated with approximately 100 g methyl bromide/m^2 under plastic. The plastic was removed 1 week after the fumigant was applied, and plots were allowed to aerate for 3 weeks before infestation with nematodes and *P. lilacinus.*

Meloidogyne incognita race 1 (E 904, International *Meloidogyne* Project nematode culture collection) (16) was reared on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse at 25–27 C for 60 days. Nematode inoculum consisted of eggs extracted from the tomato roots with 0.5% sodium hypochlorite solution (NaOCl) (4).

A *P. lilacinus* isolate from the International Potato Center, Lima, Peru (10) was cultured on potato dextrose agar (PDA) and incubated at 25 C for 10 days. Commercial wheat kernels soaked in tap water (300 cm^3 wheat in 250 ml water v/v) were placed in heat-resistant bags (20 × 30 cm) and autoclaved for 30 minutes at 121 C on 2 consecutive days. The fungal colonies were flooded with sterile distilled water, and the agar surface was gently rubbed to release the spores. Five milliliters of spore suspension (3.5×10^7 spores/ml) treated with Tween 20 were injected with a sterile hypodermic syringe into the autoclaved bag containing wheat kernels. The infested wheat medium was incubated at 25 C for 21 days resulting in a fungal inoculum level of about 3.5×10^9 spores/g wheat kernels.

The *Paecilomyces*-semi-selective medium (PSM) consisted of the following in distilled water: streptomycin sulfate, 50 mg/liter; chlorotetracycline hydrochloride, 50 mg/liter; dichloran 75 WP, 50 mg/liter; Ox gall (Bile bovine), 250 mg/liter. The basal medium was PDA. Soil dilutions for *P. lilacinus* assays on this medium were made by suspending the equivalent of 10 g oven-dry soil in 95 ml sterile tap water, followed by further dilutions (11). The plates were incubated at 25 C for 3–4 days, and the numbers of colonies per gram of soil were counted.

Ten egg masses were randomly collected from each root system, surface sterilized with 0.5% NaOCl for 30 seconds, rinsed twice in sterile distilled water, and placed on a petri dish containing PSM medium. Dishes were incubated at room temperature (25 C), and the numbers of egg masses infested with *P. lilacinus* were estimated after 3–4 days incubation.

Inoculum densities of P. lilacinus: The protective effects of two inoculum levels of *P. lilacinus* against *M. incognita* on tomato were compared in microplots. Inoculum of the fungus was prepared for each plot by incorporating the appropriate amount of infested wheat containing 3.5 × 10 fungal spores/g into 2,250 g of a steam sterilized mixture of soil and sand (1:1 v/v). The inoculum was stored in plastic bags for 24 hours at room temperature, and plots were infested by placing the wheat–soil mixture into a 15-cm-d well in the center of each one. On 30 June 1984, 5-week-old Rutgers tomato seedlings were transplanted into each microplot. Also at this time, the center well in each plot was infested with a suspension of 5,000 *M. incognita* eggs that had been extracted as previously described. Tomato plants were fertilized as needed with VHPF (Miller Chemical Corp., Hanover, PA) which was supplemented with $MgSO_4$ and KNO_3.

On 15 August 1984 and 25 October 1984, estimates of population levels of the fungus and nematode were determined from soil samples (five 2.5-cm-d cores, 15–20 cm deep) taken from treated and untreated plots. Nematodes were extracted from a 200-cm^3 soil sample per plot by a combination of elutriation and modified centrifugal flotation (4). The root fraction trapped on the 500-μm sieve was processed by the NaOCl method to estimate the number of *M. incognita* eggs (4).

Paecilomyces lilacinus was isolated from soil by the dilution plate technique in combination with the semiselective medium. Soil samples were stored in plastic bags in a refrigerator for 1 week, then air dried at room temperature (25–26 C) for 24 hours. Ten grams of soil were diluted 10^{-4} in distilled water, and a 1-ml aliquot of the dilution was transferred to each of four petri dishes containing the semiselective medium at about 45 C. Assay plates were incubated at 25 C and *P. lilacinus* colonies were counted on the fourth day.

