Supplement to Journal of Nematology 26(4S):600–605. 1994.

Efficacy of *Paecilomyces lilacinus* in Suppressing *Rotylenchulus reniformis* on Tomato[1]

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Abstract: Effects of rice-cultured *Paecilomyces lilacinus* on *Rotylenchulus reniformis* were studied in both greenhouse and field microplot tests with 'Rutgers' tomato. Numbers of *R. reniformis* were reduced ($P \leq 0.05$) by *P. lilacinus*, with suppression in the initial greenhouse test ranging from 46 to 48% for two rice + *P. lilacinus* treatments; the rice-only treatment caused a nonsignificant reduction of 25%. In the second greenhouse test, total *R. reniformis* numbers were restricted ($P \leq 0.05$) by 41% by the rice + *P. lilacinus* treatment, whereas the rice-only treatment had a slight negative effect (16% inhibition, NS). Total numbers of *R. reniformis* were suppressed 59 and 36% at midseason and harvest, respectively, in microplots infested with *P. lilacinus*. The fungus was recovered from egg masses via isolations in the second greenhouse test. Shoot and fruit growth of Rutgers tomato were restricted by *R. reniformis* in the initial greenhouse test irrespective of *P. lilacinus* treatment, but this nematode did not affect fresh shoot weights in the second greenhouse test. The nematode also limited shoot growth of Rutgers tomato in microplots, and *P. lilacinus* suppressed *R. reniformis* numbers sufficiently to prevent related impairment of shoot and fruit growth. This study indicated that *P. lilacinus* has detrimental effects on *R. reniformis* population development under both greenhouse and field microplot conditions.

Key words: biological control, *Lycopersicon esculentum*, nematode, *Paecilomyces lilacinus*, reniform nematode, *Rotylenchulus reniformis*, tomato.

The fungus *Paecilomyces lilacinus* has shown potential as a biological control agent for sedentary plant-parasitic nematodes (6). This fungus is an egg parasite of sedentary nematode species; eggs of these nematodes are found either in egg masses or cysts and thus are more vulnerable to fungal attack (6). *Paecilomyces lilacinus* has been effective in controlling species of *Meloidogyne* (7), *Tylenchulus* (6), *Globodera* (4,8), and *Nacobbus* (6). Reddy and Khan (11) reported that *P. lilacinus* reduced total populations of *R. reniformis* on 'Pusa Ruby' tomato (*Lycopersicon esculentum*).

The objectives of our experiments were twofold: first, to determine whether *P. lilacinus* would suppress *R. reniformis* on tomato under greenhouse conditions; and second, to evaluate the efficacy of this fungus as a biocontrol agent for *R. reniformis* under field microplot conditions.

Received for publication 18 May 1993.

[1] The research reported in this publication was funded in part by the North Carolina Agriculture and Life Sciences Research Service, North Carolina State University, Raleigh, and USAID/USDA/OICD Grant 58-319R-1-008.

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The authors thank Dr. J. P. Noe for supplying us with the population of *Rotylenchulus reniformis* and Dr. S. R. Koenning for his statistical advice.

Materials and Methods

First greenhouse experiment. The reproductive and damage potentials of *R. reniformis* were determined on 'Rutgers' tomato in soil infested with *P. lilacinus* under greenhouse conditions. An isolate of *P. lilacinus* obtained from the International Potato Center, Lima, Peru (6), was established in microplots near Clayton, North Carolina, and later re-isolated from soil (3). The procedure used to isolate and grow *P. lilacinus* on rice grains was previously described (3). The experiment included five treatments: control (no nematode eggs, no rice grains, and no fungus); *R. reniformis* eggs only; *R. reniformis* eggs + 10 g rice grains (rice-only); *R. reniformis* eggs + 5 g rice grains colonized by *P. lilacinus*; and *R. reniformis* eggs + 10 g colonized rice. Treatments were randomized in a complete block design with five replications. Each rice amendment was mixed with a loamy sand soil (85% sand, 10% silt, and 5% clay) and placed in a 15-cm-d (1,700 cm^3 volume) clay pot. Three weeks later, Rutgers tomato plants (2–3 leaf stage) were transplanted into soil in pots and those pots receiving *R. reniformis* were infested with 5,000 eggs. Extracted with a 4-minute exposure to 1% NaOCl (1) from

'Better Boy' tomato grown for approximately 115 days in a greenhouse with temperatures averaging from 26–30 C. Experimental units were watered twice daily and fertilized once a week with Peter's 20-20-20 (N-P-K) (W. R. Grace & Co., Fogelsville, PA).

