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Survival of *Paecilomyces lilacinus* in Selected Carriers and Related Effects on *Meloidogyne incognita* on Tomato[1]

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Abstract: Laboratory and microplot experiments were conducted to determine the influence of carrier and storage of *Paecilomyces lilacinus* on its survival and related protection of tomato against *Meloidogyne incognita*. Spores of *P. lilacinus* were prepared in five formulations: alginate pellets (pellets), diatomaceous earth granules (granules), wheat grain, soil, and soil plus chitin. Fungal viability was high in wheat and granules, intermediate in pellets, and low in soil and chitin-amended soil stored at 25 ± 2 C. In 1985 *P. lilacinus* in field microplots resulted in about a 25% increase in tomato yield and 25% gall suppression, compared with nematodes alone. Greatest suppression of egg development occurred in plots treated with *P. lilacinus* in pellets, wheat grain, and granules. In 1986 carryover protection of tomato against *M. incognita* resulted in about a threefold increase in tomato fruit yield and 25% suppression of gall development, compared with plants treated with nematodes alone. Higher numbers of fungus-infected egg masses occurred in plots treated with pellets (32%) than in those treated with chitin-amended soil (24%), wheat (16%), granules (12%), or soil (7%). Numbers of fungal colony-forming units per gram of soil in plots treated with pellets were 10-fold greater than initial levels estimated at planting time in 1986.

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

Root-knot nematodes are important plant pathogens affecting crop production throughout the world (10,13). Recent problems in the use of chemical pesticides have enhanced the development of biocontrol methods for integrated management of plant-parasitic nematodes with various types of antagonistic organisms (1,10,13,15). The action of fungal parasites of eggs in reducing the nematode population is a promising form of crop protection against *Meloidogyne* spp. *Paecilomyces lilacinus* (Thom) Samson was described as an effective fungal parasite of eggs of *Meloidogyne incognita* (Kofoid & White) Chitwood and *Globodera pallida* (Stone) Behrens (13). Attempts to control various nematodes with this fungus has given inconsistent results (1,10,13,19).

New methods are needed for *P. lilacinus* to be used more effectively against *Meloidogyne* spp. and other plant-parasitic nematodes. One tactic would be to apply biocontrol agents in a manner similar to nematicides. Delivery of *P. lilacinus* into soil has been limited to traditional methods such as infested grains or in aqueous spore suspensions (1,10,13). Commercial production of a material named BIOCON has been initiated in the Philippines (13). Royal 350, a commercial product containing *Arthrobotrys irregularis* strain 1141b was applied on rye grains (140 g/m^2) to soil as a means to control *Meloidogyne* sp. in tomato fields (7).

Formulation, method and time of application, and selection of biological control agents play an important role in the successful introduction of antagonists and subsequent biocontrol of certain plant parasites (8,9,11,14). Methods used to deliver biological control agents to soil include carriers such as diatomaceous earth granules (2), bark pellets (21), vermiculite or clay such as pyrax (11), and Laponite gel with germinated seedlings (8). Recently, alginate pellets with pyrophyllite clay filler containing fungal spores and bacterial cells have been formulated (11,22). These pellets are biodegradable, relatively uniform in size, and convenient for storing, ship-

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ping, and delivering antagonists into the soil. There is little available information, however, on the effects of formulation of *P. lilacinus* on its viability during storage, establishment, survival, or impact on control of nematodes when delivered into the soil.

The objectives of this research were to 1) determine the effects of carriers and length of storage on viability of *P. lilacinus*; 2) elucidate the efficacy of carriers of this fungus on the protection of tomato against *M. incognita*; 3) determine fungal survival after introduction into soil; and 4) determine the residual effects of *P. lilacinus* on *M. incognita*.

Materials and Methods

Effects of carrier and length of storage on fungal viability: The isolate of *P. lilacinus* used was from the International Potato Center, Lima, Peru (13). The fungus was introduced into microplots at Central Crops Research Station near Clayton, North Carolina, and reisolated from infected egg masses of *M. incognita* on tomato roots. The fungus was cultured on potato dextrose agar (PDA), at 25 C for 14 days, then added to the carriers.

