Journal of Nematology 21(2):164–172. 1989.

Growth of Isolates of *Paecilomyces lilacinus* and Their Efficacy in Biocontrol of *Meloidogyne incognita* on Tomato[1]

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Abstract: The potential of 13 *Paecilomyces lilacinus* isolates from various geographic regions as biocontrol agents against *Meloidogyne incognita,* the effects of temperature on their growth, and the characterization of the impact of soil temperature on their efficacy for controlling this nematode were investigated. Maximum fungal growth, as determined by dry weight of the mycelium, occurred from 24 to 30 C; least growth was at 12 and 36 C. The best control of *M. incognita* was provided by an isolate from Peru or a mixture of isolates of *P. lilacinus.* As soil temperatures increased from 16 to 28 C, both root-knot damage caused by *M. incognita* and percentage of egg masses infected by *P. lilacinus* increased. The greatest residual *P. lilacinus* activity on *M. incognita* was attained with a mixture of fungal isolates. These isolates effected lower root-galling and necrosis, egg development, and enhanced shoot growth compared with plants inoculated with *M. incognita* alone.

Key words: bioassay, biocontrol, *Meloidogyne incognita, Paecilomyces lilacinus,* residual effect.

Biological control of plant-parasitic nematodes, including *Meloidogyne* spp., has potential (6,14,16,20). The discovery of fungi parasitic on the eggs of nematodes has been recent (11,14,16,21). *Paecilomyces lilacinus* (Thom) Samson is an effective parasite of the eggs of *Meloidogyne incognita* (Kofoid and White) Chitwood and *Globodera pallida* (Stone) Behrens (14). This fungus, found in most agricultural soils (19), also may infect adult stages of plant-parasitic nematodes (14). *P. lilacinus* grows well in temperature ranges between 15 and 30 C, but optimal growth occurs between 25 and 30 C, similar to its hosts (14). Efficacy of a Peruvian isolate in controlling *Meloidogyne, Globodera, Tylenchulus, Nacobbus,* and other nematode species affecting important crops under diverse climatic and soil conditions in more than 60 countries has varied greatly (14). Although significant control has been achieved, inconclusive results were obtained by some investigators in Brazil, Costa Rica, Malaysia, and the United States (14).

The general effects of temperature and other environmental factors on the reproduction of *Meloidogyne* spp., including those on tomato, have been determined (3,4,7,8,17,22,23). Information is needed, however, about the influence of environmental factors on the efficacy of *P. lilacinus* as a biocontrol agent on *M. incognita.* The objectives of this research were to 1) elucidate the effects of temperature on growth of different isolates of *P. lilacinus,* 2) determine the potential of individual isolates and a mixture of isolates of *P. lilacinus* as biocontrol agents against *M. incognita* under different soil temperatures, and 3) characterize any effects of soil temperature on the residual efficacy of single isolates or a mixture of *P. lilacinus* in controlling *M. incognita* in tomato.

MATERIALS AND METHODS

Influence of temperature on growth of Paecilomyces lilacinus: Each of 13 isolates of *P. lilacinus* from a wide range of geographic regions (Table 1) was cultured on potato dextrose agar (PDA) at 24 C. All stock cultures were stored at ca. 5 C. Most isolates sporulated within 3–5 days on PDA, and

Received for publication 22 January 1988.

[1] Paper No. 11419 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. This research was supported in part by the Latin American Scholarship Program of American Universities (LASPAU) and by the International Potato Center (CIP) grant provided to Dr. J. N. Sasser at North Carolina State University, Department of Plant Pathology.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the product named nor criticism of similar ones not mentioned.

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The authors thank D. W. Byrd, Jr., D. W. Corbett, Kathy Forrest, Evelyn B. Reller, Marvin Williams, Katherine Kershaw, and the staff at Central Corps Research Station for technical assistance.

by 7 days mycelial growth and spores almost covered the surface of the medium in 9-cm-d dishes. Growth of each isolate was assessed by seeding 20 ml potato dextrose broth (pH 5.5 in a 125-ml Erlenmeyer flask) with a 5-mm-d disc cut from the advancing edge of 7-day-old *P. lilacinus* cultures on PDA. The flasks were incubated at 12, 16, 20, 24, 28, 30, 32, 34, or 36 C in the dark for 7 days. All flasks were manually agitated every 24 hours to maintain adequate aeration. After incubation the fluid was removed from the cultures using Whatman 50 (2–3 μm retention) filter paper in a Buchner funnel with aspiration. Filter paper and fungal samples were placed on aluminum weighing dishes in a drying oven at 80 C for 8–10 hours and then weighed. There was one flask per isolate at each temperature; the test was run three times.