Plants were harvested 70 days after nematode inoculation. Fresh shoot, fruit, and root weights, number of vermiform nematodes in soil, eggs in soil (2), and number of eggs on roots (1) were determined. Data were analyzed statistically, with the general linear models (GLM) and correlation (CORR) procedures of SAS (13). Treatment means were compared by single degree of freedom contrasts.

Second greenhouse experiment: The effects of *P. lilacinus* on *R. reniformis* were again evaluated on Rutgers tomato under greenhouse conditions. This test was conducted to verify earlier results, as well as to reisolate *P. lilacinus* from infected egg masses. The inocula for *P. lilacinus* and *R. reniformis* were prepared in the same manner as for the first experiment. The *P. lilacinus* concentration was determined to be 2.0×10^3 cfu/gram of colonized rice. The experiment involved four treatments: control (no nematode eggs, no rice, and no fungus); *R. reniformis* eggs only; *R. reniformis* eggs + 10 g rice grains (rice-only); and *R. reniformis* eggs + 10 g rice grains colonized by *P. lilacinus*. Treatments were randomized in a complete block design with five replications. Amendments were mixed with a loamy sand soil (85% sand, 10% silt, and 5% clay) and placed in a 15-cm-d (1,700 cm^3 volume) clay pot. Three weeks later, Rutgers tomato seedlings (2–3 leaf stage) were transplanted into soil in pots and pots receiving *R. reniformis* were infested with 10,000 eggs. Plants were grown at temperatures averaging between 25 and 29 C, watered and fertilized as described for the first experiment.

Plants were harvested 70 days after nematode inoculation. Fresh shoot and root weights, numbers of vermiform nematodes in soil (2), eggs in soil (2), and numbers of eggs on roots (1) were determined. Five egg masses were collected from each root system infected with *R. reniformis*, surface sterilized with 0.5% NaOCl for 30 seconds, rinsed twice in sterile tap water, and uniformly distributed on one Petri dish containing *Paecilomyces*-semi-selective medium (3). Dishes were incubated at room temperature (approximately 25 C) for 5 days before infected egg masses were counted, and the number of egg masses infected by *P. lilacinus* were expressed as the percentage of egg masses infected (3). Data were analyzed with the GLM procedure of SAS (13), and treatment means were compared by single-degree-of-freedom comparisons.

Microplot experiment: During 1992, a microplot experiment was conducted at the Central Crops Research Station, near Clayton, North Carolina, to determine whether *P. lilacinus* would limit *R. reniformis* reproduction and (or) damage to Rutgers tomato. Fiberglass microplots were 76-cm-d and 50–55 cm in depth. Soil in microplots was a Fuquay sand (94.0% sand, 5.5% silt, and 0.5% clay). Microplots were fumigated with ca. 98 g a.i. methyl bromide + 2 g a.i. chloropicrin/m^2 in November before spring planting. The test was a 2 × 2 factorial with *R. reniformis* (2,500 nematodes/500 cm^3 soil) or no nematodes and two levels of *P. lilacinus* (0 to 20 g/microplot) (the rice-only treatment was omitted since it was not significantly different from the *R. reniformis*-only treatment for total numbers of nematodes in the greenhouse tests). Treatments were arranged in randomized complete blocks with four replicates. Rice grains supporting *P. lilacinus* were prepared as in the first greenhouse experiment. Two weeks before microplot infestation with *R. reniformis*, 20 g of *P. lilacinus*-colonized rice (1.67×10^3 cfu/500 cm^3 soil) were incorporated 20 cm deep into the soil of each microplot that received the fungal treatment. Better Boy tomato roots infected with *R. reniformis* were cut into 1-cm long pieces and mixed with infested soil in which these tomatoes were grown. Vermiforms and eggs in soil were determined by centrifugal flotation (2), and

numbers of eggs on roots were determined by the NaOCl method (1). Two liters of infested soil and root fragments contained 450,000 nematodes (eggs and vermiforms) which approximated 2,500 nematodes/500 cm^3 soil in microplots. The no-nematode treatment received 2 liters of a moist, sterile soil mix (85% sand, 10% silt, and 5% clay).