Spores of *P. lilacinus* were formulated in alginate pellets, diatomaceous earth granules, soil, or soil plus chitin, or they were grown on sterile wheat grains. Alginate pellets with and without this fungus were prepared as previously described (11,22), except 10 g of sodium alginate and 100 g pyrax were added to 1 liter sterile distilled water and mixed in a sterilized blender for 30 seconds. Two hundred milliliters of the alginate-pyrax mixture, adjusted to pH 7, were transferred into a 500-ml wide-mouth erlenmeyer flask with a coupling system. The mixture was amended with 20 ml of an aqueous spore suspension of a 14-day-old PDA culture of *P. lilacinus* (5.2×10^7 spores/ml). The final mixture was added dropwise into 500 ml of gellant solution 0.1 M calcium gluconate (Sigma, Cat. No. 6-4615 D-gluconic acid). Each droplet entering the solution gelled, forming a distinct, spherical pellet. This process was facilitated by use of a four-gang aquarium valve attached to the apparatus previously described for preparing pellets (11). Pellets were collected on a cheesecloth, dried overnight in a laminar air flow hood, and stored in plastic bags at room temperature (24–26 C). The moist pellets were 3 mm d; after drying pellets were 1 mm d. The air-dry weight was 400 pellets/g.

Pellets were assayed by dilution plating as follows: one gram of air-dried pellets was dissolved in 100 ml of a mixture of 8.7×10^{-2} M KH_2PO_4 and 3.0×10^{-2} M Na_2HPO_4 for about 5 hours. Dilutions of the homogenate were prepared by dispensing 1 ml of the spore suspension into 9 ml sterile distilled water and mixing on a wrist action shaker for about 1 minute; 1-ml aliquants were dispensed in petri dishes and mixed with about 20 ml molten agar (40–45 C) of semiselective medium for *P. lilacinus* (6).

Wheat medium with and without *P. lilacinus* was prepared separately by autoclaving 300 cm^3 wheat in autoclaving bags (Fisher Scientific Co.) containing 250 ml distilled water at 121 C for 30 minutes for 2 consecutive days. Inoculum of the fungus was collected from 14-day-old cultures on PDA. Ten milliliters of an aqueous spore suspension of *P. lilacinus* was injected with a sterilized syringe through the bag onto the autoclaved seeds. Cultures were incubated at room conditions (25 ± 2 C) for 20 days. Bags were shaken by hand for about 5 minutes every day to homogenize the inoculum on the wheat seeds.

Diatomaceous earth granules with and without *P. lilacinus* were prepared separately by a modification of procedures previously described (2). Three hundred cubic centimeters of diatomaceous earth granules (Celatom MP-78) in autoclaving bags were impregnated with 150 ml of a 10% solution (v/v) of molasses at pH 5.0 containing 3 g KNO_3 and 3 g KH_2PO_4 per liter of solution. The mixture was autoclaved and allowed to cool for 24 hours. The granules then were spread in polypropylene plastic pans with covers and inoculated with a minced 14-day-old PDA culture of

P. lilacinus. Granules were incubated at 24 C for 5 days. Clumps of granules were crushed, and granules were air dried with frequent stirring and stored at room conditions (25 ± 2 C) for 20 days.

Soil inoculum of *P. lilacinus* was prepared by dispensing 20 ml of an aqueous spore suspension made from a 14-day-old PDA culture of this fungus onto 500 cm^3 steamed sandy soil (75 C for 90 minutes) contained in autoclaving bags. The soil–fungus preparation was mixed thoroughly for about 5 minutes daily and stored at room conditions for 20 days. Control soil and fungal soil preparation were compared to determine the influence of length of storage and temperature on the viability of *P. lilacinus* in soil. On 20 July 1984, 10 ml of a spore suspension (3.2×10^8) were added to 250 cm^3 steamed-sterilized greenhouse soil (1:1 sand : soil) contained in a 500-ml glass jar. Soil was incubated at room temperature (24–26 C) for 14 days and then refrigerated at 5 C. Samples, taken on 20 June 1985 (almost 1 year after soil infestation) were assayed by dilution culture on the semiselective medium. The *P. lilacinus* population (2.3×10^6 CFU/g soil) was still viable and could be easily and quickly recovered from soil cultures.

Soil plus chitin preparations with and without *P. lilacinus* involved the same procedure described for soil preparation, except chitin was added to soil. Practical grade chitin (poly-N-acetylglucosamine from crab shells (Sigma Chemical Co.) was added to a 100-ml spore suspension at a rate of 3% w/v. Twenty milliliters of chitin–spore suspension were added to 500 cm^3 steamed sandy soil contained in autoclaving bags. This soil–fungus preparation amended with chitin was stored at room conditions (25 ± 2 C) for 20 days.

