EVALUATION OF 20 SPECIES OF FUNGI FROM BRAZIL FOR BIOCONTROL OF *MELOIDOGYNE INCOGNITA* RACE 3

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ABSTRACT

Santos, M. A., S. Ferraz, and J. J. Muchovej. 1992. Evaluation of 20 species of fungi from Brazil for biocontrol of *Meloidogyne incognita* race 3. Nematropica 22:183–192.

Twenty species of fungi isolated from root and soil samples collected in 50 localities in Brazil were tested on agar as potential biological control agents against second-stage juveniles (J2), eggs, and egg masses of *Meloidogyne incognita* race 3. *In vitro* tests with eggs and egg masses showed isolates of *Paecilomyces lilacinus, Arthrobotrys conoides, A. musiformis, A. robusta, Monacrosporium ellisosporum, Dactylaria thaumasia, Cylindrocarpon* sp., and *Trichoderma harzianum* to be efficient parasites. Substantial variability in virulence among isolates of the same species was observed. Juveniles were parasitized by nematode-trapping fungi but not by opportunistic or ectoparasitic fungi. The more promising isolates of *M. ellipsosporum, P. lilacinus, F. oxysporum, T. harzianum, A. robusta,* and *D. thaumasia* were further evaluated against *M. incognita* race 3 in greenhouse pots. Each fungus and the nematode, alone or in combination, were mixed into soil that either had been fumigated or had not been fumigated, and after 10 days one tomato seedling was transplanted to each pot. After 60 days, *M. ellipsosporum* was the only fungus to show potential in the control of *M. incognita*. Numbers of galls and egg masses in roots, and juveniles in the soil were significantly reduced.

Key words: Arthrobotrys conoides, Arthrobotrys musiformis, Arthrobotrys robusta, biological control, *Meloidogyne incognita, Monacrosporium ellipsosporum.*

RESUMEN

Dos Santos, M. A., S. Ferraz y J. J. Muchovej. 1992. Evaluación de 20 especies de hongos de Brasil para el control biológico de *Meloidogyne incognita* raza 3. Nematrópica 22:183–192.

Se evaluaron en agar 20 especies de hongos aislados de muestras de raíces y suelo recolectadas en 50 localidades de Brasil, como agentes de control biológico frente a segundos estadíos juveniles, huevos y masas de huevos de *Meloidogyne incognita*. Ensayos *in vitro* mostraron que aislados de *Paecilomyces lilacinus, Arthrobotrys conoides, A musiformis, A robusta, Monoacrosporium ellipsosporum, Dactylaria thaumasia, Cylindrocarpon* sp. y *Trichoderma harzianum* fueron parásitos eficientes. Se observó una variabilidad considerable entre aislados de la misma especie. Estadíos juveniles fueron parasitados por hongos atrapadores de nematodos pero no por hongos parásitos facultativos o ectoparásitos. Los aislados más interesantes, *M. ellipsosporum, P. lilacinus, F. oxysporum, T. harzianum, A. robusta* y *D. thaumasia*, se evaluaron nuevamente frente a *M. incognita* raza 3 dentro de macetas en invernadero. Cada hongo y el nematodo, solo o en combinación se mezclaron con suelo fumigado y sin fumigar. Luego, a los 10 días se trasplantó una plántula de tomate en cada maceta. Después de 60 días, *M. ellipsosporum* fue el único hongo que mostró capacidad para el control de *M. incognita*. El número de agallas y masas de huevos en las raíces fueron significamente reducidos.

Palabras clave: Arthrobotrys conoides, Arthrobotrys musiformis, Arthrobotrys robusta, control biológico, *Meloidogyne incognita, Monacrosporium ellipsosporum.*

INTRODUCTION

The control of soil-borne plant-parasitic nematodes using trapping or parasitic fungi is of great interest because the use of nematicides or soil sterilants brings about various ecological and toxicological dangers (5). The initial screening of fungi may be done *in vitro* to permit the evaluation of a large number of fungi, which can then be studied in the greenhouse or in the field. *In vitro* screen-

ing of nematode trapping or parasitic fungi tends to overestimate control potential as it does not provide the nematode with opportunities to escape that would occur naturally.

