EFFECTIVENESS OF DIFFERENT ISOLATES OF *PAECILOMYCES LILACINUS* AND AN ISOLATE OF *CYLINDROCARPON DESTRUCTANS* ON THE CONTROL OF *MELOIDOGYNE JAVANICA*

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ABSTRACT

Freitas, L. G., S. Ferraz, and J. J. Muchovej. 1995. Effectiveness of different isolates of *Paecilomyces lilacinus* and an isolate of *Cylindrocarpon destructans* on the control of *Meloidogyne javanica*. Nematropica 25:109-115.

Nineteen isolates of *Paecilomyces lilacinus* from different countries and different regions of Brazil and one isolate of *Cylindrocarpon destructans* were evaluated *in vitro* for parasitism of eggs of *Meloidogyne javanica*. The isolates varied in their ability to colonize eggs. In greenhouse studies with tomato plants, isolates of *P. lilacinus* and *C. destructans* reduced the number of galls of *M. javanica* but differed in effectiveness. The isolates of *Paecilomyces lilacinus* from France and Italy were the most effective in both *in vitro* and greenhouse experiments.

Keywords: biocontrol, biological control, *Cylindrocarpon destructans*, *Meloidogyne javanica*, nematophagous fungi, *Paecilomyces lilacinus*, root-knot nematode.

RESUMEN

Freitas, L. G., S. Ferraz y J. J. Muchovej. 1995. Efectividad de diferentes aislamientos de *Paecilomyces lilacinus* y un aislamientos de *Cylindrocarpon destructans* en el control de *Meloidogyne javanica*. Nematropica 25:109-115.

Diez y nueve aislamientos de *Paecilomyces lilacinus* de diferentes paises y regiones de Brasil mas un aislamiento de *Cylindrocarpon destructans* fueron evaluados *in vitro* por su parasitismo en huevos de *Meloidogyne javanica*. Los aislamientos variaron en su habilidad para colonizar huevos. En estudios bajo invernadero con plantas de tomate, aislamientos de *P. lilacinus* y *C. destructans* redujeron el numero de agallas de *M. javanica* pero diferenciaron en su eficacia. Los aislamientos procedentes de Francia e Italia fueron los mas efectivos en los dos experimentos: *in vitro* e invernadero.

Palabras clave: biocontrol, control biológico, *Cylindrocarpon destructans*, hongos nematofagos, *Meloidogyne javanica*, nematodo agallador, *Paecilomyces lilacinus*.

INTRODUCTION

Many studies have been conducted to determine the potential use of various types of nematode biocontrol agents (9,10,12,13,15). Fungi have been the organisms studied most often as natural antagonists of nematodes (3). *Paecilomyces lilacinus* (Thom) Samson was described as an effective parasite of eggs of *Meloidogyne* spp. (8) but attempts to control various nematodes with this fungus have given inconsistent results (2). The specificity of an antagonist is critical for the successful biocontrol of certain plant parasitic nematodes (2); therefore, variability among iso-

lates is a important factor to be considered in the development of an effective biocontrol agent.

Cylindrocarpon destructans (Zin) Scholten, another soilborne fungus, has been reported as a pathogen of species of *Heterodera* and *Globodera* (12). In 1991, an isolate of this fungus was found parasiting females of *Meloidogyne* sp. in roots of peach (*Prunus persica*) in Pelotas, Rio Grande do Sul, Brazil (M. L. Mendes, personal communication).

The objectives of this work were to test the pathogenicity of *C. destructans* to *M. javanica* (Treub) Chitwood and to evaluate the effectiveness of *P. lilacinus* isolates from different geographic regions under standard environmental conditions as biocontrol agents of *Meloidogyne javanica.*

MATERIALS AND METHODS

Colonization of nematode eggs in vitro: The pathogenicity of 19 isolates of *P. lilacinus* from 4 different countries and from different regions of Brazil and one isolate of *C. destructans* to *M. javanica* eggs was studied *in vitro* (Table 1). A population of *M. javanica* was extracted from field soil in Minas Gerais state, Brazil, and maintained in the greenhouse for use in the experiment. Fifty, first-generation egg masses of the nematode were removed from roots of tomato (*Lycopersicon esculentum*) cv. Rutgers and transferred to a beaker containing 50 ml of sterile water. The beaker was agitated to promote washing of the egg masses. The water was discarded and the process repeated 5 times. The egg masses were then surface disinfested with a solution of mercuric chloride (1 g/L of ethanol at 30%) for 2 min and then washed in sterile water and treated with 0.5% sodium hypochlorite to dissolve the gelatinous matrix. Sterile water was added to the beaker to provide a concentration of 1 000 eggs/ml.