The effects of carriers and length of storage on the viability of *P. lilacinus* were determined over a period of 8 weeks. Forty-gram portions of air-dried pellets, 100 g granules, 100 g wheat, 200 g soil, and 200 g soil plus chitin containing this fungus were placed separately in plastic bags and incubated at room temperature (25 ± 2 C). They were sampled after 1, 7, 14, 28, and 56 days of storage. Samples were randomly taken, and colony-forming units of *P. lilacinus* per gram of carrier (CFU/g) were determined by dilution plating on semiselective media. For each fungus-treated carrier, 10-fold dilutions were performed with two dishes per dilution per replicate. The procedure was replicated twice.

The design of this experiment was a split-plot with carrier as the whole plot and time of storage as the subplot. There were two replications of carrier and two dishes per replication. Data of the CFU/g carrier were transformed to $\log_{10} Y$ prior to statistical analysis to normalize the variances. Regression analysis was performed to compare the relationship between the viability of the fungal propagules (response of dependent variable) and length of storage of each formulation (independent variables).

Efficacy of carriers of P. lilacinus: Two experiments were conducted in field microplots. On 3 July 1985, the microplots established (3) in a Varina sandy loam (89% sand, 9.5% silt, 1.5% clay, 0.8% organic matter; pH 6.0; CEC 4.4 meq/100 cm^3, base saturation 82% of CEC) at Central Crops Research Station near Clayton, North Carolina. Plots were fumigated with methyl bromide (0.10 kg/m^2) 4 weeks before planting tomato (*Lycopersicon esculentum* Mill.).

Meloidogyne incognita race 1 (E589) from the Crop Nematode Research and Control Project at Raleigh, North Carolina, was reared on Rutgers tomato in a greenhouse (26–28 C) for 60 days. Nematode eggs were extracted by the NaOCl technique (4) and adjusted to 500 eggs/500 cm^3 soil. Each microplot was infested with 42,000 eggs which were incorporated into 42,000 cm^3 of soil in the center of the 76-cm-d plot.

The preparation of *P. lilacinus* was described in the formulation section. The fungal formulation was adjusted to equal a rate of 18 g fungus-infested wheat per 0.4778-m^2 plot (1.5 kg/40 m^2) (17). *P. lilacinus* formulations were prepared 13 June 1985 and stored at room temperature. Before field application, viability of the dried

TABLE 1. Concentration of viable spores of *Paecilomyces lilacinus* in different carriers used for field application in 1985.

Carrier	CFU/g†	g/plot
Wheat	3.1×10^7	18
Pellets	2.9×10^7	19
Granules	3.3×10^7	17
Soil	5.5×10^6	101
Soil + chitin	5.6×10^6	100

The amount of spores was standardized in each carrier equal to 1.5 kg of wheat per 40 m^2 (0.4 ton/ha) (plot = 0.4778 m^2).

† CFU/g = colony-forming units per gram of formulation.

preparations was tested 7 days after preparation by separately placing 10 subsamples of each formulation on two dishes containing the semiselective medium. The standardization of inoculum was performed 14 days after preparation of formulations by the dilution technique. Two 1-g subsamples of each material were diluted to 10^{-4} and 10^{-5}. A 1-ml suspension was placed in each of five dishes of the semiselective medium. All plates were incubated in the laboratory at 25 ± 2 C for 4 days before counting *P. lilacinus* colonies. Concentrations of viable spores and the amount of inoculum used varied with the formulation (Table 1).

Except for the controls, each plot was infested with *M. incognita* and the fungal formulations were added at transplanting in the center of the microplot. Two 13-week-old Rutgers tomato plants were transplanted in each plot on 3 July 1985. The experimental design was a randomized complete block with 12 treatments (Table 2) and 9 replicates.

To assess nematode populations, composite soil samples (ten 2.5-cm-d cores per plot) were collected from the upper 20 cm of the root zone. Juveniles were extracted from soil samples by elutriation and centrifugation (4). Roots from the 425-μm-mesh sieve were treated with NaOCl to extract eggs (4).