The objective of this study was to evaluate *in vitro* the potential of 50 isolates of nematophagous fungi against vermiform *Panagrellus* sp. and juveniles, eggs, and egg masses of *Meloidogyne incognita* race 3. The fungi were obtained from soil and root samples from different localities in Brazil. Six species of these fungi selected *in vitro*, were further evaluated under greenhouse conditions for the control of *Meloidogyne incognita* race 3.

MATERIALS AND METHODS

The fungi, 15 isolates of nematode trapping fungi, 25 of ectoparasitic fungi, and 10 of opportunistically parasitic fungi were isolated from 50 localities in Brazil (13). These were maintained in culture on potato-dextrose agar (PDA) until used.

Meloidogyne incognita race 3 was provided by the Institute of Agronomy of Paraná, Brazil (IAPAR) and increased on tomato (*Lycopersicon esculentum*) cv. Rutgers under greenhouse conditions for 45 days.

In vitro *studies:* Egg masses of *M. incognita* were hand picked from tomato roots, placed in a beaker and rinsed five times with sterile distilled water. The rinse water was discarded and 50 ml of 1 000 ppm $HgCl_2$ in ethanol was added. After treatment for 2 min, the mercuric chloride-ethanol solution was discarded and the egg masses were rinsed five times with sterile distilled water. These egg masses were then used directly or were used as sources of eggs and second-stage juveniles (J2). Eggs in initial stages of embryonic development were obtained by treating egg masses with a 0.5% NaOCl solution (8). As soon as the gelatinous matrix dissolved, sterile water was added to the beaker and the suspension was calibrated to 1 000 eggs/ml. To obtain juveniles, egg masses were poured into hatching chambers (8) and maintained for 24 hr. Sterile water was then added to collect juveniles released from the eggs. The final suspension was adjusted to 300 J2/ml. An unidentified *Panagrellus* sp. was cultured on oatmeal in petri plates. The nematodes were harvested by adding water to the petri plate lid, onto which many of the nematodes had migrated.

Petri plates were prepared containing water agar (2%) with an 8-mm disk of 15-day-old fungal colony placed in the center. For *Panagrellus* sp., 1 000 non-axenized nematodes were added per plate. For *M. incognita*, 1 000 eggs/plate (two replicates), 3 egg masses/plate (three replicates), or 300 J2/plate (three replicates) were added. The *Panagrellus* sp. and the *M. incognita* eggs and J2 were spread uniformly over the entire surface of the agar plates, whereas egg masses were placed near the disc. Controls consisted of plates to which one disc of PDA without fungus was added and to which then were added *Panagrellus* sp., or *M. incognita* eggs, egg masses, or J2. Plates were incubated at 28 C in the dark for up to 10 days. Evaluations of parasitism and predation were done daily. Also recorded was the time required for trap formation. For *Panagrellus* sp. and J2 of *M. incognita*, the occurrence of predation or parasitism was determined directly on the water agar.

After 10 days, eggs were collected at random from the each plate and transferred to microscope slides with a drop of 0.05% cotton blue in lactophenol, and the percentage of parasitized eggs was determined. For egg masses, one mass from each replicate was selected and placed on a microscope slide with a drop of 0.05%

cotton blue in lactophenol. The occurrence of fungal colonization of the gelatinous matrix and the number of parasitized eggs in each egg mass were observed. The fungus was reisolated and compared with the original isolate.

Greenhouse study: Arthrobotrys robusta (isolate 32), *D. thaumasia* (isolate 41), *M. ellipsosporum* (isolate 2), *P. lilacinus* (isolate 4), *F. oxysporum* (isolate 9), and *T. harzianum* (isolate 30), were grown on potato dextrose agar amended with peptone (PDA-P, PDA + 10 g peptone/L) for 15 days. Sterile water was then added to each plate, the conidia were dislodged, and their concentrations were determined using a hemacytometer. The final suspension of each fungus was adjusted to 1.5 x 10^5 conidia/ml. Because of the high sporulation capacity by *P. lilacinus, F. oxysporum,* and *T. harzianum,* only one plate of each was needed, whereas three plates were required for *D. thaumasia.* For *M. ellipsosporum* and *A. robusta,* 24 plates were needed.