The experimental design was a split-plot with temperatures as whole plots and isolates as subplots. Linear fungus weight (dependent variable) was regressed against temperature (independent variable) for all isolates together and for each one individually, using linear and quadratic models. The coefficient of determination (R^2) and plots of standardized residuals vs. predicted values from regression analysis were used to evaluate goodness of fit to a model.

P. lilacinus control of M. incognita in tomato: *Meloidogyne incognita* race 1 (E 589-Crop Nematode Research and Control Project, Raleigh, NC) was reared on tomato, *Lycopersicon esculentum* Mill cv. Rutgers, in a greenhouse (26–28 C) for 60 days. Nematode inoculum consisted of eggs extracted by the NaOCl technique (2). Approximately 5,000 eggs in an aqueous suspension were placed in a depression in the center of each 15-cm-d pot containing 1,500 cm^3 of sterilized soil–sand (1:1) mixture.

Isolates of *P. lilacinus* selected for this study were 1) #2 (PL-85-1) from Peru, 2) #3 from Florida, 3) #8 from New York, and 4) #11 from California (Table 1). An aqueous conidial suspension from a 14-day-old PDA-grown culture of each fungus isolate (1.7×10^7 spores/ml in test 1; 1.2×10^7 spores/ml in test 2) was mixed separately in a steam-sterilized soil mix. Fifty milliliters conidial suspension was added to 1,500 cm^3 soil mix in sterile plastic bags and thoroughly mixed. Two days later, 100 cm^3 *P. lilacinus*-infested soil was introduced into each 15-cm-d plastic pot in temperature tanks where appropriate. A 4-week-old Rutgers tomato seedling was transplanted into each pot.

This experiment was performed in a greenhouse with water-bath tanks that maintained soil temperatures at 16, 20, 24, 28, 32, or 36 C. It was run 1 November 1985 to 7 January 1986 and repeated 22 May to 2 August 1986. Each week 100–150 ml nutrient solution (2.5 g Peters professional water soluble fertilizer 20-10-20 in 1 liter water) was added to each pot. Treatments were 1) *M. incognita* eggs (MI); 2) MI + *P. lilacinus* (PL) isolate 2; 3) MI + PL isolate 3; 4) MI + PL isolate 8; 5) MI + PL isolate 11; 6) MI + mixture of isolates 1, 3, 8, and 11; and 7) control (no nemas, no fungus). Each treatment was applied to soil in three 15-cm-d plastic pots with sealed bottoms containing steam-sterilized soil. The pots were placed randomly in each temperature tank. Temperatures were assigned at random to each tank in each trial. This experiment had a split-plot design with six temperatures as whole plots and three replicates of the seven treatments completely randomized within each temperature.

At the end of each test, shoot and root fresh weights, root gall indices (0–100) (3), root necrosis indices (0–100), nematode eggs per 10 g roots, juveniles per 500 cm^3 soil, eggs per 500 cm^3 soil, and percentage of egg masses infected by *P. lilacinus* were recorded. Final population densities of juveniles in the soil were determined after thoroughly mixing the soil from each pot. A 500-cm^3 subsample was processed by a combination of elutriation and centrifugal flotation (2). Eggs from soil samples were extracted from the root fraction caught on a 425-μm-pore sieve during extraction of juveniles. Eggs also were extracted separately from root fragments in the soil sam-

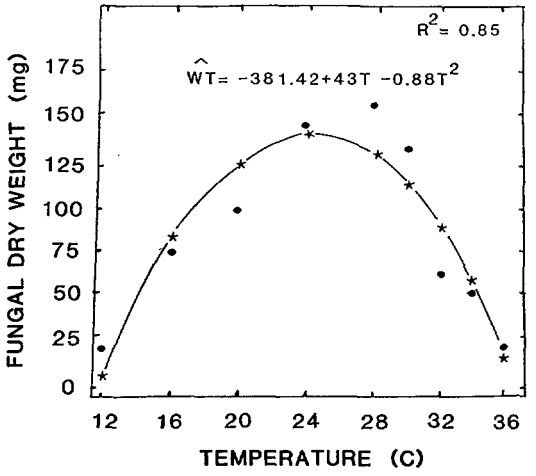

FIG. 1. Growth of *Paecilomyces lilacinus* isolates at different temperatures, expressed as dry weight (mg of mycelium) ● = observed values. * = predicted values.

ple and from 10 g roots by the NaOCl methods (2).

