

EFFECTIVENESS OF SPECIES OF *GLIOCLADIUM*, *PAECILOMYCES*, AND *VERTICILLIUM* FOR CONTROL OF *MELOIDOGYNE ARENARIA* IN FIELD SOIL

R. Rodríguez-Kábana, G. Morgan-Jones, G. Godoy, and B. O. Gintis
Department of Botany, Plant Pathology and Microbiology, Auburn University, Alabama Agricultural Experiment Station, Auburn, Alabama 36849, U.S.A.

Accepted:

19.X.1984

Acceptedo:

ABSTRACT

Rodríguez-Kábana, R., G. Morgan-Jones, G. Godoy, and B. O. Gintis. 1984. Effectiveness of species of *Gliocladium*, *Paecilomyces*, and *Verticillium* for control of *Meloidogyne arenaria* in field soil. *Nematopica* 14:155-170.

The potential of several species and isolates of *Gliocladium*, *Paecilomyces*, and *Verticillium*, grown on autoclaved oat kernels, for use as biocontrol agents against *Meloidogyne arenaria*, was evaluated in two greenhouse experiments. The colonized oats were added to soil infested with *M. arenaria* at rates of 0-1.0% (w/w). Unamended soil and soil treated with uncolonized autoclaved oats were included in the experiments as controls. After treatment, soil was seeded with 'Summer Crookneck' squash (*Cucurbita pepo*) and allowed to develop for 6 weeks in a greenhouse. All amendments with uncolonized oats reduced the number of galls/g of root. Additional reductions in numbers of galls were observed in soil amended with oats colonized with 2 isolates of *Gliocladium roseum*, one isolate of *G. catenulatum*, one isolate of *Paecilomyces lilacinus*, and one isolate each of *Verticillium lamellicola* and *V. chlamydosporium*. Amendments with either of two other isolates of *P. lilacinus* or with one isolate of *P. nostocoides* were ineffective in reducing root galling by *M. arenaria*. All isolates and species of *Gliocladium*, all isolates of *P. lilacinus* but one, and the single isolate of *P. nostocoides* were recovered at the end of the experiments from soils to which they were added; *V. chlamydosporium* and *V. lamellicola* were not recovered from soil amended with oat cultures of these fungi. The most common fungal species isolated from *M. arenaria* females obtained from root galls was *Fusarium oxysporum*. Species of *Paecilomyces* or *Verticillium* were never found associated with the females, whereas *Gliocladium* spp. were isolated from females obtained from galls in roots of plants from soils treated with *Gliocladium*-colonized oats. The results indicated the effectiveness of fungi for control of *M. arenaria* depends on the species and individual isolates within a species.

Additional key words: population dynamics, nematode ecology, nonchemical control, methods, peanut root-knot nematode, organic amendments.

RESUMEN

Rodríguez-Kábana, R., G. Morgan-Jones, G. Godoy y B. A. Gintis. 1984. Eficacia de

especies de *Gliocladium*, *Paecilomyces* y *Verticillium* para combatir *Meloidogyne arenaria* en un suelo de campo. Nematrópica 14:155-170.

Se efectuaron dos experimentos de invernadero para evaluar el potencial de varias especies y cepas de *Gliocladium*, *Paecilomyces* y *Verticillium* para el combate biológico de *Meloidogyne arenaria*. Para este propósito se inocularon con los hongos granos de avena previamente esterilizados al autoclave. Una vez colonizados los granos se les añadieron a un suelo infestado con *M. arenaria* a razón de 0-1.0% (p/p). En cada experimento también se incluyeron un tratamiento con granos de avena esterilizados pero sin inocular, así como otro con suelo sin enmienda alguna. Una vez efectuados los tratamientos se sembraron todos los suelos con semillas de calabacín (*Cucurbita pepo*). A las plantas resultantes, se les permitió desarrollar por 6 semanas en un invernadero. Las enmiendas con granos de avena sin inocular resultaron en bajas en el número de agallas/gm de raíz. Los tratamientos con granos colonizados con 2 cepas de *Gliocladium roseum*, o con una cepa de *G. catenulatum*, así como con una de *Paecilomyces lilacinus* o una de *Verticillium lamellicola* y otra de *V. chlamydosporium* resultaron en bajas en el número de agallas/gm de raíz más pronunciadas que las obtenidas con la avena sin colonizar. Enmiendas con avena colonizada con dos otras cepas de *P. lilacinus* o con una de *P. nostocoides* fueron inefectivas para disminuir el agallamiento causado por *M. arenaria*. Todas las cepas y especies de *Gliocladium*, así como todas menos una de las de *P. lilacinus*, y la de *P. nostocoides* fueron recobradas al final del experimento de los suelos a los que habían sido añadidas; no se recuperaron sin embargo las cepas de *V. chlamydosporium* o de *V. lamellicola* de suelos enmendados con avena colonizada por estos hongos. La especie fungosa aislada con más frecuencia de las hembras de *M. arenaria* provenientes de las agallas en las raíces fue *Fusarium oxysporum*. No se observó la presencia de especies de *Paecilomyces* o de *Verticillium* en las hembras del nematodo aunque sí se obtuvieron cepas de *Gliocladium* spp. de las hembras extraídas de agallas de raíces de plantas provenientes de suelos tratados con avena colonizada por especies de *Gliocladium*. Los resultados obtenidos señalan que la eficacia de los hongos para combatir *M. arenaria* depende no sólo de la especie sino también de la eficacia de las cepas dentro de cada especie.