Eggs of *M. incognita* were extracted from tomato roots 45 days after inoculation by cutting the root system in 1–2 cm pieces and chopping the pieces in a blender with 0.5% aqueous NaOCl for 20 s. The suspension was then poured through a 200-mesh (75 μm pore diam) sieve stacked above a 500-mesh (29 μm pore diam) sieve. The eggs retained on the 500-mesh sieve were washed to remove residual NaOCl and transferred to a beaker (1), and the suspension was adjusted to 1 000 eggs/ml.

The soil used was a sandy clay loam with pH = 4.9, 1.5% organic matter, 4.3 ppm P, 54 ppm K, and 0.1, 2.4, and 0.5 eqmg/100 cm^3 soil of Al^{+++}, Ca^{++}, and Mg^{++}, respectively. Half of this soil was treated with methyl bromide (90 cm^3/m^3 soil).

Treatments consisted of each fungus and the nematode, alone or in combination, in soil that had been fumigated, and also in soil that had not been fumigated. Controls consisted of fumigated and non-fumigated soil with no organisms added.

Soil was inoculated with fungi and nematodes by placing 1.5 kg soil in a large plastic bag, adding 50 ml tap water and mixing. Ten ml of a suspension of fungal spores (1.5 × 10^6 conidia/pot) was added to the soil and mixed thoroughly. Thereafter, 10 ml of egg suspension (10^4 eggs/pot) were added; the soil was again mixed, and then transferred to clay pots of 2-L capacity. In the treatments with only fungi, 60 ml tap water and 10 ml conidia suspension were added. In treatments with only nematodes, 60 ml tap water and 10 ml egg suspension were added. The control treatments received 70 ml of tap water. A completely randomized design was used and each treatment was replicated six times.

The soil was kept moist and the pots were incubated under greenhouse conditions for 10 days. This simulated field conditions where the fungus is present before planting. After this period one 21-day-old tomato seedling (cv. Rutgers) was transplanted to each pot.

The pots were watered daily. After transplanting, 200 ml/pot of a modified Hoagland's solution (16) was applied weekly. The micronutrients B, Cu, and Mo were supplied as 0.88 g $CuSO_4 \cdot 5H_2O$, 0.99 g $H_2MoO_4 \cdot H_2O$ and 2.6 g H_3BO_3 per liter. Iron was supplied as Fe-EDTA at 7.54 g/L. The averages of the maximum and minimum temperatures, during the study, were 35.2 and 20.8 C, respectively.

Sixty days after transplanting, the top of each tomato plant was cut at ground level, placed in a paper bag, and oven-dried at 85 C to constant weight. The root system was gently separated from the soil and washed. The soil of each pot was mixed and a 150-cm^3 soil sample was col-

lected and processed by centrifugal flotation to determine the number of J2 (6).

The roots from treatments that did not contain nematodes were dried and weighed. In treatments that had been inoculated with nematodes, 10 egg masses were removed randomly to evaluate egg parasitism (7) by checking for the presence of fungal mycelium. The roots were then placed in agueous phloxine B solution (15 mg/L) to stain the remaining egg masses. Also, the number of galls per root system was determined. The roots were then dried and weighed.

Statistical analyses were done using SAEG (2) and when necessary data were transformed to log(X) or $X^{1/2}$.

RESULTS

In vitro *study:* All isolates of nematode-trapping fungi attacked *Panagrellus* sp., but within different time frames (Table 1). Three different time frames were found among the six isolates of *Arthrobotrys musiformis*. The percentage of parasitism of eggs was high with most isolates of nematode-trapping fungi (Table 1), although variability occurred within the same species. No parasitism was observed in the controls, indicating that the original egg masses removed from tomato roots were not parasitized, nor did the soil contain egg parasitizing fungi.

Table 1. *In vitro* colonization of eggs and egg masses of *Meloidogyne incognita* race 3 by nematode trapping fungi and time for trap formation when in the presence of *Panagrellus* sp.