Egg masses of selected treatments of the first test were assayed for *P. lilacinus* (5). This assay included surface sterilization of roots with 0.525% NaOCl. Colonies were counted and their diameters were measured.

Analyses of variance were performed to test 1) the effects of temperature on *P. lilacinus*, 2) the interaction of temperature and the fungus on plant growth, and 3) the efficacy of *P. lilacinus* to control *M. incognita*. Data for treatments with zero means (uninoculated) were omitted from the analysis of variance because they had zero variance. Comparisons of treatment means were performed by Duncan's multiple-range test.

Residual efficacy of P. lilacinus to control M. incognita in tomato: A bioassay method was used to measure the residual nematode-biocontrol potential of different *P. lilacinus* isolates exposed to soil temperatures of 16, 20, 24, 28, 32, and 36 C from the experiment conducted in water-bath tanks. This experiment was established in a greenhouse (25 ± 2 C) 8 February and terminated 8 April 1986. For bioassays, roots were surface sterilized in 0.525% NaOCl for 1 minute, rinsed under a stream of cold water for several minutes to remove residual NaOCl, wrapped in moist paper towel, sealed in plastic bags, and stored at 5 C for 4 weeks. Five egg masses then were aseptically removed from the stored galled roots (from each pot of the previous experiment) and placed in a depression made in the center of each 10-cm-d pot containing soil. The soil used for this bioassay was the same type used for the previous temperature–tank experiment. Four-week-old Rutgers tomato seedlings were transplanted singly into depressions in the soil in each of 126 pots. The 10-cm-d pots were placed inside 15-cm-d pots to minimize contamination.

There were 42 treatments (seven treatments at six temperatures from the previous test) replicated three times in a completely randomized design. Plants were watered as needed and fertilized in the same manner as in the experiment in the temperature tanks. Data recorded were shoot fresh weight, root gall indices (0–100), root necrosis indices (0–100), juveniles per 500 cm^3 soil, and number of eggs per 500 cm^3 soil.

Analyses of variance were performed on the data, and Duncan's multiple-range test was used to compare means. Data for treatments with zero means were omitted from the analysis of variance because of their zero variance.

RESULTS

Influence of temperature on growth of P. lilacinus: Temperature differentially affected the growth of *P. lilacinus* isolates ($P = 0.01$). Mean fungal dry weight was greatest at 24 to 30 C (Fig. 1). Although there were striking differences in fungal dry weight among isolates (Tables 1, 2), the maximum yield in dry weight for all isolates occurred at 24–30 C. The least growth of all isolates occurred at 12 and 36 C. Isolates 11 and 12 grew well at 16 and 34 C (Table 2). The general response of fungal growth (WT) of all isolates as a function of temperature (T) was approximated by a quadratic growth curve (Fig. 1).

P. lilacinus biocontrol of M. incognita in tomato: Soil temperature greatly influenced

TABLE 1. Isolates of *Paecilomyces lilacinus* and mean dry weights of fungal growth in liquid culture in controlled growth chambers.

Code no.	*P. lilacinus* isolate	Source	Fungal dry weight (mg)
1	PL 84-1	International Potato Center, Lima, Peru (P. Jatala).	66
2	PL 85-1	Recovered through bioassay from infected egg masses of *M. incognita*, from soil infested with PL 84-1, Clayton, NC.	63
3	FL H-10	University of Florida, Gainesville, FL (D. W. Dickson).	106
4	AL 85-1	Auburn University, AL (R. Rodríguez-Kábana).	70
5	PL #1	Beltsville, MD (R. Sayre).	52
6	NRRL - 895	Beltsville, MD (R. Sayre).	71
7	RS 986	Japan. USDA—Insect Pathology Research Unit, Boyce Thompson Institute (R. A. Humber, Insect Mycologist). Host: *Bombix mori.*	69
8	RS 410	New York (R. A. Humber). Host: *Diapresa* sp.	106
9	CAL-1	California (J. Gaspar), "Grape Isolate."	71
10	CAL-2	California (J. Gaspar), Mel-pack isolate.	94
11	CAL-3	California (J. Gaspar), grape.	127
12	CAL-4	California (J. Gaspar), citrus orchard.	142
13	CAL-5	California (J. Gaspar), citrus.	65
LSD ($P = 0.05$) = 22			