Fruit were harvested and weighed at 1-week intervals during the harvest season. Shoot weights were determined at the final harvest. Midseason and end of season soil samples were taken and consisted of 15 cores (2.5-cm-d × 20 cm deep) for each microplot. Numbers of nematodes were determined from 500-cm^3 subsamples. Soil samples were processed by elutriation and centrifugation to extract vermiforms (2). Roots were collected during elutriation, and numbers of eggs on roots were determined (1). Data were analyzed using the GLM and CORR procedures of SAS (13).

Results and Discussion

First greenhouse experiment: Total numbers of *R. reniformis* (eggs and vermiforms) were reduced ($P \leq 0.05$) when treatments included *P. lilacinus* (Table 1). This fungus reduced ($P \leq 0.05$) the total numbers of *R. reniformis*, with a suppression ranging from 46–48% for the two rice + *P. lilacinus* treatments compared with the *R. reniformis*-only treatment (Table 1).

Numbers of *R. reniformis* eggs on roots were also suppressed by *P. lilacinus* compared with either the *R. reniformis*-only or rice-only treatments. Both *P. lilacinus* treatments reduced nematode eggs on Rutgers tomato roots ($P \leq 0.05$). Numbers of *R. reniformis* eggs on roots were reduced 65% for *R. reniformis* + 5 g infested rice and 63% for *R. reniformis* + 10 g infested rice. Numbers of eggs in soil were slightly lower ($P \geq 0.05$; NS) for the rice-only and rice + *P. lilacinus* treatments compared with the *R. reniformis*-only treatment, possibly indicating that other organisms growing on the rice, besides *P. lilacinus*, were detrimental to eggs in the soil or that the decomposition of rice produced some substance that inhibited nematode development (5,9). No treatments suppressed numbers of vermiforms found in the soil; however, there was a slight, but nonsignificant, decrease for numbers of vermiforms in the *P. lilacinus* treatments (Table 1). The absence of differences between the *P. lilacinus* treatments for all responses (Table 1) indicated that there was not a dosage

Table 1. Impact of *Paecilomyces lilacinus* on *Rotylenchulus reniformis* reproduction on 'Rutgers' tomato in a greenhouse.

Treatment	Fruit and shoot weight (g)	Rotylenchulus reniformis numbers (in 1,000's)			
		Eggs on roots	Eggs in soil	Vermiforms in soil	Total number nematodes
1. Control (no eggs, no rice, or no fungus)	289	—	—	—	—
2. 5,000 eggs	177	51	61	62	174
3. 5,000 eggs + 10 g rice	207	52	32	47	131
4. 5,000 eggs + 5 g rice w/*P. lilacinus*	196	15	36	40	91
5. 5,000 eggs + 10 g rice w/*P. lilacinus*	185	20	41	33	94
CV (%)	18	66	83	46	49
Linear contrasts†					
1 vs. 2 + 3 + 4 + 5	***	—	—	—	—
2 vs. 4 + 5	NS	*	NS	NS	*
3 vs. 4 + 5	NS	*	NS	NS	NS

Data are means of five replications of one plant each. * and *** indicate significance at $P \leq 0.05$ and 0.001, respectively. NS = not significant.

† Treatment 2 vs. 3 + 4 + 5, 2 vs. 3, and 4 vs. 5 were not significant for all variables listed.

effect for *R. reniformis* control by this fungus.

Rotylenchulus reniformis suppressed shoot and fruit growth of Rutgers tomato in the greenhouse; there was a significant difference between the control and all treatments that included *R. reniformis* for shoot and fruit weights (Table 1). Tomato growth did not differ between the *R. reniformis*-only and the treatments that included rice-only or rice + *P. lilacinus*, indicating that *P. lilacinus* did not alter the suppression of tomato growth due to *R. reniformis*. All measures of nematode reproduction were negatively correlated with shoot and fruit growth: eggs in roots ($r = -0.41$, $P = 0.06$), eggs in soil ($r = -0.61$, $P = 0.003$), vermiforms in soil ($r = -0.63$, $P = 0.002$), and total nematode numbers ($r = -0.66$, $P = 0.0009$).

Second greenhouse experiment: Eggs and vermiforms of *R. reniformis* were again suppressed by *P. lilacinus*. Total numbers of *R. reniformis* were reduced 41% when compared with the nematode-only treatment, but not when compared to the rice-only treatment (Table 2). Total numbers of nematodes for the rice-only treatment were suppressed by 16% when compared with the *R. reniformis*-only treatment, indicating that rice had a negative effect on *R. reniformis* population development.