		M. incognita			
			Egg masses		
Fungus	Isolate	Eggs[y] parasitized (%)	Colonization of matrix	Average no. of parasitized eggs	Time for trap formation[z]
Arthrobotrys robusta	1	2	+	0.3	2
	31	23	+	0	2
	32	72	+	19.3	3
	35	67	–	0	3
	38	53	+	0	2
	39	58	–	0	2
Arthrobotrys musiformis	18	1	+	0	1
	23	1	+	53.3	2
	26	8	+	3.7	3
	29	51	–	0	2
	34	50	–	0	2
	36	57	–	0	2
Arthrobotrys conoides	40	66	+	10.7	3
Dactylaria thaumasia	41	70	+	1.7	2
Monacrosporium ellipsosporum	2	68	+	0.3	3

[y]Previously separated from egg masses with 0.5% NaOCl solution.
[z]1 = > 96 hr; 2 = 48–96 hr; 3 = 24–48 hr.

Table 2. *In vitro* behavior of ectoparasitic and opportunistic fungi on *Meloidogyne incognita* race 3.

Fungus	Isolate	Eggs[z] parasitized (%)	Egg masses	
			Colonization of matrix	Average no. of parasitized eggs
Paecilomyces	4	80	+	13.7
lilacinus	50	3	+	29.7
Trichoderma hamatum	14	0	+	0
	21	6	+	0
T. aureoviride	16	12	+	0.7
T. harzianum	30	53	+	10.7
T. polysporum	42	9	+	0.3
Fusarium	8	23	+	0.7
oxysporum	9	31	+	0
	15	6	+	5.3
Fusarium	22	6	+	0
solani	25	8	+	1.3
	27	4	+	0.7
	28	4	+	35.7
	43	17	+	0.3
Fusarium equiseti	5	2	+	2.0
Acremonium	10	0	+	0
spp.	20	2	+	0
	24	5	+	0
	48	2	+	12.3
Chaetomium sp.	37	9	+	0
Penicillium	19	0	+	0
spp.	45	2	+	0
	46	0	+	0
Aspergillus sp.	33	2	+	0
A. niger	44	2	+	0
Circinella	12	1	–	0
sp.	13	2	–	0
Non sporulating	3	7	+	0
fungi	6	1	+	0
	7	1	+	0
	11	5	+	0
	17	0	+	12.3
	47	1	+	2.3
	49	7	+	0

[z]Previously separated from egg masses with 0.5% NaOCl solution.

All isolates of nematode-trapping fungi, with the exception of isolate 18 of *A. musiformis*, captured J2 of *M. incognita*. Except for some isolates of *A. musiformis* and *A. robusta* all isolates colonized egg masses. There was variation in the average number of parasitized eggs within egg masses, even though this was not correlated with the tests of individual eggs on agar.

No marked egg parasitism was observed for most of the isolates of fungi considered to be opportunistic or ectoparasitic (Table 2). All ectoparasitic and opportunistic fungi, with the exception of isolates of *Circinella* sp., colonized the gelatinous matrix (Table 2). Nevertheless, the number of parasitized eggs in egg masses was low with the exception of *Paecilomyces lilacinus* (isolate 4), *Trichoderma harzianum* (isolate 30), and *Cylindrocarpon* sp. (isolate 9). The two isolates of *P. lilacinus* showed different percentages of parasitized eggs (80% versus 3%). This also occurred with isolates of *Fusarium oxysporum* and *F. solani* and the species of *Trichoderma* (Table 2). Opportunistic and ectoparasitic fungi were unable to invade *Panagrellus* or J2 of *M. incognita*.

Greenhouse study: Of the six fungi studied, only *M. ellipsosporum* suppressed *M. incognita* race 3 by reducing ($P < 0.05$) the numbers of galls and egg masses on roots and juveniles in the soil (Table 3). The behavior of *M. ellipsosporum* was similar in fumigated and non-fumigated soil. The other fungi did not parasitize nematodes well in sterilized soil.

Monacrosporium ellipsosporum was the only fungus that colonized the gelatinous matrix and preyed upon J2. The numbers of hatched J2 in treatments receiving *M. ellipsosporum* was significantly reduced (Table 4). Other fungi were not observed colonizing the gelatinous matrix nor were eggs parasitized. Nevertheless, a smaller number of J2 was observed in natural soil than in fumigated soil, indicating probable egg and (or) juvenile parasitism.