Residual effects of P. lilacinus 1 year later: A second experiment was conducted in 1986 to determine the effects of *P. lilacinus* on both *M. incognita* and fungal survival 1 year after addition to the soil. The experiment design was similar to that used in the first experiment, except that microplots were not fumigated before planting. The experiment was initiated 16 May 1986 and terminated 5 September 1986. Plots received no nematode or fungal treatment other than those applied on 3 July 1985. Two 9-week-old Rutgers tomato plants were transplanted into each plot. Collection of soil samples from microplots and extraction of nematodes followed the same procedures described for the 1985 experiment. Initial *M. incognita* juvenile population densities were estimated from soil samples taken 1 day prior to planting (Pi), and the final population levels (Pf) were estimated at final fruit harvest.

To assess nematode and fungus population levels, composite soil samples (ten 2.5-cm-d cores) were collected from the upper 20 cm of soil in the root zone. The initial and final fungal and nematode numbers were estimated at the same time. Samples were sealed in plastic bags and stored at 5 C. One 10-g subsample of soil was diluted to 10^{-3}, and 1 ml of the dilution was dispersed evenly onto each of two petri dishes containing 15–20 ml of semiselective medium for *P. lilacinus*. All dishes were incubated in the dark at 24 C for 2 days and on a laboratory bench at 25 ± 2 C under fluorescent light for 2 additional days before counting fungal colonies. A 10-g portion of each soil sample was used to determine the moisture content. It was dried in an oven at 105 C for 24 hours. The average numbers of fungal colonies were calculated per gram of oven-dry soil.

At harvest, galled root systems from microplots were gently washed to remove soil particles, drained, wrapped in a paper towel, sealed in plastic bags, and stored at 5 C for 16 days. Ten egg masses were taken randomly from galled roots, surface sterilized with 0.5% NaOCl for about 30 seconds, rinsed twice in sterile tap water, and placed in each of two dishes containing a solidified selective medium. Each dish had 10 egg masses uniformly distributed. All dishes were incubated at 24 C in the dark for 2 days and on a laboratory bench at 25

± 2 C for 2 additional days before counting *P. lilacinus* colonies. The numbers of egg masses infected by the fungus were counted after 4 days and expressed as percentage of egg masses infected.

The randomized complete block design included 12 treatments replicated nine times. Analyses of variance incorporating linear contrasts were performed on the data. Treatment means and results of significance tests of the linear contrasts for various response variables were calculated. Data for treatments with zero means were omitted from the analysis of variance.

Results

Effects of P. lilacinus carriers and length of storage on fungal viability: The viability of fungal propagules differed with length of storage in the carriers (Fig. 1). Viability remained high in wheat grains and diatomaceous granules but decreased in alginate pellets, soil, and chitin-amended soil over time of storage. The fungal viability was least in pellets, soil, and chitin-amended soil at 7 and 14 days of storage; after 14 days it dropped sharply in soil, and chitin-amended soil. Viability was intermediate in the pellets.

Efficacy of carriers of P. lilacinus on the protection of M. incognita 1985: The carrier of the fungus differentially affected the protection of tomato against *M. incognita* (Table 2). The best protection was with *P. lilacinus* formulated in granules and on wheat, which resulted in about 25% increase in tomato yield and 25% gall suppression when compared with plots treated with nematodes alone. Despite suppression of gall development, root necrosis developed in all treatments (range of 27–53%) with no significant differences between them.

Based on linear contrasts, the influence of *P. lilacinus* on nematode reproduction also depended on the type of carrier used for its delivery to soil (Table 2). Plots treated with the fungus had lower numbers of juveniles at midseason (data not included) and at harvest than did untreated plots. Tomatoes in plots treated with *P. lilacinus* also supported less nematode egg development than tomatoes in plots treated with carriers minus the fungus. Suppression of egg development at harvest was greater in plots treated with the fungus in pellets than those treated with pellets alone. Similarly, the inhibition of egg development at harvest was greater in plots in which *P. lilacinus* was applied in granules than in those receiving granules alone. In general, despite the suppression of *M. incognita* attained by these treatments with *P. lilacinus*, levels of eggs were greater than those of juveniles in all plots.