Numbers of *R. reniformis* eggs on roots were suppressed ($P \leq 0.05$) by *P. lilacinus* compared with the *R. reniformis*-only or rice-only treatment; the fungus suppressed nematode reproduction as indicated by the lower numbers of eggs found on the roots of Rutgers tomato. Rice + *P. lilacinus* limited the number of eggs on roots ($P \leq 0.05$), whereas the rice-only treatment did not affect numbers of eggs on roots (Table 2). *Paecilomyces lilacinus* was successfully recovered from 48% of the egg masses picked from roots grown in fungus-infested soil (Table 2). Attempts to recover *P. lilacinus* from egg masses from all other treatments infected with *R. reniformis* were unsuccessful.

Numbers of eggs in soil were lower for the rice-only and rice + *P. lilacinus* treatments compared with the *R. reniformis*-only treatment, again possibly indicating that either organisms colonizing the rice or products from rice decomposition were detrimental to eggs in the soil (5,9). No treatment affected numbers of vermiforms found in the soil, although slightly fewer vermiforms were recovered in the rice + *P. lilacinus* treatment (Table 2). Restriction in numbers of vermiforms in soil could result from reduced numbers of viable eggs, since *P. lilacinus* is reported only to infect eggs (7). None of the treatments affected shoot growth of Rutgers tomato in this test.

TABLE 2. Effects of *Paecilomyces lilacinus* on *Rotylenchulus reniformis* reproduction on 'Rutgers' tomato in a greenhouse.

Treatment	Rotylenchulus reniformis numbers (in 1,000's)				Egg mass infection (%)
	Eggs on roots	Eggs in soil	Vermiforms in soil	Total number nematodes	
1. Control (no eggs, no rice, or no fungus)	—	—	—	—	—
2. 10,000 eggs	11	18	27	56	0
3. 10,000 eggs + 10 g rice	12	8	27	47	0
4. 10,000 eggs + 5 g rice w/*P. lilacinus*	6	6	21	33	48
CV (%)	38	77	56	43	75
Linear contrasts					
2 vs. 3	NS	*	NS	NS	
2 vs. 4	**	**	NS	*	
2 vs. 3 + 4	NS	**	NS	NS	
3 vs. 4	**	NS	NS	NS	

Data are means of five replications of one plant each (soil volume 1,700 cm^3). * and ** indicate significance at $P \leq 0.05$, and 0.01, respectively. NS = not significant.

Microplot experiment. Numbers of *R. reniformis*, including eggs on roots and vermiforms in soil, were suppressed ($P \leq 0.05$) by *P. lilacinus* at midseason and harvest (Fig. 1). The reduction in numbers of vermiforms in soil was probably due to the low viability of eggs infected by *P. lilacinus* (6). Differences existed ($P \leq 0.05$) between sampling dates (midseason and harvest) for numbers of eggs in roots, vermiforms in soil, and total nematode numbers. *Rotylenchulus reniformis* numbers increased from midseason to harvest, and *P. lilacinus* did not restrict *R. reniformis* numbers sufficiently to prevent this increase. Nonetheless, the suppression of total *R. reniformis* numbers was 59 and 36% at midseason and harvest, respectively (Fig. 1), indicating that the fungus had a detrimental effect on reniform nematode population development.

Fresh fruit weights were not affected by *R. reniformis*; however, shoot growth was restricted, and *P. lilacinus* limited the effect of shoot-growth suppression by *R. reniformis*. Shoot weights were negatively correlated with eggs on roots and vermiforms in soil at midseason and harvest (Table 3), indicating that *R. reniformis* suppressed shoot growth of Rutgers tomato. In treatments receiving *P. lilacinus*, there was no relationship between fruit or shoot weights and numbers of *R. reniformis* at either sampling date; however, in the treatments that did not receive *P. lilacinus*, significant negative correlations existed between fruit or shoot weights and numbers of *R. reniformis* at both sampling dates (Table 3). The only correlations that were not significant were fruit weights with vermiforms in the soil at both sampling dates. Thus *P. lilacinus* suppressed *R. reniformis* numbers sufficiently to allow increased shoot and fruit weights.