An 8-mm-diam disk containing each fungal isolate from a 15-day-old potato dextrose agar (PDA) culture was transferred to the center of each of 3 Petri dishes containing 2% water-agar. One ml of the egg suspension was then spread on the surface of the water-agar of each Petri dish. The *P. lilacinus* isolates were inocubated at 28°C and *C. destructans* at 20°C for 12 days. The temperatures were previously determined as optimum for mycelial growth of each species (7). One hundred eggs were then randomly collected from each Petri dish. The eggs were placed on a microscope slide in a drop of lactophenol with 0.05% cotton blue and observed at 100 × with a light microscope. Percentage colonization was obtained by counting the number of eggs with mycelium in their interior. Each treatment was replicated 3 times.

Control of M. javanica in Tomato Roots in the Greenhouse: Rice (*Oryza sativa* L.) grains were used as a carrier for *P. lilacinus* and *C. destructans* in the greenhouse experiment. Eighty g of rice were added to a 200-ml flask and washed twice. The flasks were then filled with tap water and allowed to stand for 2 min. The excess water was decanted and the flasks autoclaved for 20 min at 120°C. After cooling to room temperature, each flask was inoculated with an 8-mm-diam PDA disk culture of one of the fungal isolates. The isolates of *P. lilacinus* and *C. destructans* were incubated at 24°C and 20°C, respectively, for 15 days in the dark. The flasks were shaken vigorously each day to promote aeration and uniformity of the carrier.

A mixture of 2 parts soil and one part sand was used in the experiment. The soil used in the mixture had a pH of 6.2; 3.1 ppm P; 59 ppm K; and 0, 2.1, and 0.4 meq of $Al^{3}+$, $Ca^{2}+$, and $Mg^{2}+$ per 100 cm^{3},

Table 1. *In vitro* colonization of *Meloidogyne javanica* by 19 isolates of *Paecilomyces lilacinus* and 1 isolate of *Cylindrocarpon destructans*.

Isolate Number	Species	Researcher Source	Geographic Region	Colonized eggs (%)	SD[z]
2	*P. lilacinus*	Cayrol, J. C.	Italy	100.0	0.0
3	*P. lilacinus*	Jatala, P.	Peru	100.0	0.0
6	*P. lilacinus*	Carneiro, R. M. D. G	France	76.7	8.6
1	*P. lilacinus*	Cayrol, J. C.	France	68.7	15.9
13	*P. lilacinus*	Castro, M. E. A.	Brazil, SP, Campinas	58.0	7.5
19	*P. lilacinus*	Castro, M. E. A.	Brazil, SP, Campinas	37.3	5.7
5	*P. lilacinus*	Santos, M. A.	Brazil, RS, Pelotas	35.7	5.9
15	*P. lilacinus*	Castro, M. E. A.	Brazil, MG, Araguari	33.3	6.4
18	*P. lilacinus*	Freihs, L. G.	Brazil, MG, Vicosa	32.0	7.5
10	*P. lilacinus*	Castro, M. E. A.	Brazil, SP, Campinas	24.7	3.8
14	*P. lilacinus*	Castro, M. E. A.	Brazil, MG, Araguari	24.3	5.5
7	*P. lilacinus*	Freire, F. C. O.	Brazil, PA, Castanhal	21.7	2.1
8	*P. lilacinus*	Santos, M. A.	Brazil, PR, Atalaia	19.7	4.0
20	*C. destructans*	Mendes, M. L.	Brazil, RS, Pelotas	18.0	5.6
9	*P. lilacinus*	Castro, M. E. A.	Brazil, MG, Itamarandiba	17.0	7.0
4	*P. lilacinus*	Dalla Pria, M.	Brazil, SE, Boquim	12.0	2.6
11	*P. lilacinus*	Castro, M. E. A.	Brazil, SP, Campinas	9.0	2.6
16	*P. lilacinus*	Castro, M. E. A.	Brazil, MG, Itamarandiba	8.0	2.0
17	*P. lilacinus*	Castro, M. E. A.	Brazil, MG, Itamarandiba	7.0	3.6
12	*P. lilacinus*	Castro, M. E. A.	Brazil, SP, Campinas	2.0	2.0

[z]SD = Standard deviation

respectively. The soil was treated with methyl bromide (90 cm^3/m^3 substrate) for 96 hr in a closed container, and then spread in a thin layer over a bench in the greenhouse. The treated substrate was allowed to aerate for 1 week.