In general, plant development was less in natural than in fumigated soil (Table 5). Dry matter production of plants in the treatments without fungi and with only fungi were similar, indicating that the isolates of fungi tested did not affect plant development. Dry matter production of plants in the treatment with *M. ellipsosporum* plus nematodes was similar to that in the treatments which did not contain nematodes, indicating that the plant was benefited by nematode control. The greater root weight in natural soil for this treatment resulted from a better developed root system rather than from an increased weight of galls on roots. In treatments receiving nematodes, roots were heavy due to the presence of many galls (Table 5).

DISCUSSION

Considerable variability was found among isolates. This variability could be due to selective adaptation to various edaphic factors at their geographic origin such as soil type or ambient temperature. We note, however, that *P. lilacinus* isolate 4 from Pelotas, Rio Grande do Sul, gave better results than isolate 50 from Atalaia, Paraná even though the average soil temperature at Atalaia is higher than at Pelotas and closer to the temperature that was used in the *in vitro* tests. Other factors may also be important. Freire and Bridge (3), working with *P. lilacinus* and *Verticillium chlamydosporium*, considered that the gelatinous matrix may facilitate parasitism due to aggregation of the eggs. This, however, was not indicated by our results, as most fungi colonized the gelatinous matrix, while few colonized

Table 3. Number of galls and egg masses per root system of tomato cv. Rutgers, and second-stage juveniles per 150 cm^3 soil of *Meloidogyne incognita* race 3, 70 days after soil infestation with six species of fungi under greenhouse conditions.

Treatment	Isolate	Galls		Egg masses		Second-stage juveniles	
		Fumigated soil	Non-fumigated soil	Fumigated soil	Non-fumigated soil	Fumigated soil	Non-fumigated soil
Monacrosporium ellipsosporum	2	527 B[y] a[z]	326 A a	183 C a	118 C a	141 B a	107 C a
Dactylaria thaumasia	41	966 A a	446 A b	547 AB a	108 B b	3 016 A a	272 B b
Arthrobotrys robusta	32	950 A a	443 A b	545 B a	313 A b	3 435 A a	865 AB b
Paecilomyces lilacinus	4	893 A a	514 A b	626 AB a	308 A b	2 911 A a	1 038 AB b
Fusarium oxysporum	9	895 A a	347 A b	668 AB a	210 AB b	4 334 A a	754 AB b
Trichoderma harzianum	30	1 015 A a	456 A b	778 A a	340 A b	3 702 A a	717 AB b
Control		1 069 A a	535 A b	709 AB a	350 A b	2 751 A a	2 179 A b

Data are averages of six replications.
[y]Averages followed by the same upper case letter in columns do not differ at $P = 0.05$ by Duncan's multiple-range test.
[z]Averages followed by the same lower case letter within rows do not differ at $P = 0.05$ by the Student's *t*-test.

Table 4. Number of second-stage juveniles of *Meloidogyne incognita* race 3 hatched from 10 egg masses randomly collected from root systems of tomato cv. Rutgers, and plated on water agar in petri plates for 10 days.

Treatment	Fumigated soil	Non-fumigated soil
Monacrosporium ellipsosporum	2 035 C[y] a[z]	2 005 B a
Dactylaria thaumasia	4 465 AB a	3 728 A a
Arthrobotrys robusta	5 333 A a	4 243 A a
Paecilomyces lilacinus	4 577 AB a	3 978 A a
Fusarium oxysporum	3 933 C a	3 740 A a
Trichoderma harzianum	4 637 AB a	3 392 A a
Control	4 757 AB a	3 568 A a

Data are averages of six replications.
[y]Averages followed by the same upper case letter within columns do not differ at $P = 0.05$ by Duncan's multiple-range test.
[z]Averages followed by the same lower case letter within rows do not differ at $P = 0.05$ by the Student's *t*-test.

eggs. Stirling and Mankau (15) found that *Dactylella oviparasitica* infected more eggs in small egg masses (300–400 eggs) than in large egg masses (1 000–1 500 eggs). Preliminary studies indicated that our population of *M. incognita* produced about 400–500 eggs per egg mass. Based on this and the large variability of the results, the size of the egg mass may be a relatively unimportant factor governing the percent colonization that occurs.