Means of 27 observations (nine temperatures × three flasks per isolate).

the efficacy of the different isolates of *P. lilacinus* as biocontrol agents of *M. incognita* (Fig. 2). There were significant differences in gall indices, root necrosis, and percentages of egg masses infected by *P. lilacinus* due to temperature and isolate effects and the interaction of these two variables. Differences in numbers of eggs were limited to temperature (data not included). There were significant differences in shoot and root fresh weights due to treatment effects only. The best control of *M. incognita* on tomato was attained with fungus isolate 2 or a mixture of *P. lilacinus* isolates which resulted in about a 12% increase in shoot weight and 20% suppression of gall development, compared with plants inoculated with nematodes alone (Table 3, Fig. 2). With the exception of isolate 8, all plants treated with individual or mixed isolates showed increased shoot growth and inhibited root-gall development, compared with those treated with *M. incognita* alone.

The influence of each isolate and the mixture of those of *P. lilacinus* as biocontrol agents of *M. incognita* was temperature

TABLE 2. Influence of temperature on fungal growth in potato dextrose broth of selected isolates of *Paecilomyces lilacinus*, expressed in fungal dry weight (mg).

Code no.	*P. lilacinus* isolate	Temperature								
		12	16	20	24	28	30	32	34	36
1	PL 84-1	17	47	77	120	137	110	23	33	27
2	PL 85-1	27	40	73	103	133	107	30	33	17
3	FL H-10	13	87	110	187	177	153	100	107	20
5	PL #1	23	57	63	73	103	67	33	30	20
8	RS 410	23	113	150	173	197	233	27	23	10
11	CAL-3	23	117	167	207	200	187	116	97	27
12	CAL-4	17	130	150	197	213	280	146	117	27
LSD ($P = 0.05$) = 22										

Values are means of three tests. Information on each fungal isolate is listed in Table 1.