Treatments consisted of the addition of 5 g of rice infested with each of the following isolates into a 8.5-kg lot of soil: *P. lilacinus* isolates from Italy, France, Peru and Brazil (Vicosa, Minas Gerais) at 1.1×10^9, 1.4×10^9, 1.0×10^9, and 1.3×10^9, respectively. The spore density of each isolate in the medium was obtained by adding 1 g of infested rice into each of three 50-ml test tubes with 20 ml of water and a drop of Tween 80. The tubes were shaken for 5 minutes, and 5 spore samples from each tube were counted with the use of a haemocytometer. *Cylindrocarpon destructans* from Brazil was introduced into soil in the form of mycelia in rice grains (5 g) since no spores were produced. The control was prepared with noninfested, sterilized rice. The infested or noninfested rice was mixed thoroughly into the soil in plas-

tic bags. Four-liter pots received 2.5 kg of different inoculum mixtures and were irrigated for 10 days to permit the establishment of the fungi. After this period, 10 ml of a suspension of 5 000 *M. javanica* eggs was added to the substrate, and one 21-day-old 'Rutgers' tomato seedling was planted per pot. The plants were fertilized weekly, alternating N,P,K (4-14-8) at 2 g/pot and Hoagland's solution at 100 ml/pot (17). Each of the fungal isolates were tested in 7 replicates. The pots were maintained in a greenhouse in a completely randomized design. The temperature during the experiment ranged from 17.0 - 36.5°C.

The first evaluation of the experiment was made 80 days after the seedlings were planted. Fresh foliage including immature fruit was weighed. The root systems were removed from the pots and washed. Twenty egg masses were collected from each root system to evaluate the percentage of egg masses colonized by the fungi. The root systems were weighed and stained in a phloxine B solution (15 mg/L of water). The number of galls and egg masses, obtained from a 3-g subsample of roots, were recorded.

Second-stage juveniles (J2) of *M. javanica* were extracted from 100 cm^3 soil samples from each pot by centrifugal flotation (11). Other 21-day-old seedlings of 'Rutgers' tomato were transplanted into the pots to be used in a bioassay to indicate the number of ineffective J2 present in the soil. These plants were irrigated and fertilized as before. After 30 days, the shoot and root systems were weighed, and the galls were counted as described above.

Data were subjected to analyses of variance using SAEG software (6). Tukey's honestly significant difference test was used to determine differences between means at $P \leq 0.05$.

RESULTS AND DISCUSSION

The percentage of *M. javanica* eggs colonized by *P. lilacinus* varied considerably (Table 1). *Paecilomyces lilacinus in vitro* isolates from Italy and Peru colonized 100% of the eggs and were selected for further study in the greenhouse. The isolate from France colonized more than 70% of the eggs and also was selected for greenhouse studies. The other isolates from Brazil colonized from 2 to 69% of the eggs including 18% colonization by *C. destructans*. These data indicate different isolates of the same fungal species may differ greatly in their ability to colonize *M. javanica* eggs under standardized conditions. Similar variability also has been observed by Cabanillas *et al.* (2) and Santos (13). If a larger number of isolates from different regions of Brazil had been tested *in vitro* for egg colonization, some isolates that have colonization potentials similar or superior to those of the best isolates used in this trial may have been detected.

In the greenhouse, *P. lilacinus* isolates from Italy, Peru, and France reduced ($P \leq 0.05$) the number of galls per root system, the number of galls per g of root, and the number of *M. javanica* egg masses when compared to the control (Table 2). The *P. lilacinus* isolate from Viçosa, MG (Brazil) and *C. destructans* were not different from the control. Cabanillas *et al.* (2), working with *P. lilacinus*, also found significant differences in aggressiveness among isolates.

In the second planting, number of galls per root system were significantly lower following treatment with all *P. lilacinus* isolates and *C. destructans* compared to the control (Table 3). The *P. lilacinus* isolates from Italy, Peru, and France also reduced ($P \leq 0.05$) the number of galls per g of root. The *P. lilacinus* isolate from Brazil (Viçosa, Minas Gerais) and *C. destructans*

Table 2. Effect of 4 isolates of *Paecilomyces lilacinus* and 1 isolate of *Cylindrocarpon destructans* introduced into the soil on rice grains on the control of *Meloidogyne javanica* in 'Rutgers' tomato after 80 days in a greenhouse test.