Since the opportunistic and ectoparasitic fungi do not produce structures for the capture of nematodes, the constant movement of nematodes on an agar surface should interfere with sustained contact and penetration of the fungi into live juveniles (3). On the other hand, dead juveniles would facilitate penetration by fungi. Therefore, the possibility exists that many ectoparasitic fungi detected in previous studies and tested here are saprophytic rather than parasitic. There is evidence that many egg parasites produce enzymes with chitinolytic activity (5), which are thought to be required for penetration of the chitinous egg shell. Although chitin is not present in the cuticle of juvenile nematodes, other specific enzymes may be essential to penetrate the cuticle of living juveniles.

Nicolay and Sikora (11) noted that a parasitic fungus may not be observed in eggs due to the digestion of their contents, thus leaving an empty egg shell. Determining whether an empty egg shell is the result of parasitism, physiological death, or eclosion is difficult. Our use of newly formed eggs in *in vitro* evaluations indicates that fungi attacking these eggs probably are parasitic rather than saprophytic (14).

The number of galls, egg masses per root system, and the number of J2 detected were less in non-fumigated than fumigated soil. This suggests that in the absence of the natural complement of soil microflora, the fungi that were introduced utilized food sources other than nematodes, or were unable to compete with other fungi and bacteria recolonizing the soil. Prior to parasitism and predation, introduced predacious and facultatively parasitic fungi must be able to grow and colonize the soil or rhizosphere, which requires energy (9), and therefore must be able to compete for nutrients with the native flora. If the native flora has enhanced competitive saprophytic ability, the introduced flora may turn to other sources of nutrients. Saprophytically growing predacious fungi submitted to nutritional stress have been shown to change their behavior and prey on

Table 5. Dry matter production of tops and roots of tomato cv. Rutgers (in grams per plant) at 81 days of age after infestation of soil with fungi and *Meloidogyne incognita* race 3.

	Tops		Roots	
Treatment	Fumigated soil	Non-fumigated soil	Fumigated soil	Non-fumigated soil
Monacrosporium ellipsosporum + N[y]	9.90 A[z]	3.20 BCDE	2.72 ABCD	1.51 AB
Paecilomyces lilacinus + N	8.63 A	1.63 EF	4.25 AB	1.02 ABCDE
Fusarium oxysporum + N	7.93 A	1.88 DEF	4.31 AB	0.87 BCDE
Trichoderma harzianum + N	7.51 A	1.43 F	4.27 AB	0.99 ABCDE
Arthrobotrys robusta + N	8.56 A	1.76 EF	3.87 ABC	1.34 ABCD
Dactylaria thaumasia + N	7.94 A	3.01 BCDEF	3.99 ABC	1.60 A
Monacrosporium ellipsosporum	7.37 A	5.15 A	2.24 DE	0.86 BCDE
Paecilomyces lilacinus	7.87 A	4.75 AB	1.22 E	0.80 CDE
Fusarium oxysporum	9.80 A	3.85 ABC	2.54 BCDE	0.69 E
Trichoderma harzianum	8.29 A	3.26 BCDE	1.77 DE	0.73 E
Arthrobotrys robusta	7.80 A	4.05 AB	1.60 DE	0.97 ABCD
Dactylaria thaumasia	9.42 A	4.02 AB	2.44 CDE	0.76 DE
N	10.20 A	2.17 CDEF	4.84 A	1.44 ABC
Control	9.19 A	3.50 ABCD	2.82 BCDE	0.66 E

Data are averages of six replications.

[y]N = inoculated with *M. incognita* as well as fungi indicated.

[z]Averages followed by the same upper case letter in columns do not differ at $P = 0.05$ by Duncan's multiple-range test.

nematodes, until the nutrient deficiency is removed (4,17). Alternative explanations for smaller numbers of nematodes in non-fumigated that in fumigated soil, include the possible presence of indigenous nematode antagonists, and the smaller root systems of plants growing in non-fumigated soil.

Soil microflora may also compete with plants for nutrients (12) and can liberate toxic metabolites affecting plant growth (10). This type of reaction is implicated by the greater dry matter production of plants in fumigated than in non-fumigated soil, whether or not nematodes were also present.

Although *Monacrosporium ellipsosporum* isolate 2 did not excel when the parameters of average number of parasitized eggs within egg masses and time for trap formation were observed *in vitro*, it was the best isolate tested in the pot experi-

ment. Since its performance did not appear to be affected by fumigation of the soil, it would be a good candidate for field studies.

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