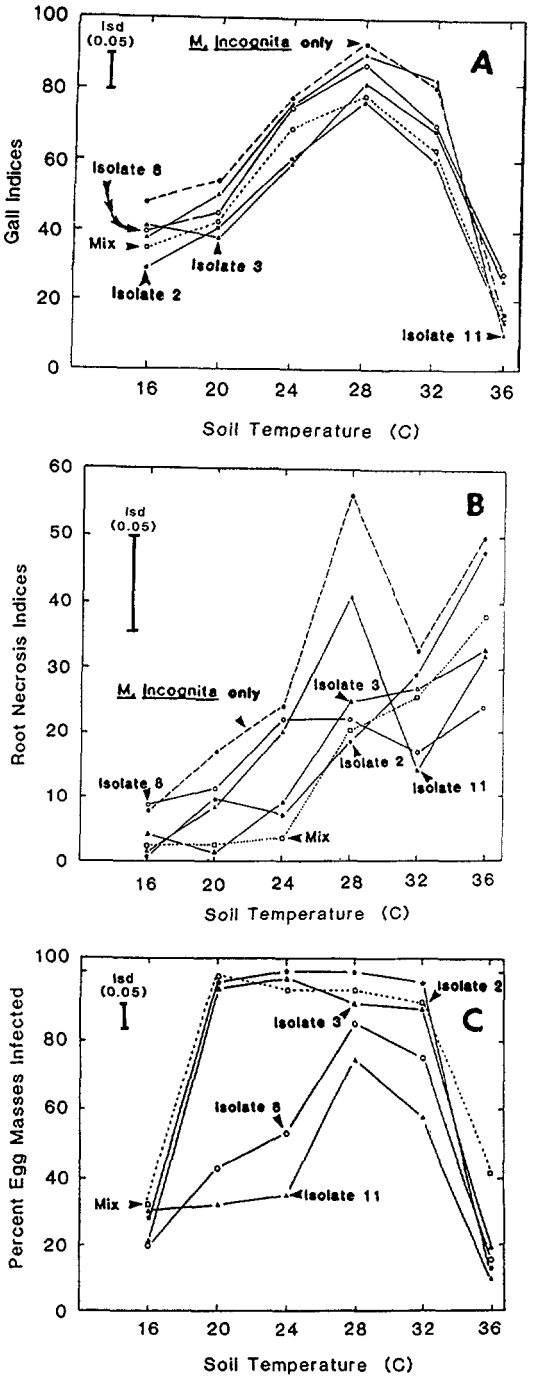

FIG. 2. Impact of *Paecilomyces lilacinus* isolates on root-knot and related disease on tomato at soil temperatures. All pots inoculated with 5,000 eggs. A) Gall development. B) Associated root necrosis. C) Effects of temperature on isolates infecting egg masses.

dependent. Adverse temperature effects on root-knot disease and plant growth were observed, especially at 16 and 36 C (Fig. 2A, B). Soil temperatures of 16, 20, 24, and 28 C resulted in progressively increased root-knot damage (Fig. 2A, B), but this was paralleled by an increase in egg-mass infection by *P. lilacinus* (Fig. 2C). The greatest percentage of egg mass infection generally occurred with isolates 2 and 3 and the mixture of selected ones (Table 4). Colony diameter in the bioassays was greater for isolate 2 and the mixture of isolates 2, 3, 8, and 11 than for isolates 3, 8, or 11 alone at each soil temperature (data not included).

Residual efficacy of P. lilacinus to control M. incognita in tomato: Gall indices, root necrosis, and numbers of juveniles of *M. incognita* per 500 cm^3 soil differed ($P = 0.01$) among *P. lilacinus* isolate treatments and temperatures (Table 5, Fig. 3). Although the influence of isolates ($P = 0.01$) and temperature ($P = 0.05$) on number of eggs per 500 cm^3 soil were both significant, the interaction between isolates and soil temperature ($P = 0.05$) was not. The greatest residual nematode control was attained with the mixture of fungus isolates, which resulted in 76% gall suppression, 90% root necrosis inhibition, and 84% suppression of nematode reproduction (eggs), compared with that on tomato plants inoculated with *M. incognita* alone (Table 5, Fig. 3A–C). Most plants treated with egg masses previously exposed to individual isolates or a mix of isolates of *P. lilacinus* resulted in a significant increase in shoot growth and limited galling and nematode reproduction, compared with those treated with nematodes alone ($P = 0.01$) (Table 5).

Generally, at each soil temperature, isolates 2 and 3 of *P. lilacinus* controlled *M. incognita* best, as judged by increase in shoot weight, gall suppression, inhibition of root necrosis, and suppression of egg development (Fig. 3). Use of the mixture of isolates of *P. lilacinus* at each soil temperature gave the best nematode control. This control resulted in a 21% increase in shoot fresh weight and suppression of gall indices

TABLE 3. Tomato growth, disease responses, and effects of different isolates of *Paecilomyces lilacinus* on *Meloidogyne incognita*.

Treatment	Fresh wt (g/plant)		Root gall indices†	Root necrosis†	Nematodes (in 1,000s)/500 cm³ soil		Eggs (in 1,000s)/ 10 g roots	Infected egg masses (%)
	Shoot	Root			Eggs	Juveniles		
M. incognita (MI)	49 d	13.7 c	61 a	31 a	33 a	0.7 a	128 a	0
MI + *P. lilacinus* (PL)-2	55 b	16.3 a	47 d	19 bc	22 d	0.2 c	48 d	73 ab
MI + PL-3	52 c	14.3 bc	53 c	17 bc	26 c	0.4 b	66 c	70 b
MI + PL-8	50 cd	13.9 c	57 b	17 bc	28 c	0.6 a	109 b	49 c
MI + PL-11	52 c	14.7 b	58 b	20 b	30 b	0.4 b	119 a	40 d
MI + mixture of PL-isolates 2, 3, 8, and 11	55 b	16.3 a	50 c	15 c	23 d	0.4 b	69 c	75 a
Control (no nemas, no fungus)	68 a	16.9 a	0	0	0	0	0	0

Data for treatments with zero means were omitted from the analysis of variance. Means within a column with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test (means taken over six temperatures and three applications).

† Root gall and necrosis indices: 0 = healthy and 100 = 100% of root affected.

(46%), root necrosis (90%), egg numbers in soil (84%), and numbers of *M. incognita* juveniles in soil (98%), compared with plants inoculated with nematodes alone (Fig. 3).