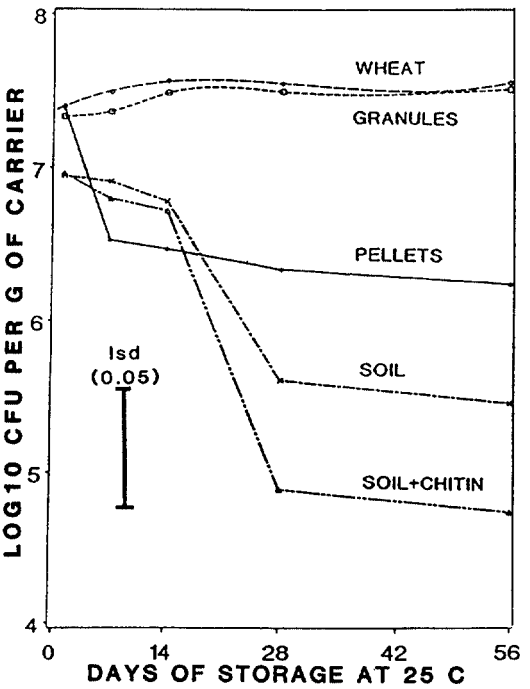

FIG. 1. Effects of carriers and length of storage at 25 ± 2 C on viability of *Paecilomyces lilacinus*. CFU = colony-forming units.

Residual effects of P. lilacinus 1 year after delivery into soil: The efficiency with which formulations of *P. lilacinus* protected tomato against *M. incognita* declined 1 year after its introduction into the soil (Table 3). Nevertheless, the residual populations of this fungus in the soil increased by the end of the second tomato growing season. In the second growing season the tomato yield decreased by 75% or more, gall indices increased slightly, and root necrosis increased 15 to 43% compared with the results obtained in the previous year. At

TABLE 2. Comparative efficacy of carriers of *Paecilomyces lilacinus* in limiting damage to tomato by *Meloidogyne incognita* in microplots, 1985.

Treatment	Tomato yield (g/plot)	Gall indices (0–100)	Root necrosis (0–100)	Final nematode numbers (in 1,000s)		
				Roots (eggs per 10 g)	Soil (500 cm³)	
					Eggs	Juveniles
1. Pellets (fungus) + *M. incognita* (MI)	4,991	79	43	87	19	1.6
2. Wheat (fungus) + MI	6,171	63	27	63	27	2.5
3. Granules (fungus) + MI	6,403	62	33	58	36	2.9
4. Soil (fungus) + MI	5,451	76	37	80	55	3.1
5. Soil + chitin (fungus) + MI	5,159	84	37	82	36	2.5
6. Pellets + MI	4,907	82	46	113	64	4.8
7. Wheat + MI	4,567	81	32	149	50	5.0
8. Granules + MI	5,016	91	53	126	73	6.6
9. Soil + MI	4,811	79	39	128	67	6.9
10. Soil + chitin + MI	5,535	86	41	106	46	5.9
11. *M. incognita* only	4,919	88	43	121	79	17.9
12. Control (no nematode eggs, no fungus)	7,746	0	0	0	0	0
CV (%)	28	20	62	39	66	154
Linear contrasts†						
12 vs. 1–11	**					
11 vs. 1–10, 12	NS	NS	NS	NS	**	**
1–5 vs. 6–10	*	**	NS	**	**	NS
4 vs. 1–3, 5	NS	*	NS	NS		
5 vs. 1–4	NS	NS	NS	NS		
2 vs. 1, 3	NS	NS	NS	NS		
1 vs. 3	NS	*	NS	NS		
1 vs. 6	NS	NS	NS	NS	**	NS
2 vs. 7	*	*	NS	**	NS	NS
3 vs. 8	NS	**	NS	**	*	NS
5 vs. 10	NS	NS	NS	NS	NS	NS

Data in body of table are means of nine replicates. For root gall and necrosis indices, 0 = healthy and 100 = 100% of root affected.

† 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.

harvest, some tomato plants were affected by bacterial wilt caused by *Pseudomonas solanacearum*. Despite this poor performance, biocontrol of *M. incognita* was better in plots treated with formulations of *P. lilacinus* than in those treated with carriers alone. Differences ($P = 0.05$) were detected in yield, root weight, gall indices, and root necrosis. The best protection against *M. incognita* was obtained in plots treated with *P. lilacinus* granules which resulted in about threefold increase in tomato yield, 26% suppression of gall development, 47% suppression of root necrosis, and 12% fungus-infected egg masses compared with those treated with nematodes alone. There were no differences in yield in plots treated with *P. lilacinus* on granules, wheat, or soil. Similarly, there were no differences in gall development on plants treated with this fungus on granules, wheat, pellets, or soil. The highest percentage of egg-mass infection was in plots where *P. lilacinus* was applied in pellets (32%); it was significantly higher than in plots treated with granules with the fungus (12%) according to the linear contrasts (Table 3). Fungus infection of egg masses also was found in plots where *P. lilacinus* was applied in chitin-amended soil (24%), on wheat (16%), in granules (12%), and in soil (7%) (Table 3).