At harvest roots were dug, gently washed, visually rated for galls on a visual scale of 0 to 100 (100 = maximum galls), wrapped with paper towels, and stored in plastic bags at 13 C for 1 week. Ten egg masses from each root system were surface sterilized with 0.5% NaOCl for 30 seconds, rinsed twice in sterile tap water, and placed on solidified semiselective medium in petri dishes.

The treatments used in this study were 1) 5,000 eggs + 10 g fungus-infested wheat, 2) 5,000 eggs + 10 g autoclaved wheat, 3) 5,000 eggs + 20 g fungus-infested wheat, 4) 5,000 eggs + 20 g autoclaved wheat, 5) 5,000 eggs, and 6) control (no nematode eggs, no fungus). Treatments were replicated five times in a randomized complete block design.

Variables recorded to evaluate the effects of inoculum densities of *P. lilacinus* on the control of *M. incognita* were number of juveniles at midseason (Pm), number of *P. lilacinus* spores per gram of soil at midseason and at harvest, yield (11–25 October 1984), fresh root weight, gall indices (0–100), number of eggs and juveniles at harvest (Pf), and the percentage of egg masses infected with *P. lilacinus* at harvest.

Time of fungus application: Inoculation methods were similar to those used in the inoculum density study. The treatments were as follows: 1) 20 g fungus-infested wheat applied 10 days before planting; 2) 20 g fungus-infested wheat applied at transplanting; 3) 20 g fungus-infested wheat applied at midseason (45 days after planting); 4) combining 1 and 2; 5) combining 1 and 3; 6) combining 2 and 3; 7) 5,000 eggs only; 8) control (no nematode eggs, no fungus); and 9) 20 g autoclaved wheat, 10 days before planting. Treatments were replicated five times in a randomized complete block design.

Management, harvest, and parameters estimated to determine the influence of time of fungus application on control of *M. incognita* were similar to those described for the inoculum density study. The statistical analysis consisted of an analysis of variance of the data obtained for plant growth, nematode, and fungus populations. Treatment means were compared by linear contrasts.

Results and Discussion

Inoculum densities of P. lilacinus: Both rates of *P. lilacinus* limited the damage caused by *M. incognita* race 1 and increased the yield of tomato (Table 1). All treatments containing wheat kernels infested with *P. lilacinus* and autoclaved wheat alone had significantly higher yields than the control that had neither nematodes nor fungus. Treatments with 10 g and 20 g fungus-infested wheat resulted in threefold and fourfold increases in tomato yields, respectively, compared with tomato plants inoculated with nematodes alone. Yields of tomato from the two rates of fungus were not different. Microplots treated with fungus-infested wheat had significantly greater yields and root weights than plots treated with only autoclaved wheat (Table 1).

Gall development and reproduction of *M. incognita* were suppressed by both levels of fungus-infested wheat and autoclaved wheat alone, compared with plots treated with nematodes alone (Table 1). There were no significant differences between the fungus-infested wheat and autoclaved wheat. Gall development was suppressed more by 20 g fungus-infested wheat (36% gall inhibition) than by 10 g fungus-infested wheat (20% gall inhibition).

The recovery of the fungus from infested soil by the dish-count method at midseason resulted in 6,000 and 7,000 spores/g soil from plots treated with 10 and 20 g

TABLE 1. Plant growth, yield, and disease factors as influenced by different levels of inoculum of *Paecilomyces lilacinus* to control *Meloidogyne incognita* on tomato.