Palabras claves adicionales: dinámica poblacional, ecología de los nematodos, metodología, nematodos noduladores, enmiendas orgánicas, combate sin nematicidas.

INTRODUCTION

A ubiquitous mycoflora, taxonomically diverse but restricted in number of species, can be found associated with eggs, females, or cysts of *Heterodera glycines* Ichinohe and of *Meloidogyne* spp. in agricultural soils (2, 3, 6, 9, 10, 13). Many of the taxa in this specialized mycoflora are capable of colonizing nematode eggs in vitro (4). A possibility thus exists of using these fungi as biological agents for control of plant parasitic nematodes. *Paecilomyces lilacinus* (Thom) Samson and *Verticillium chlamydosporium* Goddard were successfully used to control *Meloidogyne* spp. under greenhouse and/or field conditions (6, 7, 8). They were also shown, in ultrastructural studies, to be effective in destroying eggs of *Meloidogyne arenaria* (Neal) Chitwood (11, 12). Although a substantial body of literature has now accumulated documenting the constant asso-

ciation of some fungal species with nematode reproductive structures, little is known of the relative potential of individual taxa for use as biocontrol agents. Such potential would in part be dictated by factors such as competitive ability in soil and capacity to build up inoculum under a range of environmental conditions. Likewise there is little, if any, published information on variability within species and whether or not selection pressures have led to emergence of biotypes having biocontrol capacity not common to all isolates. In this paper the results of greenhouse studies designed to determine the biocontrol potential of several isolates and species in the genera *Gliocladium* Corda, *Paecilomyces* Bainier, and *Verticillium* Nees, are presented.

MATERIALS AND METHODS

Soil for the experiments was obtained from a peanut (*Arachis hypogaea* L.) field at the Wiregrass Substation near Headland, Alabama. The field was under continuous peanut culture for the preceding 7 years and was heavily infested with *M. arenaria*. The soil was a sandy loam with less than 1.0% (w/w) organic matter and pH of 6.2. The soil was sieved (1-mm mesh) and mixed with builder's sand (\leq mm mesh) in a 50% (v/v) ratio, henceforth referred to as soil. The soil was apportioned in 1.0-kg amounts into polyethylene bags where it was thoroughly mixed with oat inoculum colonized with various fungi to receive either 0, 0.25, 0.5, or 1.0% (w/w) of the amendment. The amended soil was placed into 10-cm-diam (1.2 L) cylindrical polyvinyl chloride (PVC) pots. The pots with the soil were placed in a greenhouse and kept moist (60% field capacity) for 10 days before each were planted with 5 'Summer Crookneck' squash (*Cucurbita pepo* L.) seeds. Plants were allowed to develop for 6 weeks, after which the experiments were terminated.

Fungal isolates for the experiments were obtained in previous studies from cysts or eggs of *H. glycines* or *M. arenaria*. Fungal cultures were maintained on chitin agar (4). Inoculum was prepared following a modification (6) of the method of Epps *et al.* (1). Oats were boiled in water for 20 min, strained, and apportioned into 250-ml Erlenmeyer flasks to give 50 cm³ oats/flask. The flasks with oats were closed with styrofoam stoppers and sterilized by autoclaving for 30 min followed by a second autoclave treatment of equal duration 14 hr later.

The sterilized flasks were each inoculated with a 1-cm-diam agar disk from the periphery of actively-growing 3- to 7-day-old chitin agar cultures of the appropriate fungal species. Inoculated flasks were placed in an incubator (25C) in which the fungi were allowed to grow with periodic shaking of the flasks until the surface of all oat kernels were colonized. The time required varied with the fungal species: 4-7 days for species of

Gliocladium and *Paecilomyces* and 10 days for *Verticillium* spp. The colonized oats were spread on aluminum foil and were allowed to dry in an air conditioned room at 25-26C. Once dried, the oats were placed in polyethylene bags and stored at 4C in the dark until needed.