Type of fungal carriers also had a major effect on nematode reproduction and the development of fungal populations in the soil (Table 3). Numbers of eggs and juveniles at planting and at harvest were less in plots that had received various formulations of *P. lilacinus* than in the controls.

TABLE 3. Influence of formulations of *Paecilomyces lilacinus* on the control of *Meloidogyne incognita* on tomato and fungal activity after 1 year of its delivery into the soil, 1986.

Treatment	Tomato yield (g/plot)	Gall indices (0–100)	Root necrosis (0–100)	Infected egg masses (%)	Numbers of nematodes/500 cm³ soil				*P. lilacinus* (CFU/g soil—in 1,000s)	
					Planting		Final harvest			
					Eggs	Juveniles	Eggs (in 1,000s)	Juveniles	Planting	Harvest
1. Pellets (fungus) + *M. incognita* (MI)	528	61	42	32	235	119	202	3.0	2.0	20.6
2. Wheat (fungus) + MI	674	69	54	16	174	77	259	4.6	0.9	2.1
3. Granules (fungus) + MI	977	56	49	12	368	93	280	3.7	0.6	6.6
4. Soil (fungus) + MI	710	67	57	7	586	93	293	3.3	1.7	9.8
5. Soil + chitin (fungus) + MI	541	74	66	24	500	57	286	3.0	1.6	13.1
6. Pellets + MI	640	83	68	0	777	199	412	2.3	0	0
7. Wheat + MI	420	89	83	0	797	189	313	3.8	0	0
8. Granules + MI	581	84	68	0	705	153	306	3.0	0	0
9. Soil + MI	479	91	87	0	722	157	459	2.7	0	0
10. Soil + chitin + MI	500	91	81	0	1,002	133	511	4.0	0	0
11. *M. incognita* only	252	95	96	0	910	132	537	4.5	0	0
12. Control (no nematodes, no fungus)	1,979	0	0	0	0	0	0	0	0	0
CV (%)	54	16	32	97	83	124	53	78	131	142
Linear contrasts†										
12 vs. 1–11	**									
11 vs. 1–10, 12	**	**	**		NS	NS	**	NS		
1–5 vs. 6–10	*	**	**		**	*	**	NS		
4 vs. 1–3, 5	NS	*	*	NS	NS	NS	NS	NS	NS	NS
5 vs. 1–4	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
2 vs. 1, 3	NS	*	NS	NS	NS	NS	NS	NS	NS	*
1 vs. 3	*	NS	NS	*	NS	NS	NS	NS	NS	*
1 vs. 6	NS	**	*		*	NS	*	NS		
2 vs. 7	NS	**	**		*	NS	NS	NS		
3 vs. 8	*	**	NS		NS	NS	NS	NS		
5 vs. 10	NS	**	NS		*	NS	*	NS		

Data in body of table are means of nine replicates; J2s = juveniles; CFU/g = colony forming units per gram of dry weight soil.
† Data for treatments with zero means were omitted from the analysis of variance. *, ** indicate significant differences at $P = 0.05$ and 0.01, respectively; NS = no significant difference.

Greatest numbers of eggs at harvest were in plots inoculated with *M. incognita* alone. Egg suppression at harvest was slightly higher in plots that had received the fungus in pellets (62%) than those that were treated with wheat (52%), granules (48%), soil (45%), or chitin-amended soil (47%).

The *P. lilacinus* populations increased from 556 to 2,000 CFU/g soil at planting to 2–11 times as many at harvest (Table 3). At harvest, *P. lilacinus* populations in plots treated with pelleted fungus were 20,556 CFU/g soil.

Discussion

Results of our experiments showed that the type of carrier used in the introduction of *P. lilacinus* to soil influenced its survival during storage, its establishment in field soil, and subsequent protection of tomato against *M. incognita*. The differences in survival of the fungus on carriers can be attributed to the availability of nutrients, length of storage, favorable temperature, and other growth factors (14). The addition of nutrients along with the propagules may enhance spore germination during assays. Some biocontrol fungi proliferate abundantly in various natural soils when propagules are in contact with a food base that allows the antagonist to grow through the soil (17). The addition of a food base along with the propagules may stimulate spores to germinate, but growth may not occur, possibly because the organic substrates are colonized and exploited more rapidly by pathogenic or saprophytic fungi possessing higher competitive saprophytic ability than the antagonists.