Treatment	Fruit yield (g/plot)	Root weight (g/plot)	Gall indices (0–100)	Rf†	Fungus recovery‡	Bio-assay§
1. 5,000 eggs + (10 g wheat + fungus)	2,850	115	80	0.7	6,000	43
2. 5,000 eggs + (10 g wheat)	1,949	86	92	3.1	0	0
3. 5,000 eggs + (20 g wheat + fungus)	3,116	119	64	0.5	7,000	48
4. 5,000 eggs + (20 g wheat)	1,511	102	68	1.4	0	0
5. 5,000 eggs	781	71	100	22.3	0	0
6. Control (no nematode eggs, no fungus)	2,019	106	0	0.0	0	0
CV (%)	26	22	15	154	117	64
Linear contrasts‖						
6 vs. 1–5	NS	NS				
5 vs. 1–4	**	**	**	**		
1, 3 vs. 2, 4	**	*	NS	NS		
1 vs. 3	NS	NS	NS	NS		
2 vs. 4	NS	NS	**	NS		
1 vs. 2	**	NS	NS	NS		
3 vs. 4	**	NS	NS	NS		

All data are means of five replicates.
† *M. incognita* reproductive factors at final harvest (no. juveniles and eggs/500 cm^3 of soil).
‡ Fungus recovery at midseason (no. spores/g soil).
§ % egg masses infected with *P. lilacinus* at final harvest.
‖ Data for treatments with zero means were omitted from the analysis of variance. *, ** indicate significant difference at $P = 0.05$ and 0.01, respectively. NS = no significant difference at $P = 0.05$.

fungus-infested wheat, respectively. Forty-three and forty-eight percent of egg masses were infected in plots treated with 10 and 20 g fungus-infested wheat, respectively.

Decomposition products from wheat kernels may have contributed to nematode suppression in these experiments. Organic matter is known to stimulate growth of predacious fungi (6), but these effects tend to be short lived (J. P. Noe, unpubl.). Organic matter may also have stimulated plant growth and offset nematode damage (12), or decomposition of organic matter may have produced nematicidal breakdown products (10). In our studies, wheat substrate probably enhanced the growth of *P. lilacinus* and also allowed colonization by other saprophytic fungi. Gall development on tomato appeared to be influenced by inoculum density. Similar results have been obtained in earlier greenhouse experiments (1).

Since yields of tomato attained with fungus-infested wheat were greater than with autoclaved wheat alone, *P. lilacinus* probably accounted for most of the control obtained, and decomposition products from wheat had a lesser effect. The use of the two levels, 10 and 20 g, of fungus-infested wheat (ca. 0.208 and 0.416 ton/ha as row treatments) may be evaluated economically. Presently, these rates are less than most others used in similar biocontrol studies against these nematodes. *Arthrobotrys irregularis* strain 1141 b (Royal 350) is applied on rye grains at 140 g/m^2 (1.4 ton/ha) to soil infested with *Meloidogyne* spp. and limits damage to tomatoes (12). *P. lilacinus* applications on wheat grain at a rate of 1.5 kg/40 m^2 (0.375 ton/ha) also are effective against these nematodes (10). To keep costs of supplies, storage space, and delivery at an economical level, however, applications of biological control agents in excess of 100–200 kg/ha should be avoided (2).

Time of application of P. lilacinus: The best protection against *M. incognita* on tomato was attained with two applications of *P. lilacinus,* 10 days before transplanting and at transplanting. This treatment resulted in about a twofold increase in tomato yield and a 56% suppression of galling, compared with plots treated with nematodes alone (Table 2). Root-galling was suppressed and yield increased in all plots treated with *P. lilacinus* 10 days before

TABLE 2. Efficacy of the fungus *Paecilomyces lilacinus* at different times of application in the soil to control *Meloidogyne incognita* on tomato in microplots.