Two experiments were conducted in the greenhouse. One experiment studied the efficacy of 2 isolates of *Gliocladium roseum* Bainier, and one isolate each of *G. catenulatum* Gilman and Abbott and *Verticillium lamellicola* Gams. All isolates of these fungi had been obtained from females or cysts of *H. glycines*. Colonized oats bearing each fungus were added to the soil to give 0.25 or 0.50% (w/w) of inoculum. Controls with uncolonized autoclaved oats were also added at the same rates. Each treatment of the experiment was represented by 10 replications (pots) arranged in a completely randomized design.

A second experiment was conducted to assess the efficacy of 3 isolates of *P. lilacinus*, and one isolate each of *P. nostocoides* Dunn and *V. chlamydosporium*. One isolate of *P. lilacinus*, from a parasitized insect in Ecuador, was obtained from J. D. Harper (Auburn University), a second isolate from colonized *M. arenaria* eggs, and a third from a colonized cyst of *H. glycines*, the latter both from Alabama. The type isolate of *P. nostocoides*, obtained from a cyst of *Heterodera zae* from a cotton field soil in Maryland, U.S.A., was provided by M. T. Dunn (Mycogen Corp.), and an isolate of *V. chlamydosporium* was recovered from colonized eggs of *M. arenaria* in Alabama. In this experiment each fungus was added to the soil on colonized oats at rates of 0.5 and 1.0% (w/w) but other details of the experiment were as described for the first, including controls.

At the termination of the experiment the root systems of each squash plant were carefully separated from the soil and the number of galls induced by *M. arenaria* counted. The height of shoots and the fresh weights of shoots and roots were also recorded.

The number and identity of species of fungi in the soil was also determined by plating on Rose Bengal chitin-agar as described previously (5). The number and identity of the fungi in *M. arenaria* females in squash roots was determined following removal from root tissue, and placement, after repeated washing with sterile water containing 150 $\mu\text{g/ml}$ of streptomycin sulfate, on acidified chitin agar containing streptomycin sulfate (3). A total of 8 females were chosen at random from root systems of each pot, resulting in 80 females per treatment.

All data were subjected to analysis of variance for completely randomized experiments; also factorial analysis was performed to determine the effect of amendment levels on the variables (14). Fisher's least significant differences (FLSD) were used for comparison of means (14).

RESULTS

Experiment 1. Addition of uncolonized oats to soil infested with *M. arenaria* resulted in reductions in the number of galls per g of squash root (Fig. 1A) compared to unamended soil. Amendments with oats colonized by species of *Gliocladium* or by *V. lamellicola* resulted in additional reductions in the number of galls when compared to the reduction obtained with corresponding rates of uninoculated oats. For each fungal species or isolate, the 0.25% amendment rate was as effective as the 0.50% level in reducing galling reaction. The addition of uncolonized oats to the soil at the 0.50% rate resulted in a lower number of galls than that observed in roots from soil treated with 0.25%. Differences between fungal isolates in reducing gall numbers were not significant.

Addition of uninoculated oats or of the *V. lamellicola* amendment did not result in any change in the number of fungal propagules in the soil (Fig. 1B). However, amendments with *Gliocladium*-colonized oats resulted in high numbers of propagules of that genus/g soil. Highest fungal occurrence rates were recorded for soil with *G. catenulatum* or *G. roseum* II. Soil that received the 0.25% rate contained as many propagules as those with the 0.50% level. There were no differences in numbers of fungal propagules between soil amended with *G. roseum* II and those that received *G. catenulatum*. Soil receiving the 0.25% rate of the *G. roseum* I amendment had lower numbers of fungal propagules than those that received the highest rate of the amendment. The predominant fungal species isolated from soil amended with *G. roseum* I, *G. roseum* II, or *G. catenulatum* were the *Gliocladium* species originally added to the soil in the amendment. Thirty to 40% of the fungal species isolated from all soils amended with the *Gliocladium* spp. were identified as the species originally added to the soil in the corresponding amendment. *V. lamellicola* was not observed in dilution plates at the end of the experiment.

A range of 3-10 fungal species were isolated from *M. arenaria* females, depending on the type of amendment. The species most frequently isolated from females are listed (Table 1). *Fusarium oxysporum* Schlecht. was most abundant in females from the uninoculated oat treatments and in those with *V. lamellicola*, *G. roseum* I, or the 0.5% rate of *G. roseum* II. *Gliocladium* species were predominant only in females from plants grown in *Gliocladium*-treated soil. *V. lamellicola* was not isolated from females in roots from soil amended with this fungus.