Treatments	Dry shoot wt. (g)	Dry root wt. (g)	Galls/root system	Galls/g root	Egg masses/ root system	J2 No./ 100 cm^3 soil[y]	J2 No. hatched *in vitro*	Egg masses colonized (%)
PI[w]-Italy	130.6 a[x]	55.0 a	315.6 c	5.9 c	87.0 b	300.7 b	857.7 cd	35.7 ab
PI-Peru	142.0 a	47.9 ab	375.0 bc	8.1 bc	96.4 b	348.9 ab	429.3 e	59.3 a
PI-France	116.3 a	54.4 a	330.6 c	6.4 c	102.6 b	273.3 b	1125.5 c	20.7 b
PI[v]-Brazil	134.3 a	53.6 ab	520.4 a	10.0 abc	140.4 ab	325.6 ab	617.9 de	62.9 a
Cd[z]	116.3 a	36.8 b	457.9 ab	12.8 a	136.1 ab	59.1 ab	1729.1 b	17.9 b
Control	126.4 a	43.8 ab	466.9 a	11.6 ab	228.3 a	412.0 a	2330.0 a	—

[y]J2 = second-stage juveniles.
[w]PI = *Paecilomyces lilacinus.*
[x]Means in rows followed by the same letter are not significantly different at $P \leq 0.05$ according to Turkey's honestly significant differences test. Average of 7 replications.
[v]Isolate no. 18 from Vicosa, Minas Gerais, Brazil.
[z]Cd = *Cylindrocarpon destructans.*

did not differ from the control in the number of galls per g of root. The increase in effectiveness may be explained by the systematic reduction of nematode inoculum by the fungi during each nematode cycle (16). The persistence of the fungus, after a single inoculation, in succeeding generations of nematodes is consistent with the findings of Strattner (16) and Jatala (10).

The greater number of galls per root system and galls per g of root following treatment with the *P. lilacinus* isolate from Brazil (Viçosa, Minas Gerais) and *C. destructans* compared to other isolates of *P. lilacinus* may indicate they require a longer period of time to establish (5). This may be especially true of *C. destructans*, since its inoculum was composed of mycelia only. According to Strattner (16), the number of first-generation eggs produced in soil infested or not infested with *P. lilacinus* may be the same, but 80 to 90% of the eggs produced in the infested soil are infected by the fungus. Therefore, a progressive nematode reduction in subsequent generations should occur. Unlike chemical nematicides, introduction of antagonistic microorganisms may not immediately reduce the nematode population, but once established, the microorganisms may act to induce soil suppressiveness for long periods without new applications.

The number of *M. javanica* juveniles in the soil were 13 to 34% less in soil infested with *P. lilacinus* treatments than in the control (Table 2). However, the J2 reductions ($P \leq 0.05$) were significant only in pots with the isolates from France and Italy. Juvenile deformations may be induced by *P. lilacinus* at all embryonic developmental stages (4) and may impede root penetration by the juveniles. However, most of the deformations are not visible when observing the juveniles under the light microscope. Even juveniles with normal appearances may be damaged by exogenous compounds from *P. lilacinus* (10,14). Consequently, the best biological indicator of the number of infective juveniles remaining in the soil is the number of galls formed in tomato

Table 3. Effect of 4 isolates of *Paecilomyces lilacinus* and 1 isolate of *Cylindrocarpon destructans* introduced into the soil on rice grains on the control of *Meloidogyne javanica* in a second planting of 'Rutgers' tomato after 30 days in the greenhouse.[z]

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Number of galls per root system	Number of galls per g of root
P. lilacinus	79.90 ab	27.29 a	365.14 d	13.90 b
P. lilacinus	81.76 ab	24.84 a	379.43 cd	15.93 b
P. lilacinus	76.42 ab	27.65 a	444.14 bc	17.22 b
P. lilacinus	81.47 ab	24.33 a	502.86 b	20.99 ab
C. destructans	64.79 b	22.18 a	428.00 bcd	19.18 ab
Control	84.85 a	23.54 a	587.43 a	26.07 a

[z]Means in columns followed by the same letter are not significantly different at $P \leq 0.05$ according to Turkey's honestly significant difference test; average of 7 replications.

roots in subsequent plantings (Table 3). *Paecilomyces lilacinus* colonized 21 to 63% of the egg masses removed from tomato plants and *C. destructans* colonized 18%. This resulted in 22 to 81% fewer juveniles hatching from fungal infected egg masses than from noninfected egg masses (Table 2).

The results of this study indicate that the *in vitro* test may be useful as an easy screening procedure to reduce the number of isolates tested in greenhouse or field experiments. It is interesting that fungal isolates from distant geographic regions were more effective in controlling nematodes than the local isolates under the condition of these tests. The isolate of *C. destructans* studied was, under these conditions, not effective or less effective than *P. lilacinus* in controlling *M. javanica*. Since *in vitro* temperature requirements for both fungal species were different, pot tests conduced at relatively high temperature may have influenced the results. It is possible that *C. destructans* is a weak parasite or saprophyte that may become a pathogen of *M. javanica* only under certain conditions.

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