Treatment	Fruit yield (g/plot)	Root weight (g/plot)	Gall indices (0–100)	Rf†	Fungus recovery‡	Bio-assay§
1. 5,000 eggs + 20 g wheat + fungus, 10 days before planting	2,081	101	80	0.75	2,000	19
2. 5,000 eggs + 20 g wheat + fungus, planting time	2,273	116	44	0.40	6,000	22
3. 5,000 eggs + 20 g wheat + fungus, midseason	1,583	90	100	40.20	51,000	53
4. 5,000 eggs + 1 and 2	2,608	104	44	0.27	2,000	22
5. 5,000 eggs + 1 and 3	1,903	99	84	3.21	14,000	57
6. 5,000 eggs + 2 and 3	2,493	113	60	0.32	54,000	57
7. 5,000 eggs, 10 days before planting	1,207	84	100	175.42	0	0
8. Control	2,238	97	0	0	0	0
9. 5,000 eggs + 20 g wheat, 10 days before planting	1,511	102	68	1.58	0	0
CV (%)	29	14	25	37	66	52
Linear contrasts‖						
7 vs. 1–6, 9	**	**	**	**		
9 vs. 1–6	*	NS	NS	NS		
8 vs. 1–9	NS	NS	—	—		
1–2 vs. 4–6	NS	NS	NS	**		

All data are means of five replicates.
† *M. incognita* reproductive factors at final harvest (no. juveniles and eggs/5,000 eggs).
‡ Fungus recovery at midseason (no. spores/g soil).
§ % egg masses infected with *P. lilacinus* at final harvest.
‖ Data for treatments with zero means were omitted from the analysis of variance. *, ** indicate significant difference at $P = 0.05$ and 0.01, respectively; NS = no significant difference at $P = 0.05$.

transplanting, at planting, or the combination of these two applications. Midseason applications alone were not effective. Plants in plots treated with two applications of the fungus had greater yields and root weights, compared with one application, but differences were not significant according to linear contrasts (Table 2).

Recovery of the fungus from the soil at harvest was greater in plots treated at midseason (5.1×10^4 spores/g soil), at transplanting + midseason (5.4×10^4 spores/g soil), and 10 days before planting + midseason (1.4×10^4 spores/g soil), than in plots treated 10 days before planting (2.0×10^3 spores/g soil), at planting (6.0×10^3 spores/g soil), and 10 days before planting + planting (2.0×10^3 spores/g soil). Similarly, the bioassay test for *P. lilacinus* resulted in a greater percentage of fungus-infected egg masses in plots treated at midseason only or at midseason plus an early application than in plots treated with the fungus 10 days before planting and (or) at planting time.

The protective efficacy of *P. lilacinus* against *M. incognita* on tomato depended on the time of application. The nematode reproductive factor was less than one in all plots treated with one or two fungal applications except for the one at midseason. This result was reflected in higher yields, and limited gall indices and reproductive factors in plots treated before and (or) at transplanting than plots treated at midseason. This difference in effectiveness on control of *M. incognita* can be attributed to the fact that much of the nematode increase and damage occurred before the midseason application of the fungus. Since *P. lilacinus* is an egg parasite of these and other plant-parasitic nematodes (8,10), the stage of egg development is probably important when timing the delivery of the fungus into the soil. *P. lilacinus* probably will be more effective against *M. incognita* during early rather than advanced stages of egg development, especially as the first-stage juveniles appear. This assumption may help explain differences in the results

obtained when the fungus is delivered 10 days before planting and when it is delivered at planting.

The importance of the stage of the nematode life cycle in which parasitism occurs and its impact on their populations should be considered in future investigations (14). A single application of *P. lilacinus* may be sufficient to establish the fungus in soil (10), but our results indicate that effective biological control will require more than one application at the proper time. This issue is particularly important for *M. incognita* and other plant parasites that exhibit high reproductive capacity. *P. lilacinus* apparently is more effective in protecting tomatoes against this nematode when it is delivered before transplanting and (or) at transplanting time than at midseason. Midseason applications did not provide effective control as did the earlier applications, but the higher percentage of infected egg masses resulting from midseason applications could reduce the initial level of nematode inoculum in the subsequent growing season. The protective efficacy of *P. lilacinus* should not be measured on the basis of the results at the end of one growing season but on the performance through subsequent growing seasons.

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