Factorial analysis of the data on shoot height (Table 2) revealed no significant interaction between amendment rate and the type of amendment. Shoot heights of plants growing in soil with the 0.5% rate were greater than those receiving the 0.25% treatment. Also, shoot heights of

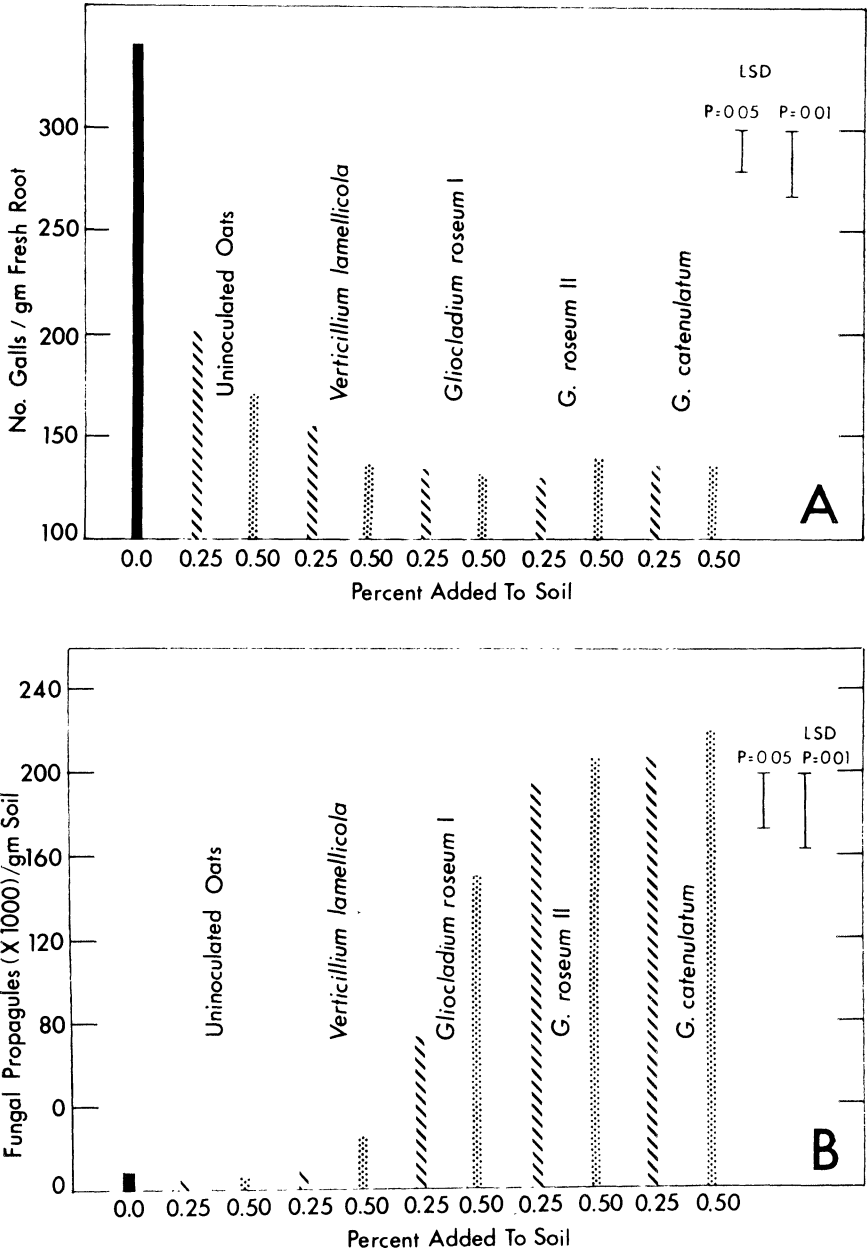


Fig. 1. Effect of amending soil with oat cultures of 4 isolates of nematode-egg-destroying fungi on (A) galling of squash roots by *Meloidogyne arenaria* and (B) soil fungal populations.

Table 1. Predominant fungal species isolated from *Meloidogyne arenaria* females obtained from galls in roots of squash growing in amended soil (Experiment 1).

Type of Amendment	Percent Added	Fungal Species	Frequency (%)
Control		<i>Paecilomyces lilacinus</i>	22.5
		<i>Exophiala jeanselmii</i>	11.3
		<i>Pseudopapilospora kendrikii</i>	10.0
Uninoculated oats	0.25	<i>Fusarium oxysporum</i>	16.3
Uninoculated oats	0.50	<i>Fusarium oxysporum</i>	26.3
		<i>Exophiala dermatiditis</i>	20.0
<i>Verticillium lamellicola</i>	0.25	<i>Fusarium oxysporum</i>	35.0
<i>Verticillium lamellicola</i>	0.50	<i>Fusarium oxysporum</i>	35.0
<i>Gliocladium roseum</i> I	0.25	<i>Fusarium oxysporum</i>	31.3
		<i>Gliocladium roseum</i>	10.0
<i>Gliocladium roseum</i> I	0.50	<i>Fusarium oxysporum</i>	39.0
		<i>Gliocladium roseum</i>	19.0
<i>Gliocladium roseum</i> II	0.25	<i>Exophiala</i> spp.	13.7
		<i>Gliocladium roseum</i>	12.5
<i>Gliocladium roseum</i> II	0.50	<i>Fusarium oxysporum</i>	30.0
		<i>Gliocladium roseum</i>	18.8
<i>Gliocladium catenulatum</i>	0.25	<i>Gliocladium catenulatum</i>	12.5
<i>Gliocladium catenulatum</i>	0.50	<i>Penicillium steckii</i>	26.2
		<i>Gliocladium catenulatum</i>	17.5