DISCUSSION

Results of this study generally corroborated previous findings on the effects of temperature on the growth of *P. lilacinus*, but the optimum temperature of given isolates ranged from 24 to 30 C. Although most isolates had similar growth patterns, some produced almost threefold greater mycelium than others. The moderate differential growth patterns of the isolates used in the mixture of *P. lilacinus* probably were partially responsible for its enhanced biocontrol of *M. incognita*, compared with single isolates. The isolates of *P. lilacinus* differed greatly in their temperature responses in controlling *M. incognita* in tomato. The differential effects of temperature on the residual biocontrol activity of these isolates were especially striking. In addition to fungal growth, synthesis of enzymes such as chitinase that may promote nematode colonization is probably affected by temperature (20). Isolate 2, reisolated from microplots infested with isolate 1, probably was less sensitive to fungistasis and lysis when added to soil than was conidia from pure cultures (10).

The effects of temperature on infection of eggs by fungal parasites are difficult to interpret. The residual biocontrol activity of *P. lilacinus* isolates suggests that temperature probably has an important impact on their survival. Temperatures that limit nematode egg hatch may increase parasitism by fungi unable to kill mobile juveniles (16). Although *P. lilacinus* grew most rap-

TABLE 4. Influence of soil temperature on the efficacy of *Paecilomyces lilacinus* (PL) isolates as antagonists of *Meloidogyne incognita* as measured through bioassays.

P. lilacinus isolate	Egg masses (%) infected with *P. lilacinus*					
	16 C	20 C	24 C	28 C	32 C	36 C
PL-2	28	98	100	100	97	13
PL-3	22	98	98	92	90	18
PL-8	22	43	53	85	75	15
PL-11	30	32	35	75	57	10
Mixture of PL-isolates 2, 3, 8, and 11	31	98	95	95	90	42
LSD ($P = 0.05$) = 9.6						

These values are means of three replications per treatment. Egg masses of *M. incognita* were plated on semiselective media and incubated at 25 ± 2 C for 5 days. Examination of egg masses for number and size of *Paecilomyces lilacinus* colonies completed the bioassay test. Data on colony diameter not included (5).

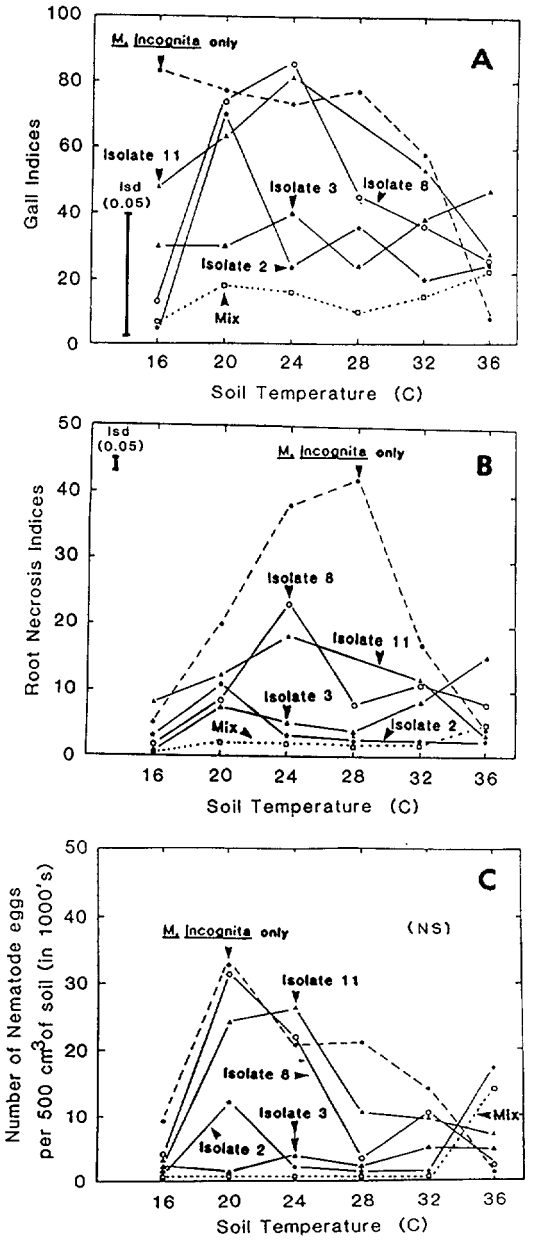

FIG. 3. Residual biocontrol activity of *Paecilomyces lilacinus* against *Meloidogyne incognita* on tomato after growth at six soil temperatures. A) Suppression of gall development. B) Inhibition of root necrosis. C) Suppression of nematode egg production.