Other problems associated with the use of nutrient amendments with biocontrol agents result from stimulation of indigenous pathogens and hence an increase in disease. Excess nutrients (molasses) in granules containing *Trichoderma harzianum* can be used rapidly by the pathogen *Phytophthora cinnamomi*, especially in wet soils when growth of the antagonist is suppressed by lack of oxygen, resulting in increased disease (14). Although high survival of a potential biocontrol agent is desirable for efficacy (5,15), there is no experimental evidence to indicate the relationship between survival of *P. lilacinus* in various formulations and biological control.

In the present studies, the greatest percentage of loss in survival of *P. lilacinus* in pellets occurred during the first 2–4 weeks. Viability of conidia of various biocontrol fungi in alginate pellets formulated with a pyrophyllite clay decline by 10–100% over a 4-week period at room temperature, but some conidia still survived after 12 weeks (11). Similarly, viability of *Trichoderma* and *Gliocladium* in alginate pellets at 25 C remained high (> 70%) after 1 week, but declined to less than 10% after 24 weeks. Despite loss in propagule viability in stored pellets, numbers of CFU produced after adding these pellets to soil were comparable with those produced from freshly prepared pellets (16). Temperature during storage is an important factor in fungal survival in formulations. Conidia of *Talaromyces flavus* in alginate-bran pellets stored for 15 weeks survived better at 5 and 15 C than at 25 C (17). Accordingly, the viability of *P. lilacinus* was high in soil cultures stored at 5 C for more than 1 year.

Results from field tests show that diatomaceous earth granules, wheat, and alginate pellets were the most satisfactory carriers for *P. lilacinus* activity against *M. incognita*. Although *P. lilacinus* became established in all treated plots, the efficiency of biological protection declined by the end of the second growing season. Carriers with a nutrient base appeared to enhance *P. lilacinus* survival and antagonistic activity, and possibly allowed the colonization of other saprophytic micro-organisms.

Decomposition products from the nutrient base may also contribute to nematode suppression. Organic matter is known to stimulate the growth of predacious fungi, but it may also stimulate plant growth and offset nematode damage (15). During decomposition, organic matter may produce nematicidal breakdown products (18). Therefore, addition of large quantities of organic matter to soil may reduce nema-

tode numbers, but such effects are short lived unless added to soil repeatedly. Similarly, more than one application of *P. lilacinus* may be needed at the proper times to maintain effective biocontrol of nematodes (6,19).

Little is known about the fate of propagules of *P. lilacinus* added to soil in diverse environments. Our data show that some *P. lilacinus* added to soil as conidia without nutrient-supplying amendments survived more than 1 year. Length of survival, however, could depend on the isolate used or soil conditions. The *P. lilacinus* isolate used for the preparation of our formulations has the potential and aggressiveness to colonize and establish in natural environments. This isolate had been used to infest field soil infested with root-knot nematodes, then recovered from infected eggs.

Nematode reproductive potential should be considered in evaluating the utility of antagonists (15). In the second growing season, population levels of *M. incognita* increased 500× over those at planting time, whereas the numbers of *P. lilacinus* (CFU/g soil) increased only 10× (in pellets). Therefore, this fungus needs to be very efficient to reduce populations of nematodes such as *M. incognita* with a high reproductive capacity. Consequently, parasites or predators that attack adult stages of nematodes should be evaluated because they are more likely to suppress nematode multiplication than those that attack juveniles or eggs (15). The populations of *P. lilacinus* added to soil in the carriers in our study did not reach sufficient numbers to give immediate and economic control. A biocontrol fungus must survive several seasons and build up in soil to an optimum level to control *M. incognita*. Edaphic factors such as soil moisture affect nematode and fungal activity (12) and are likely to impact the efficacy of *P. lilacinus* as a biocontrol agent. More information is needed on inoculum level of *P. lilacinus* necessary to effectively control root-knot nematodes for various field conditions.

Several carriers can be utilized to deliver fungi to soil, but some of these require large quantities of material or are impractical. Alginate pellets show promise as a carrier of *P. lilacinus* and should be considered for commercial production because of their potential versatility in biological control. The advantages of pellets as carriers for biocontrol agents have been described by other investigators (9,11,16,22). The development of optimum delivery systems for biocontrol agents should aid in the advancement of biocontrol research and its integration into management systems.

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