all plants from the amended soils were greater than those grown in unamended soil. Comparisons of the overall effect of the treatments on shoot height, irrespective of the effect of rate, revealed that plants corresponding to uninoculated oats or *V. lamellicola* treatments had the largest shoots and those from pots with either *G. roseum* I or *G. roseum* II the smallest; *G. catenulatum* amendments resulted in plants with shoots intermediate in height.

Table 2. Effect of amending *Meloidogyne arenaria*-infested soil with four cultures of nematode destroying fungi on growth of squash.

	Shoot Height (cm)		Fresh Weights (g)			
	0.50 ^w		Shoot		Root	
	0.25	0.50 ^w	0.25	0.50	0.25	0.50
Uninoculated oats ^s	11.7	12.9	3.01	4.88	1.47	1.63
<i>Verticillium lamellicola</i>	12.1	12.6	3.48	4.24	1.45	1.58
<i>Gliocladium roseum</i> I	10.0	10.8	1.78	2.37	0.94	0.80
<i>G. roseum</i> II	10.1	11.1	2.26	2.97	1.23	1.55
<i>G. catenulatum</i>	11.1	11.3	2.68	2.55	1.10	1.33
Unamended soil		9.7	1.74			0.45
LSD (P=0.05):		0.8	0.52			0.21
LSD (P=0.01):		1.1	0.69			0.27

^sUninoculated autoclaved oats were used as substrate on which the fungi were grown before addition to the soil.

^wAmendments were added to the soil at rates of 0.25 and 0.50% (w/w).

With only one exception (*G. roseum* I at the 0.25% rate) all amendments resulted in increased shoot weights (Table 2). Factorial analysis of the shoot weights indicated a significant interaction between rates and the type of treatment. The heaviest shoots were those of plants from the uninoculated oats and the *V. lamellicola* treatments. With only one exception (*G. catenulatum*), the 0.50% rate of all treatments resulted in heavier shoots than the 0.25% level.

Plants from amended soils had heavier roots than those from control soil (Table 2). The interaction between rate and type of treatment for the root weight data was not significant. Generally, for all treatments the 0.50% rate resulted in heavier roots than the 0.25% level. The heaviest roots were present in the uninoculated oats and *V. lamellicola* treatments and the lightest in the *G. roseum* I treatment; the *G. roseum* II treatment resulted in plants with heavier roots than the *G. catenulatum* amendment.

Experiment 2. The addition of uncolonized oats at 0.50 or 1.0% to *M. arenaria*-infested soil reduced the number of galls/g of root (Fig. 2A). Factorial analysis of the gall data revealed a significant correlation between rate and type of amendment. While addition to the soil of two of the fungal amendments (*V. chlamydosporium* and *P. lilacinus* from *M. arenaria* eggs) reduced the number of galls below the number observed for the uninoculated oat treatments at equivalent rates, other fungal amendments (*P. lilacinus* from *H. glycines* and *P. nostocoides* from *H. zea*) did not result in such reduction in gall numbers. Amendment with the *P. lilacinus* of insect origin was effective only at the 1.0% rate.

Highest numbers of fungal propagules in soil (Fig. 2B) were recorded for soils treated with *P. lilacinus* from *H. glycines*. Soils that received either *P. lilacinus* from *M. arenaria* eggs or *P. nostocoides* revealed the second highest numbers of fungal propagules. Differences in numbers of fungal propagules between the remaining soils were not significant. Over 80% of fungal species in dilution plates from soils treated with *P. nostocoides* amendments, or *P. lilacinus* amendments of nematode origin, corresponded in identity with those added to the soil. *Verticillium chlamydosporium* was not observed in dilution plates of the soil amended with the fungus. The *P. lilacinus* of insect origin was found in low frequency (<10%) in dilution plates from the soil treated with this fungus.