idly between 24 and 30 C, egg masses were parasitized heavily at 20 C. More eggs probably were parasitized at temperatures lower than 24–28 C, below the optimum temperature (25 C) for hatching this nematode (15). About 50% of viable *M. incognita* eggs in egg masses became infected with *P. lilacinus* growing on an agar medium (9).

There was a loss of biocontrol effectiveness in isolates related to Peru isolate 2 when they were kept in the laboratory by periodic transfers on media in the absence of nematodes. Thus, the mere occurrence of an isolate of *P. lilacinus* on *M. incognita* in nature, or the observed invasion of nematode eggs by an antagonist in vitro, is insufficient evidence that biological control proceeds in a natural ecosystem. Mutations may explain the variable results that are being obtained in controlling *M. incognita* by an isolate of *P. lilacinus* when introduced into a new geographical area. Standard bioassays could be utilized to select the more virulent or aggressive colonizers of nematodes (20).

The rapid and high reproductive capacity of *M. incognita* and the differential temperature optima for egg hatch vs. nematode development (4,8,22) add to the difficulties of attaining adequate control by *P. lilacinus* isolates. The great reproductive capacity of *M. incognita* allows them to be successful parasites on crop plants even in the presence of many enemies (20). Furthermore, there was a striking increase in root necrosis in plants inoculated with the nematode alone as temperature increased. *Trichoderma harzianum* and *Fusarium oxysporum* f. sp. *lycopersici* were associated with this root necrosis. The interaction of *M. incognita* with associated soil micro-organisms and the resultant effects on root necrosis and wilt symptoms have been well documented (1,3,12,18). The suppression of root necrosis by *P. lilacinus* may be due to nematode control or its effects on other micro-organisms. Bacteria also may play a role in root necrosis and may affect the development of a disease complex on tomato (12). *P. lilacinus* producess a peptidal antibiotic which has wide antimicrobial activity against fungi, yeast, and gram-positive bacteria (13).

Isolate 2 of *P. lilacinus* originating from Peru, or the mixture of isolates, resulted in the best control of *M. incognita* over all soil temperatures tested. More informa-

TABLE 5. Residual effects of *Paecilomyces lilacinus* in soil from the temperature test as measured by growth of tomato, disease ratings, and numbers of *Meloidogyne incognita* eggs and juveniles.

Treatment	Fresh wt (g/plant)		Root gall indices†	Root necrosis†	Nematodes/500 cm³ soil	
	Shoot	Root			Eggs	Juveniles
M. incognita (MI)	52 c	23 a	63 a	21 a	16,295 a	1,871 a
MI + *P. lilacinus* (PL)-2	63 ab	25 a	30 d	4 bc	5,944 bc	205 b
MI + PL-3	63 ab	22 a	35 cd	7 bc	2,862 c	74 b
MI + PL-8	54 bc	23 a	46 bc	10 b	11,975 ab	184 b
MI + PL-11	59 abc	24 a	53 b	9 bc	13,616 ab	290 b
MI + mixture of PL-isolates 2, 3, 8, and 11	63 ab	22 a	15 e	2 c	2,535 c	42 b
Control (no nemas, no fungus)	68 a	24 a	0	0	0	0

Data are means for all temperatures; data for treatments with zero means were omitted from the analysis of variance. Means within a column with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

† Root gall and necrosis indices: 0 = healthy and 100 = 100% of root affected.

tion is needed, however, on ecological factors that account for variability so *P. lilacinus* or other nematode antagonists can be used more effectively against this and other plant-parasitic nematodes. Success in the future probably will result from a combination of antagonists that effectively inhibit *M. incognita* and other target organisms during different phases of their life cycles. Environmental conditions such as soil moisture or organic matter may be even more important than temperature preferences of the fungus in developing reliable biocontrols of nematodes. Unlike the fixed temperature effects, these parameters may be manipulated. Nevertheless, control agents may need to be applied in such a way to exploit temperature changes.

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