Paecilomyces lilacinus has been widely tested on many plant-parasitic nematodes, but few studies have concerned the effects on *R. reniformis* (10). Our study verifies partial control in the greenhouse and is the first to demonstrate some biological control under field microplot conditions. Thus, this fungus may have some potential as a biological control agent for *R. reni-*

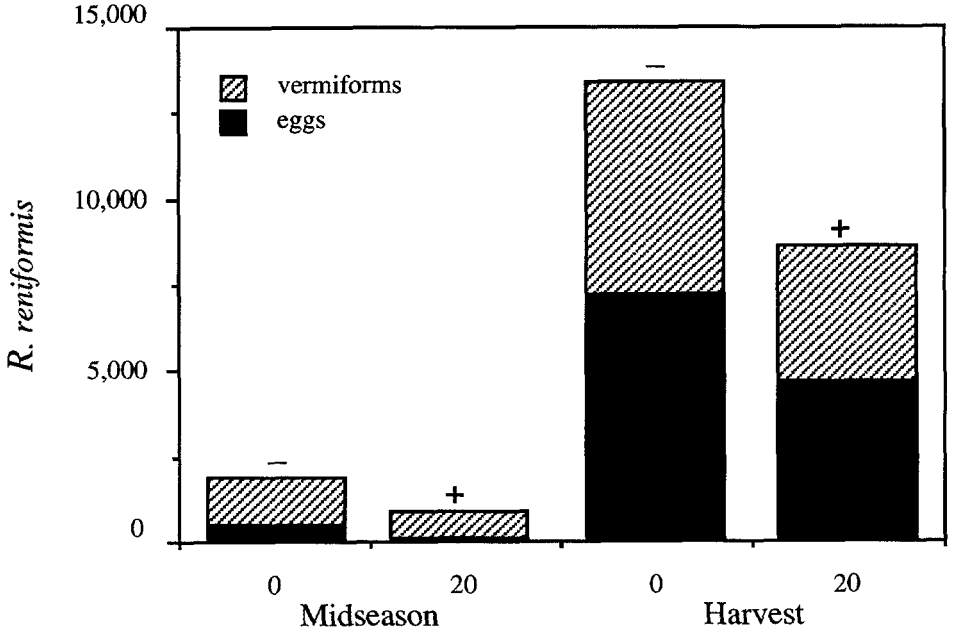

FIG. 1. Influence of *Paecilomyces lilacinus* on *Rotylenchulus reniformis* reproduction on 'Rutgers' tomato in microplots at midseason and harvest (microplots were initially infested with 2,500 *R. reniformis*/500 cm^3 soil). + = 20 g rice grains + fungus added to soil (1.67×10^3 cfu/500 cm^3 soil), and – = nothing added to soil. Treatment separation based on Fisher's LSD at $P \leq 0.05$.

TABLE 3. Correlations between 'Rutgers' tomato shoot and fruit growth with midseason and harvest counts of *Rotylenchulus reniformis* in field microplots.

	Midseason			Harvest		
	Eggs on roots	Vermiforms in soil	Total	Eggs on roots	Vermiforms in soil	Total
Shoot weight†	−0.63**	−0.65**	−0.67**	−0.65**	−0.57*	−0.62**
Shoot weight‡	−0.86**	−0.83**	−0.86**	−0.87**	−0.85**	−0.86*
Fruit weight‡	−0.77*	−0.68NS	−0.73*	−0.73*	−0.70NS	−0.72*

* and ** indicate significance at $P \leq 0.05$ and 0.01, respectively. NS = not significant.
† Correlations are based on 16 observations, including microplots infested and noninfested with *P. lilacinus*.
‡ Correlations are based on eight observations, including only those not infested with *P. lilacinus*.

formis in areas of the world where the nematode is an important plant parasite. However, the efficacy observed in these initially near-sterile environments likely would be lower in natural agroecosystems (14).

Although this paper deals with one organism for controlling *R. reniformis*, the effort is only an initial step toward developing a program in which several antagonists may be used. Most organisms used for biological control of nematodes are opportunistic parasites. They often lack the ability to become good biological control agents as they have the ability to feed on a diverse array of organic materials in the soil (14). Most studies on biological control have demonstrated that organisms have low efficacies, which make them unacceptable as sole agents for nematode control; however, integrating several organisms with other antagonists could make an excellent model for nematode control (12).

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