The number of fungal species isolated from females varied from 2-5, depending on the type of amendment. The most frequently isolated species in females in this experiment was *F. oxysporum* (Table 3); none of the fungal species added to the soils in this experiment was isolated from the females. All amendments at the 1.0% rate resulted in increased shoot height of squash plants (Table 4). However, at the 0.5% level, only the treatment with uninoculated oats or with *P. lilacinus* from *M.*

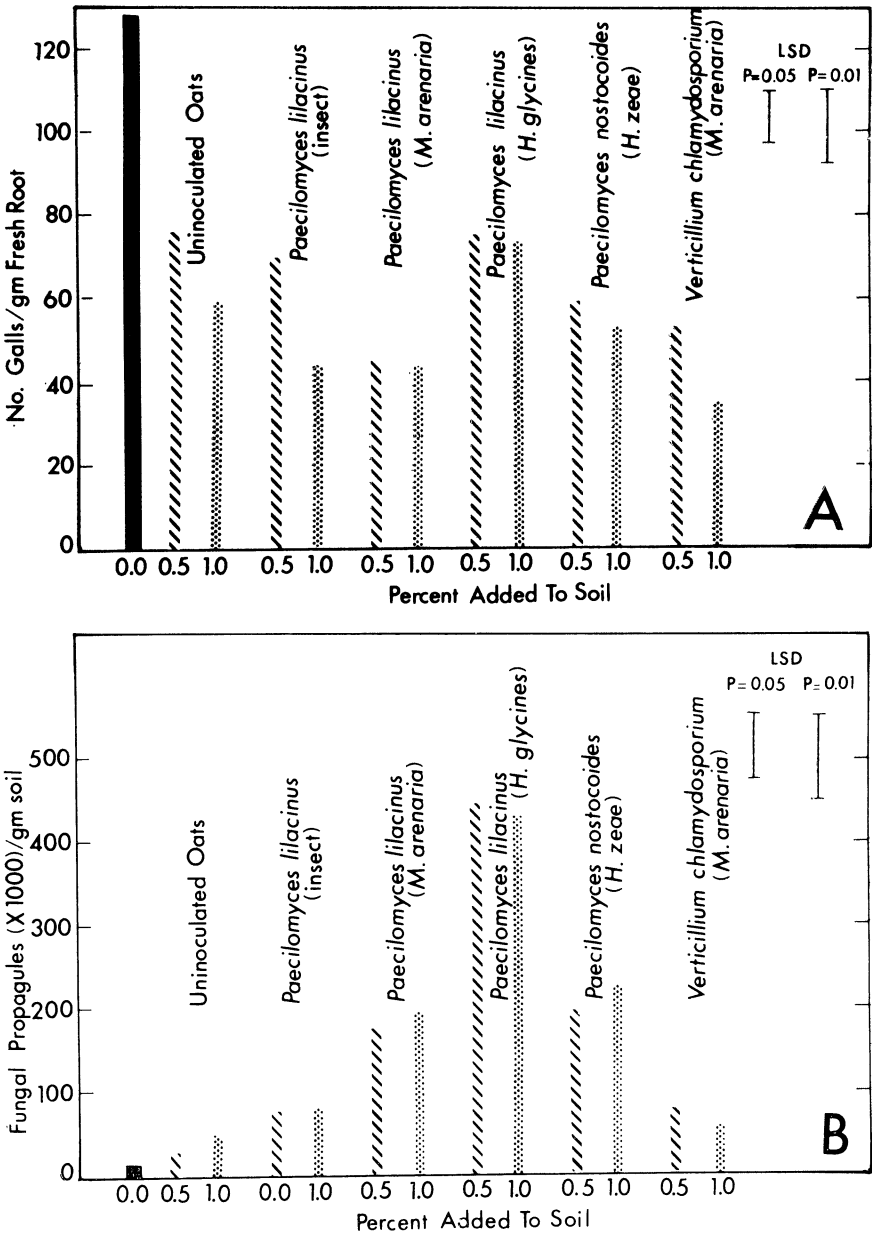


Fig. 2. Effect of amending soil with oat cultures of five isolates of nematode-egg-destroying fungi on (A) galling of squash roots by *Meloidogyne arenaria* and (B) soil fungal populations.

Table 3. Predominant fungal species isolated from *Meloidogyne arenaria* females obtained from galls in roots of squash growing in amended soil (Experiment 2).

Type of Amendment	Percent Added	Fungal Species	Frequency (%)
Control		<i>Fusarium oxysporum</i>	60
		<i>Gliocladium catenulatum</i>	14
Uninoculated oats	0.5	<i>Fusarium oxysporum</i>	76
Uninoculated oats	1.0	<i>Fusarium oxysporum</i>	49
		<i>Mucor hiemalis</i>	44
<i>Paecilomyces lilacinus</i> (inst) ^z	0.5	<i>Fusarium oxysporum</i>	92
<i>Paecilomyces lilacinus</i> (inst)	1.0	<i>Fusarium oxysporum</i>	58
		<i>Phoma americana</i>	10
		<i>Humicola fuscoatra</i>	13
<i>Paecilomyces lilacinus</i> (<i>M. a.</i>) ^z	0.5	<i>Fusarium oxysporum</i>	81
<i>Paecilomyces lilacinus</i> (<i>M. a.</i>)	1.0	<i>Fusarium oxysporum</i>	97
<i>Paecilomyces lilacinus</i> (<i>H. g.</i>) ^z	0.5	<i>Fusarium oxysporum</i>	60
<i>Paecilomyces lilacinus</i> (<i>H. g.</i>)	1.0	<i>Fusarium oxysporum</i>	87
<i>Paecilomyces nostocoides</i>	0.5	<i>Fusarium oxysporum</i>	85
<i>Paecilomyces nostocoides</i>	1.0	<i>Fusarium oxysporum</i>	75
<i>Verticillium</i> <i>chlamydosporium</i>	0.5	<i>Fusarium oxysporum</i>	91
<i>Verticillium</i> <i>chlamydosporium</i>	1.0	<i>Fusarium oxysporum</i>	95

^zinst = of insect origin; *M. a.* = isolated from eggs of *Meloidogyne arenaria*, and *H. g.* from cysts of *Heterodera glycines*.

arenaria or *V. chlamydosporium* resulted in plants with shoot height greater than those grown in unamended soil.

All amendments resulted in increased shoot weights of plants (Table 4). Factorial analysis of the shoot weights revealed no interaction between rates and the type of amendment. Plants grown in soil treated with the 1.0% level had heavier shoots than those from soils that received the 0.5% level. The analysis also revealed no differences in shoot weight in response to type of amendment.

All amendments resulted in plants with heavier roots than those grown in unamended soil (Table 4). Factorial analysis of the root weights indicated no significant interaction between rate and type of amendment. The analysis also revealed no significant effect attributable to type of amendment, but a significant increase in root weight in response to increasing amendment rate from 0.5 to 1.0%.

DISCUSSION

All fungi used in the experiments were capable of destroying eggs of *M. arenaria* *in vitro*. Although *in vitro* testing for activity against the nematode may serve for preliminary "screening purposes", it cannot provide a measure of the relative competitive ability of a fungal species in soil or even its ability to colonize eggs under natural conditions. A requirement for potential exploitation of a fungal species as a biological control agent is ability to establish itself in field soil and maintain, if not build up, its inoculum potential. The survival of a fungal species in soil depends *inter alia* on the availability of a food base from which the fungus can grow and on its capacity to successfully compete with other organisms in colonizing available substrates. Fungi were added to soil using oats as the food base from which they could grow and compete with other organisms and possibly colonize *M. arenaria* eggs or other developmental stages of the nematode. Several of the fungal species and isolates used in the experiments were capable of establishing themselves in soil and of effecting a degree of control of *M. arenaria*.

The addition to soil of the food base itself (uncolonized oats) resulted in significant control of *M. arenaria*. This is not surprising given that oats contain nitrogen at levels (2-3%) which, when added to soil at the rates used in the experiment, can be expected to stimulate general soil microbial activity resulting in some control of *M. arenaria* (5). It is also possible that oat kernels may contain nematicidal compounds or generate nematotoxicants upon their decomposition by soil microorganisms. The results do underline the need for inclusion of a "substrate or food base" check treatment when testing microbial species for activity against nematodes.

Table 4. Effect of amending *Meloidogyne arenaria*-infested soil with five cultures of nematode destroying fungi on growth of 'Summer Crookneck' squash.

	Origin	Shoot Height (cm)		Fresh Weight (g)			
		Shoot Height		Shoots		Roots	
		0.5	1.0 ^w	0.5	1.0	0.5	1.0
Uninoculated oats ^a							
<i>Paecilomyces lilacinus</i>	insect	10.2	10.0	3.14	4.17	1.17	1.67
<i>P. lilacinus</i>	<i>M. arenaria</i> eggs	8.3	9.0	2.66	3.99	1.10	1.49
<i>P. lilacinus</i>	<i>H. glycines</i> cysts	9.0	10.0	3.36	4.76	1.05	1.26
<i>P. lilacinus</i>	<i>H. zea</i> cysts	8.2	10.0	2.77	3.60	1.14	1.50
<i>P. nostocoides</i>	<i>M. arenaria</i> eggs	8.5	9.1	2.79	3.98	1.07	1.19
<i>Verticillium chlamyosporium</i>		9.0	10.1	3.08	4.89	1.20	1.15
Unamended soil			7.9		1.73		0.59
	LSD (P=0.05):		0.81		0.78		0.25
	LSD (P=0.01):		1.07		1.04		0.34

^aUninoculated autoclaved oats were used as substrate on which the fungi were grown before being added to the soil.
^wAmendments were added to the soil at rates of 0.50 and 1.0% (w/w).

The ability of a fungus species to establish itself in soil does not imply capacity to effect control of *M. arenaria*. Thus, while *P. nostocoides* and one isolate of *P. lilacinus* were recovered from soil with high frequency at the end of the experiments there was no concomitant reduction in gall numbers in the roots of plants grown in soil treated with these fungi. Also, results from the second experiment revealed that isolates of the same fungal species (*P. lilacinus*) can differ widely in their ability to establish in soil and in their capacity to control *M. arenaria*. This suggests that environmental factors or genetic differences among isolates may be decisive in determining the relative effectiveness of a particular fungal species to control a specific nematode in soil. There appears to be increasing evidence that specialized fungal biotypes are implicated in nematode pathology.

The two *Verticillium* species are puzzling in that both species were effective against *M. arenaria* although they could not be recovered from soil in the dilution plates. This phenomenon was observed before with another isolate of *V. chlamydosporium* in similar experiments in our laboratory (6). It is possible that the medium used in these studies may not be suitable to isolate *Verticillium* spp., directly from soil. In the case of *V. chlamydosporium* it is also possible that some of its reproductive structures, particularly chlamydospores, were dormant in soil at the time of determination of the level of fungal presence. This appears to be likely since its chlamydospores could be observed directly in soil amended with the fungus. There is also the possibility that the determination of levels of fungal propagules in soil was performed after the *Verticillium* spp. had declined in numbers or disappeared, implying that their activity in soil may be transient in nature. Clearly, the ecology and mode of action of *Verticillium* spp. against *M. arenaria* in soil need further study. Some doubt as to their competitive ability must remain until capacity to overcome soil fungistasis and increase biomass is demonstrated conclusively.

In contrast to *Paecilomyces* spp., all isolates of the two *Gliocladium* spp. performed well in controlling *M. arenaria* and in their ability to colonize soil and invade females. This suggests that isolates of *Gliocladium* spp. may be the more promising for development as biological control agents.

LITERATURE CITED

1. EPPS, W. M., J. C. PATTERSON, and I. E. FREEMAN. 1951. Physiology and parasitism of *Sclerotium rolfsii*. *Phytopathology* 41: 245-256.
2. GINTIS, B. O., G. MORGAN-JONES, and R. RODRIGUEZ-

- KABANA. 1982. Mycoflora of young cysts of *Heterodera glycines* in North Carolina soils. *Nematropica* 12:295-303.
3. GINTIS, B. O., G. MORGAN-JONES, and R. RODRIGUEZ-KABANA. 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soil. *Nematropica* 13:181-200.
 4. GODOY, G., R. RODRIGUEZ-KABANA, and G. MORGAN-JONES. 1982. Parasitism of eggs of *Heterodera glycines* and *Meloidogyne arenaria* by fungi isolated from cysts of *H. glycines*. *Nematropica* 12:111-119.
 5. GODOY, G., R. RODRIGUEZ-KABANA, R. A. SHELBY, and G. MORGAN-JONES. 1983. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. II. Effects on microbial population. *Nematropica* 13:63-74.
 6. GODOY, G., R. RODRIGUEZ-KABANA, and G. MORGAN-JONES. 1982. Fungal parasites of *Meloidogyne arenaria* eggs in an Alabama soil. A mycological survey and greenhouse studies. *Nematropica* 13:201-213.
 7. JATALA, P., R. KALTENBACH, A. J. DEVAUX, and R. CAMPOS. 1980. Field application of *Paecilomyces lilacinus* for controlling *Meloidogyne incognita* on potatoes. *J. Nematol.* 12:226-227.
 8. JATALA, P., R. SALAS, R. KALTENBACH, and M. BOCANGEL. 1981. Multiple application and long term effects of *Paecilomyces lilacinus* in controlling *Meloidogyne incognita* under field conditions. *J. Nematol.* 13:445.
 9. MORGAN-JONES, G., and R. RODRIGUEZ-KABANA. 1981. Fungi associated with cysts of *Heterodera glycines* in an Alabama soil. *Nematropica* 11:69-74.
 10. MORGAN-JONES, G., B. O. GINTIS, and R. RODRIGUEZ-KABANA. 1981. Fungal colonization of *Heterodera glycines* cysts in Arkansas, Florida, Mississippi and Missouri soils. *Nematropica* 11:155-164.
 11. MORGAN-JONES, G., J. F. WHITE, and R. RODRIGUEZ-KABANA. 1983. Phytonematode pathology; ultrastructural studies. I. Parasitism of *Meloidogyne arenaria* eggs by *Verticillium chlamydosporium*. *Nematropica* 13:245-260.
 12. MORGAN-JONES, G., J. F. WHITE, and R. RODRIGUEZ-KABANA. 1984. Phytonematode pathology; ultrastructural studies. II. Parasitism of *Meloidogyne arenaria* eggs and larvae by *Paecilomyces lilacinus*. *Nematropica* 14:57-71.
 13. MORGAN-JONES, G., J. F. WHITE, and R. RODRIGUEZ-

- KABANA. 1984. Fungal parasites of *Meloidogyne incognita* in an Alabama soybean field soil. *Nematropica* 14:93-96.
14. STEEL, R. G. D., and J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York. 481 pp.

Received for publication:

19.IX.1984

Recibido para publicar: