ANTAGONISM OF *PAECILOMYCES* SPP. ISOLATED FROM BANANA (*MUSA* SPP.) ROOTS AND RHIZOSPHERE AGAINST *RADOPHOLUS SIMILIS*

P. Kilama[1], T. Dubois[1*], D. Coyne[1], B. Niere[2], C. S. Gold[1] and E. Adipala[3]

[1]International Institute of Tropical Agriculture, P.O. Box 7878, Kampala, Uganda. [2]Institute for Nematology and Vertebrate Research, Federal Biological Research Centre for Agriculture and Forestry, Toppheideweg 88, 48161 Münster, Germany. [3]Department of Crop Science, Makerere University, P.O. Box 7062, Kampala, Uganda. *Corresponding author: t.dubois@cgiar.org.

ABSTRACT

Kilama, P., T. Dubois, D. Coyne, B. Niere, C. S. Gold and E. Adipala. 2007. Antagonism of *Paecilomyces* spp. isolated from banana (*Musa* spp.) roots and rhizosphere against *Radopholus similis*. Nematropica 37:215-225.

Banana (*Musa* spp.) in East Africa is threatened by pests and diseases, of which the burrowing nematode, *Radopholus similis*, is among the most serious and difficult to control. Fungal antagonists, such as *Paecilomyces* spp., can aid in controlling *R. similis*. In this study, *Paecilomyces* spp. was isolated from banana roots and rhizosphere in fields in Uganda with high and low densities of *R. similis*. Fungi were more abundant in the rhizosphere than on roots in the field with low *R. similis* density, whereas the reverse occurred in the field with high *R. similis* density. A total of 23 isolates of *Paecilomyces* spp. were collected, of which *Paecilomyces lilacinus* was the most abundant species. This is the first report showing the high presence of *Paecilomyces* spp. in the rhizosphere of bananas. Bioassays revealed that filtrates of *P. lilacinus* and *Paecilomyces marquandii* caused reversible paralysis of male and juvenile *R. similis*. *Paecilomyces lilacinus* isolate 23N5-2 caused greatest paralysis, which was not influenced by filtrate concentration.

Key words: bioassay, burrowing nematode, East African highland banana, *Musa*, *Paecilomyces lilacinus*, *Paecilomyces marquandii*, *Radopholus similis*.

RESUMEN

Kilama, P., T. Dubois, D. Coyne, B. Niere, C. S. Gold and E. Adipala. 2007. Antagonismo de *Paecilomyces* spp. aislado de raíces y rizosfera de banano (*Musa* spp.) contra *Radopholus similis*. Nematropica 37:215-225.

El cultivo de banano (*Musa* spp.) en Africa Oriental se ve afectado por plagas y enfermedades, entre las cuales el nematodo barrenador, *Radopholus similis,* es uno de los más serios y difíciles de controlar. Los hongos antagonistas, como *Paecilomyces* spp., pueden ayudar a controlar *R. similis*. En este estudio, se aisló *Paecilomyces* spp. de raíces y rizosfera de banano con densidades altas y bajas de *R. similis* en Uganda. Se encontraron los hongos en mayor abundancia en la rizosfera que en las raíces en los campos con bajas densidades de *R. similis*, mientras que lo contrario ocurrió en los campos con altas densidades de *R. similis*. Se colectó un total de 23 aislamientos de *Paecilomyces* spp., siendo *Paecilomyces lilacinus* la especie más abundante. Este es el primer registro que indica la alta presencia de *Paecilomyces* spp. en la rizosfera de bananos. Los bioensayos demostraron que los filtrados de *P. lilacinus* y *Paecilomyces marquandii* causan parálisis reversible de machos y juveniles de *R. similis*. La cepa 23N5-2 de *P. lilacinus* causó la mayor parálisis, independientemente de la concentración del filtrado.

Palabras clave: banano de Africa Oriental, bioensayo, *Musa*, nematodo barrenador, *Paecilomyces lilacinus*, *Paecilomyces marquandii*, *Radopholus similis*.

INTRODUCTION

In East Africa, banana (*Musa* spp.) is an important food staple. In Uganda, banana yield is showing a steady production decline, attributed to pests such as nematodes (Gold *et al.*, 1993; Rubaihayo and Gold, 1993; Speijer *et al.*, 1994). The burrowing nematode, *Radopholus similis*, is among the most important plant-parasitic nematodes attacking banana (Speijer and Dewaele, 1997). Plant-parasitic nematodes affect the roots, which disrupts plant anchorage, resulting in the uprooting and toppling of the plants, usually when top-heavy and bearing fruit (Gowen *et al.*, 2005).

Radopholus similis is a migratory endoparasite that completes its life cycle entirely within the root cortex. Penetration occurs mostly near the root tip, but nematodes can migrate along the entire length of the root. Females and juvenile stages are infective, while males are non-parasitic. The nematodes reproduce in cells of the cortex of the roots and rhizomes (Loos and Loos, 1960; Blake, 1961, 1966; Gowen, 1995). The nematode burrows between cortical cells, punctures cell walls with its stylet and feeds on the cytoplasm, making cavities within the roots. As the cells are destroyed and the nematode migrates, cavities coalesce to form red-brown lesions (Blake, 1961, 1966; Mateille, 1994).

The use of fungal antagonists against plant-parasitic nematodes is being explored as a management option. Several fungal species attacking plant-parasitic nematodes are now mass-produced for control (Butt *et al.*, 2001). *Paecilomyces lilacinus* is a cosmopolitan facultative pathogen of nematode females and eggs (Domsch *et al.*, 1993; Viane *et al.*, 2006). A particular isolate, *P. lilacinus* strain 251, has been commercially developed for nematode control, especially against cyst and root-knot nematodes, and is currently sold under several trade names (Kerry, 2000). The strain is currently registered in Australia, the Philippines and South Africa (Kegley *et al.*, 2007). In Africa, *P. lilacinus* strain 251 is commercialized as the wettable powder Pl Plus for use against plant-parasitic nematodes (Biological Control Products, http://www.biocontrol.co.za). *Paecilomyces lilacinus* is found both in the rhizosphere and on the root surface of the banana plant (Goos and Timonin, 1962). Mendoza *et al.* (2004) conducted promising field trials with this commercial product, indicating the great potential of *P. lilacinus* for inclusion in integrated pest management schemes against *R. similis*. In Cuba, local strains of *P. lilacinus* are currently being used in banana nurseries for control of *R. similis* (Diaz *et al.*, 1998).

No commercial product containing *Paecilomyces* spp. which could aid in control of *R. similis* has been registered in Uganda. In contrast to cyst and root-knot nematodes which are sedentary endoparasitic nematodes and which produce egg masses for fungal attack, *R. similis* does not have egg masses and is a migratory endoparasite (Luc *et al.*, 1990; Speijer and De Waele, 1997). As such, antagonism of *P. lilacinus* against *R. similis* would presumably be caused by toxins contained in the filtrate of the fungus. The objectives of our study were to isolate indigenous Ugandan *P. lilacinus* from various ecological niches (rhizosphere soil and the root surface of banana plants in fields with a high and low *R. similis* density), and test their culture filtrates for antagonistic effects against motile *R. similis* stages (males, females and juveniles) *in vitro*.

MATERIALS AND METHODS

Site Selection and Field Isolation

Fungal isolation was carried out in Namulonge, Uganda (0°32' latitude, 32°34' longitude and 1,128 m above sea

level). Soil and root samples were collected in September 2002 from fields with a high and low *R. similis* density (2,056 ± 706 and 42 ± 28 *R. similis*/100 g roots, respectively). The field with low *R. similis* density, established in November 1996, comprised 45 sucker-derived East African highland banana (AAA-EA genome group) cultivars. A total of six arbitrarily selected and healthy looking plants were sampled. The field with high *R. similis* density, established in December 1999, contained 36 tissue-cultured East African highland banana cultivars. Half of the plants had been artificially infested with *R. similis* and six plants among those were arbitrarily selected for sampling.

Serial Dilution of Soil and Root Samples

Banana roots of the selected plants (approx. 12 per plant) were excavated with attached soil from 3-5 points at a depth of 5 cm along a 30 cm radius from the pseudostem. The samples were brought to the laboratory and subsequent procedures were carried out in a laminar airflow cabinet. The soil attached to the roots was dislodged and 1 g of soil was added to 9 ml of sterile (autoclaved at 121°C for 20 min) distilled water (SDW) in a 160 × 15 mm glass test tube. Serial dilutions were made of 10^{-1} to 10^{-7}. One ml each of the 10^{-4} to 10^{-7} dilutions was transferred separately onto sterile potato dextrose agar (PDA) medium (Sigma-Aldrich, St. Louis, U.S.A.) in 100 mm diameter Petri dishes. PDA was supplemented with antibiotics (10 mg chlortetracycline, 100 mg penicillin G, 50 mg streptomycin sulphate/L PDA) to prevent bacterial growth. Petri dishes were incubated in the laboratory (approx. 25°C and a photoperiod of 12:12 h) for 4-9 days.

From each sample of excavated roots, a root of approx. 2 mm diameter was randomly selected and attached soil was scraped off. A 1 g root piece was removed, rinsed in SDW and transferred to SDW in a glass test tube from where 10^{-1} to 10^{-7} dilutions were made. Thereafter, 1 ml each of the 10^{-4} to 10^{-7} dilutions were transferred separately onto PDA, supplemented with antibiotics, in 100 mm diameter Petri dishes and incubated for 4-9 days.

Isolation and Identification of Fungal Isolates

From all fungi growing on PDA, individual isolates were obtained by transferring single colonies to synthetic nutrient agar (SNA) medium (Nirenberg, 1981) in 50 mm diameter Petri dishes. Fungal isolates were identified to the genus level according to Barnett and Hunter (1998), Domsch *et al.* (1993), Samson (1974) and Von Arx (1974). *Paecilomyces* spp. isolates were transferred onto malt extract agar (MEA) medium (Sigma-Aldrich) in 100 mm diameter Petri dishes and incubated for ≤29 days. Identification of *Paecilomyces* spp. to species level was performed based on Samson (1974).

Multi-isolate Bioassays

Of the 23 *Paecilomyces* spp. isolates obtained, five were tested against *R. similis* in a series of three repeated bioassays (henceforth referred to as bioassays 1, 2 and 3). These five isolates originated from different banana plants, and were therefore considered separately.

Isolates were grown on PDA for 7 days and, for each isolate, approx. 15 blocks (2 × 2 × 2 mm) containing fungal mycelium were transferred into 100 ml sterile potato dextrose broth (PDB) medium (Sigma-Aldrich) in 250 ml Erlenmeyer flasks. After 14 days, PDB containing fungal mycelium and spores was filtered through sterile 0.22 µm pore-sized Eagle filter paper (New Technolab Instruments,

Nashik, India). The filtrate was collected in 250 ml glass bottles.

Pure *R. similis* cultures were obtained on sterile carrot discs according to Speijer and Dewaele (1997). Nematode suspensions were diluted to 100 motile *R. similis*/ml, and 2 ml of the nematode suspension was transferred each into 30 mm diameter glass Petri dishes.

A solution of 250 µL filtrate/ml obtained from isolates 15R8-1, 22R5-2 and 23N5-2 (*P. lilacinus*), and 11N4-8 and 22R5-2 (*Paecilomyces marquandi*) was added to each of the Petri dishes after 24 h. Three control treatments were used: two control treatments consisted of 2 ml PDB solution (250 µL filtrate/ml PDB) and one control treatment consisted of 2 ml SDW. The pH of one PDB control treatments was adjusted to be higher than the highest pH value among the isolate filtrates, whereas the pH of the second PDB control treatment was adjusted to be lower than the lowest pH among the isolate filtrates. In bioassay 1, pH of fungal treatments ranged between 7.31 (isolate 23N5-2) and 8.12 (isolate 15R8-1). PDB without filtrate had a low pH of 5.20. The pH of the PDB control treatments were adjusted to 7.29 and 8.76. In bioassays 2 and 3, pH of the fungal treatments ranged between 7.60 (isolate 11N8-4) and 7.97 (isolate 15R8-1). PDB without filtrate had a low pH of 5.00. The pH of the PDB control treatments were adjusted to 5.00 and 9.59. Each treatment consisted of three 30 mm diameter glass Petri dishes. Per Petri dish, three counts of paralyzed nematodes were performed at 2 h for all bioassays. Nematodes were rinsed in tap water immediately after the 2 h counts and, with the exception of bioassay 2, nematode paralysis was again assessed after 21 h. To ensure paralysis, nematodes were gently probed with a fine needle while counted, both at 2 and at 21 h. Paralysis was defined as immobility after probing.

Multi-dose Bioassays

Isolate 23N5-2 was further tested against *R. similis* at three filtrate concentrations, 125 µl/ml, 250 µl/ml and 375 µl/ml SDW, in a series of two repeated bioassays (henceforth referred to as bioassays 4 and 5). In both bioassays, 2 ml of a suspension of 25 motile *R. similis*/ml SDW was used. Both bioassays contained four control treatments: one treatment contained only SDW and the three other treatments contained PDB media at each of the filtrate concentrations. The pH of the PDB control treatments was adjusted to that containing the filtrate of isolate 23N5-2 (pH = 8.20).

Statistics

The proportion of fungi between fields (high or low *R. similis* density) and between sampling sources (root or rhizosphere) was analyzed using chi-square tests. The proportion of *P. lilacinus* relative to other fungi between fields and sampling source was analyzed using Fisher's exact test. Nematode counts from bioassays were arcsine square-root transformed for bioassay 4 and square-root transformed for bioassays 2 and 3 prior to ANOVA. If different, means were separated by Tukey's studentized range test (SAS Institute, Cary, USA).

RESULTS

Isolation of Paecilomyces *spp.*

The majority of isolates obtained in the present study were highly sporulating, fast-growing asexual fungi, including *Paecilomyces* spp. The number of fungal isolates from the field with low *R. similis* density was similar to that from the field with high *R. similis* density ($P > 0.05$) (Table 1). However, in the field with low *R. similis* density, more fungal isolates were obtained from the rhizosphere soil than from the banana

Table 1. Number of fungal isolates obtained from banana (*Musa* spp.) root surface and rhizosphere soil taken from fields with a high and low *Radopholus similis* density in Namulonge, Uganda.

	High density field		Low density field	
Fungal taxon	Root	Soil	Root	Soil
Acremonium spp.	3	5	11	35
Arthroderma spp.	0	1	0	3
Aspergillus spp.	2	2	4	2
Athelia spp.	0	0	1	0
Fusarium spp.	2	2	6	14
Myrothecium spp.	0	0	1	0
Paecilomyces spp.	7	9	4	3
P. byssochlamydoides	1	0	0	0
P. lilacinus	4	4	3	2
P. marquandii	1	4	1	1
P. puntonii	1	0	0	0
P. viridis	0	1	0	0
Penicillium spp.	7	1	4	7
Phoma spp.	0	0	1	1
Trichoderma spp.	2	1	3	2
Unidentified	60	38	14	18
Total	83	59	49	85

root surface ($P < 0.01$), while the reverse occurred within the field with high *R. similis* density ($P < 0.05$).

Twenty-three *Paecilomyces* spp. isolates were obtained with *P. lilacinus* being most abundant. Among all fungal isolates, the relative abundance of *P. lilacinus* was not different between the two types of fields ($P > 0.05$). The relative abundance of *P. lilacinus* was also not different between the rhizosphere soil and the root surface ($P > 0.05$). Overall, *P. lilacinus* comprised 4.7% of all isolated fungi (n = 276). *Paecilomyces marquandii* was isolated several times (2.5% of all isolated fungi). Other *Paecilomyces* spp. of which only one isolate was obtained were *Paecilomyces byssochlamydoides*, *Paecilomyces puntonii* and *Paecilomyces viridis*.

Two *P. marquandii* isolates (11N4-8 and 22R5-2) and three *P. lilacinus* isolates (15R8-1, 22R5-1 and 23N5-2) were tested in bioassays 1-3. During growth in MEA, isolates 11N4-8, 22R5-2 and 23N5-2 produced exudates that changed the MEA to a bright yellow color.

Multi-isolate Bioassays

In bioassay 1, paralysis was lower after 21 h compared to 2 h ($P < 0.0001$). Within the fungal treatments, paralysis across *R. similis* stages equalled 31.1 ± 1.6% (n = 54) after 2 h, but only 18.4 ± 1.4% (n = 135) after 21 h. After 2 h, paralysis differed across treatments ($P < 0.0001$) and across *R. similis* stages ($P < 0.0001$) (Fig. 1A). Males and juveniles were paralyzed to a greater extent than females ($P < 0.05$). Male and juvenile paralysis caused by isolate 23N5-2 was higher than paralysis in control treatments ($P < 0.05$). Female paralysis caused by isolate 23N5-2, although the highest among fungal treatments, did not differ from paralysis levels observed in

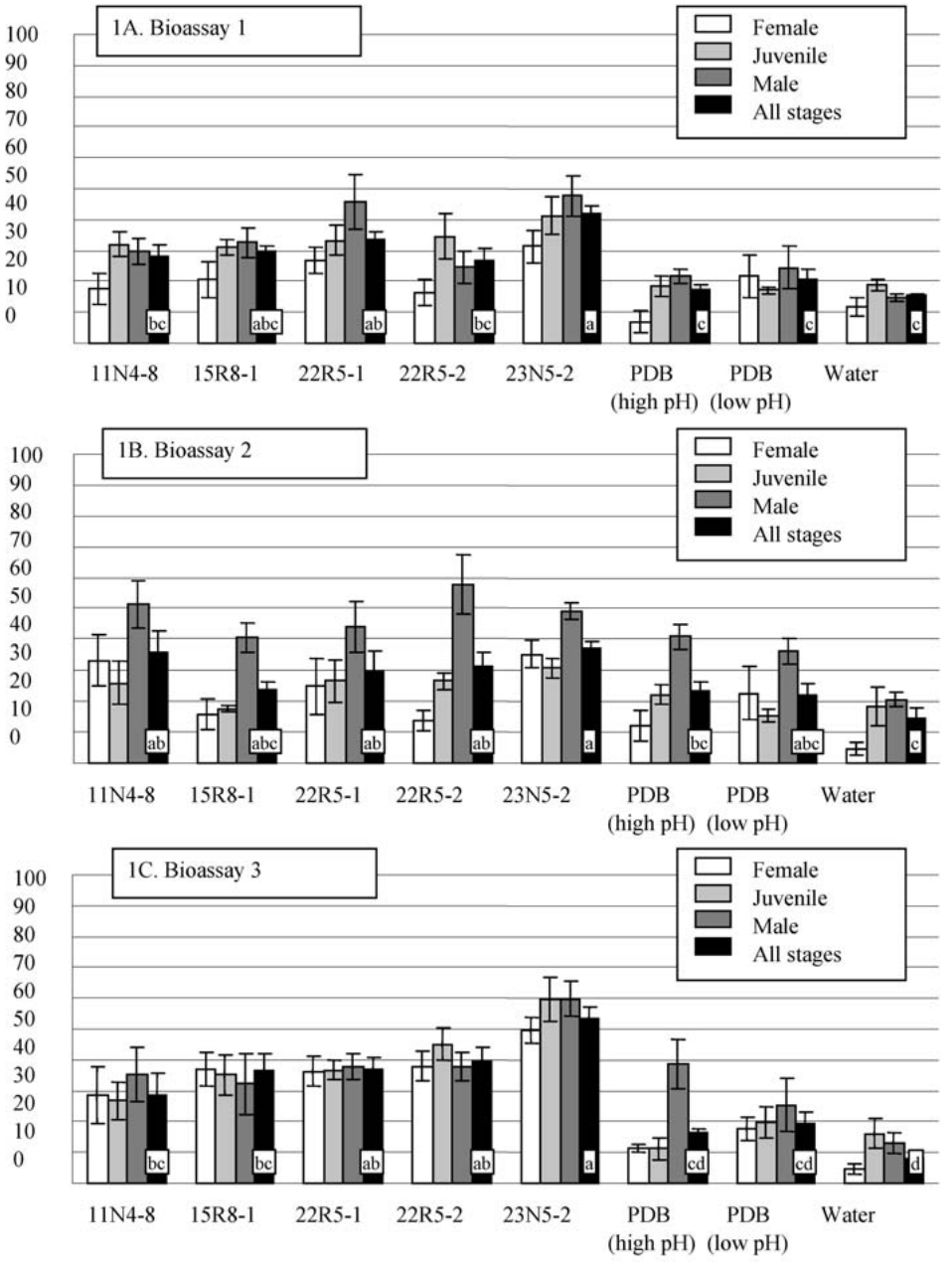

Fig. 1. Three bioassays showing percentage paralysis after 2 h of motile *Radopholus similis* stages (males, females and juveniles) treated with three culture filtrates of three *Paecilomyces lilacinus* isolates (23N5-2, 15R8-1 and 22R5-1) and two *Paecilomyces marquandii* isolates (11N4-8 and 22R5-2), obtained from banana (*Musa* spp.) rhizosphere in Namulonge, Uganda. Three control treatments were included: two potato dextrose broth treatments without filtrate with the pH value adjusted to low or high, and a water control treatment. Error bars represent standard errors of the mean. Bars depicting total (all stages) *R. similis* paralysis with the same letter are not significantly different (Tukey's studentized range test, $P \geq 0.05$).

the water and one of the PDB control treatments ($P > 0.05$). After 21 h, paralysis differed only across *R. similis* stages ($P = 0.0042$). Males (21.1 ± 2.3% (n = 72)) and juveniles (22.4 ± 1.4% (n = 72)) were paralyzed to a greater extent than females (13.8 ± 2.0% (n = 72)). No differences were noted among treatments ($P > 0.05$).

In bioassay 2, paralysis differed among treatments ($P < 0.0001$) and among *R. similis* stages ($P < 0.0001$) (Fig. 1B). Males were more paralyzed than juveniles, and juveniles were more paralyzed than females. Paralysis by isolate 23N5-2 was numerically the highest. Although paralysis by isolate 23N5-2 was higher compared to paralysis in the water and the high pH PDB control, it was not different from paralysis in the low pH PDB control.

In bioassay 3, paralysis was abnormally high for one of the control treatments (PHB with low pH after 21 h, 84.9 ± 6.1%, n = 18), creating difficulty for comparisons between 2 and 21 h. Nevertheless, within fungal treatments, paralysis after 2 h (39.7 ± 1.8, n = 90) was higher than after 21 h (31.9 ± 2.5, n = 90). After 2 h, percentage paralysis was different among treatments ($P < 0.0001$) (Fig. 1C). Three fungal isolates, 23N5-2, 22R5-2 and 22R5-1, caused higher paralysis than in any of the control treatments, with isolate 23N5-2 causing numerically the highest percentage paralysis. There was no difference in paralysis among *R. similis* stages ($P > 0.05$). After 21 h, paralysis was much higher ($P < 0.0001$) in the PDB control treatment with low pH than in any other treatment ($P < 0.05$). After omitting the latter treatment, paralysis among treatments was different ($P < 0.01$), but none of the paralysis levels in fungal treatments differed from that caused in the PDB control treatment with high pH ($P > 0.05$). Paralysis differed among *R. similis* stages ($P < 0.05$), with males (37.7 ± 4.1%, n = 41) and juveniles (36.2 ± 3.8%, n = 41) being more paralyzed than females (19.4 ± 2.3%, n = 42).

Multi-dose Bioassays

In bioassay 4, paralysis was lower after 21 h compared to 2 h ($P < 0.0001$) (Table 2). Within fungal treatments, paralysis equalled 51.9 ± 3.3% (n = 54) after 2 h, but only 21.0 ± 3.4% (n = 53) after 21 h. After 2 h, paralysis was different among treatments ($P < 0.0001$). Paralysis did not differ among *R. similis* stages ($P > 0.05$). The two highest doses of fungal culture filtrates (250 µl/ml and 375 µl/ml) induced higher paralysis than in any of the other treatments. Paralysis caused by the lowest filtrate dose (150 µl/ml) was no different from that in the control treatments. Paralysis caused by the PDB control treatments was not different from that of the water control treatment. In the same bioassay, after 21 h, paralysis differed among treatments ($P < 0.0001$) and *R. similis* stages ($P < 0.0001$). A significant interaction between treatment and *R. similis* stage ($P < 0.01$) demonstrated that each of the stages responded differently to the treatments. However, within each of the *R. similis* stages, fungal treatments were not different from control treatments. Again, after 21 h, paralysis caused by the PDB control treatments did not differ from that of the water control treatment.

In bioassay 5, paralysis of *R. similis* marginally differed among treatments ($P < 0.05$). However, this was not caused by a difference in paralysis between control and fungal treatments ($P > 0.05$). Within fungal treatments, paralysis also did not differ among filtrate concentrations or among *R. similis* stages ($P > 0.05$). Irrespective of *R. similis* stage, paralysis equalled 4.7 ± 1.2% (n = 27) for the water control treatment and 4.9 ± 1.0% (n = 81) for the PDB control treatments, whereas paralysis caused by the fungal treatments equalled 5.7 ± 0.8% (n = 81).

Table 2. Percentage paralysis (means ± SE) after 2 and 21 h of motile *Radopholus similis* stages (males, females and juveniles) treated with three culture filtrates of *Paecilomyces lilacinus* isolate 23N5-2, obtained from banana (*Musa* spp.) rhizosphere in Namulonge, Uganda.

Treatment	Concentration (µl filtrate/ ml water)	Time of assessment							
		2				21			
		Female	Juvenile	Male	Total	Female	Juvenile	Male	Total
23N5-2	325	48.8 ± 6.7 ab	60.9 ± 7.4 ab	49.4 ± 15.9 ab	52.9 ± 6.6 a	13.6 ± 2.1 a	45.4 ± 9.0 a	8.3 ± 8.3 b	23.8 ± 4.8 ab
23N5-2	250	65.8 ± 5.4 a	74.0 ± 2.4 a	73.1 ± 5.3 a	70.3 ± 2.2 a	2.7 ± 1.3 a	20.8 ± 5.2 ab	0.0 ± 0.0 b	8.2 ± 2.2 b
23N5-2	125	28.8 ± 6.0 bc	35.1 ± 4.4 bc	31.2 ± 8.5 abc	31.4 ± 2.7 b	11.7 ± 2.6 a	38.1 ± 7.4 a	53.3 ± 20.0 a	22.9 ± 3.5 a
PDB	325	16.1 ± 3.5 cd	16.1 ± 3.8 cd	18.2 ± 4.6 bc	16.6 ± 2.8 bc	2.4 ± 1.6 a	21.4 ± 6.1 ab	15.7 ± 6.7 ab	10.9 ± 2.6 b
PDB	250	13.5 ± 2.9 cd	15.3 ± 2.1 cd	15.9 ± 4.2 bc	14.7 ± 1.2 bc	4.4 ± 3.1 a	23.2 ± 3.5 ab	11.3 ± 7.9 b	12.6 ± 2.9 b
PDB	125	20.5 ± 3.7 cd	24.6 ± 6.2 cd	4.8 ± 3.4 c	20.0 ± 4.3 c	6.7 ± 3.7 a	41.6 ± 8.0 a	16.3 ± 4.6 ab	19.0 ± 4.0 ab
Water	0	10.0 ± 3.2 d	11.9 ± 4.0 d	18.8 ± 3.8 bc	12.2 ± 2.1 c	4.5 ± 2.9 a	11.3 ± 4.2 b	13.2 ± 6.0 ab	9.5 ± 1.9 b

Means in the same column followed by the same letter are not significantly different (Tukey's studentized range test, $P \geq 0.05$).

DISCUSSION

Filamentous fungi including *P. lilacinus* were isolated from fields with a high and low *R. similis* density in Uganda. In the field with high *R. similis* density, more isolates were obtained from the root surface than from the rhizosphere soil, whereas the opposite occurred in the field with low *R. similis* density. Hillocks (2001) reported that nematode invasion of the root enhances root exudation and that the resulting supply of exogenous nutrients in the form of root exudates provide the energy to overcome fungistasis in the soil, allowing resting spores of fungi to germinate. Modification of the rhizosphere nutrient pool may be a result of frequent penetration of root surfaces by *R. similis*, which would cause frequent release of root exudates onto the root surface.

The relatively high frequency of *Paecilomyces* spp. among the other isolated fungi indicated its high abundance near or on banana roots. There was no difference in abundance of *P. lilacinus* between the fields with high and low *R. similis* density. This lack of density dependence may indicate that the fungus obtained the majority of its nutrition from soil substrates, rather than from nematodes or from the release of root exudates described above. *Paecilomyces lilacinus* survives in the soil by using organic matter, such as decomposed tissues shed from roots, as the primary nutrient source and nematodes as a secondary source (Nikolay and Sikora, 1991; Sikora, 1992).

All but one of the five bioassays demonstrated that filtrates of *P. lilacinus* isolate 23N-5 caused *R. similis* paralysis. Cayrol *et al.* (1989) reported toxin production by *P. lilacinus* against 17 species of nematodes. *Paecilomyces lilacinus* toxins include paecilotoxins (Khan *et al.*, 2003), hydrolytic enzymes such as polysaccharidases, proteases and chitinases (Gupta *et al.*, 1993) and leucinostatins (Park *et al.*, 2004). The nematode-antagonistic compounds in the filtrates used the present study only induced a temporary paralysis, suggesting a presumable neurotropic action on the nervous receptors of nematodes.

After *R. similis* motile stages were rinsed with water and incubated for 21 h, paralysis had reversed and did not differ from that in the control treatments. Some of the isolates that produced exudates caused a higher level of paralysis than those not producing exudates. Filtrate concentrations had inconsistent effects across bioassays 4-5. Presumably, the filtrate concentrations contained nematode-antagonistic compounds in quantities close to threshold levels for nematode paralysis.

Across the majority of the bioassays, female *R. similis* succumbed to paralysis to a lesser extent than juveniles or males. This may be due to the stronger structural features of females compared to those of juveniles or males (Van Weerdt, 1960). For example, males are shorter than females and lack a sclerotized cephalic framework, potentially rendering females more resistant to fungal filtrates.

Two isolates of *P. marquandii* were included in bioassays 1-3. In one bioassay, *P. marquandi* isolate 22R5-1 caused higher paralysis than in any of the control treatments. *P. marquandii* has been reported to produce paecilotoxins, which have been found to be identical with paecilotoxins produced by *P. lilacinus* (Khan *et al.*, 2003). *P. marquandii* is a natural soil organism that contributes to nematode suppression (Samson, 1974; Marban *et al.*, 1992). However, few studies have reported antagonistic effects of *P. marquandii* against *R. similis*. Esnard *et al.* (1998) demonstrated that *P. marquandii*, formulated in a wheat substrate, reduced *R. similis* densities in the field in Costa Rica.

After assessing various fungal growth media, Cayrol *et al.* (1989) reported that

the pH of media affects production of toxins. Toxins were, however, reported to act upon nematodes across a wide range of pH values (Cayrol *et al.*, 1989). It was therefore important to assess the effect of pH variation among fungal filtrates by including PDB control treatments with pH values encompassing the upper and lower extreme values as that of fungal filtrates tested for bioassays 1-3. Extreme pH values clearly had an effect on nematode paralysis, as demonstrated in bioassay 2 when the pH of one of the PDB control treatments remained unadjusted, at a particularly low value, resulting in high paralysis.

Among all groups of plant-parasitic nematodes, migratory endoparasites such as *R. similis* are the most difficult to control with soil antagonists (Stirling, 1991). This study demonstrated that *Paecilomyces* spp. are present in significant numbers in banana fields in Uganda. *In vitro*, there was consistent paralysis of motile stages of *R. similis* immersed in filtrates of *P. lilacinus* isolate 23N5-2. At present, long-term effects of *P. lilacinus* filtrates against *R. similis* remain to be investigated. Isolates obtained from this study merit testing under greenhouse or field conditions to investigate whether the isolates, through antagonism of their filtrates, have promise to be further investigated for inclusion in an integrated pest management strategy against *R. similis* for resource-poor farmers in East Africa.

ACKNOWLEDGMENTS

We acknowledge the Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ) for funding this study. This work was conducted in partial fulfilment of Paul Kilama's M.Sc. thesis at the Department of Crop Protection at Makerere University. The authors wish to thank Komi Fiaboe, Christian Hillnhütter and Peter Neuenschwander for critically reviewing the manuscript prior to submission.

LITERATURE CITED

Barnett, H. L., and B. B. Hunter. 1998. Illustrated genera of imperfect fungi. 4th edition. American Phytopathological Society Press, St. Paul, MN.

Blake, C. D. 1961. Root rot of bananas caused by *Radopholus similis* (Cobb) and its control in New South Wales. Nematologica 6:295-310.

Blake, C. D. 1966. The histological changes in banana roots caused by *Radopholus similis* and *Helicotylenchus multicinctus*. Nematologica 12:129-137.

Butt, T. M., C. Jackson, and N. Magan. 2001. Introduction—fungal biological control agents: progress, problems and potential. Pp. 1-8 *in* Butt, T. M., C. Jackson, and N. Magan, eds. Fungi as biocontrol agents: progress, problems and potential. CAB International, Wallingford, UK.

Cayrol, J.-C., C. Djian, and L. Pijarowski. 1989. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. Revue de Nématologie 12:331-336.

Diaz, S., F. Modesto, and J. O. Herrera. 1998. An overview of nematological problems in Cuba. Nematropica 28:151-164.

Domsch, K. H., W. Gams, and T.-H. Anderson. 1993. Compendium of soil fungi. Vol. 1. IHW-Verlag, Eching, Germany.

Esnard, J., M. N. Marban, and B. M. Zuckerman. 1998. Effects of three microbial broth cultures and an organic amendment on growth and populations of free living and plant-parasitic nematodes on banana. European Journal of Plant Pathology 104:457-463.

Gold, C. S., M. W. Ogenga-Latigo, W. K. Tushemereirwe, I. N. Kashaija, and C. Nankinga. 1993. Farmer perception of banana pest constraints in Uganda: results from a rapid rural appraisal. Pp. 3-24 *in* Gold, C. S., and B. Gemmil, eds. Biological and integrated control of banana and plantain pests and diseases. Proceedings of a research coordination meeting. Cotonou, Benin, November 12-14, 1991. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Goos, R. D., and M. I. Timonin. 1962. Fungi from the rhizosphere of bananas in Honduras. Canadian Journal of Botany 40:1371-1377.

Gowen, S. R. 1995. Pests. Pp. 382-402 *in* Gowen, S. R., ed. Bananas and plantains. Chapman and Hall, London, UK.

Gowen, S. C., P. Quénéhervé, and R. Fogain. 2005. Nematode parasites of bananas and plantains. Pp. 611-643 *in* M. Luc, R. A. Sikora, and J. Bridge,

eds. Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edition. CAB International, Wallingford, UK.

Gupta, S. C., T. D. Leathers, and D. T. Wicklow. 1993. Hydrolytic enzymes secreted by *Paecilomyces lilacinus* cultured on sclerotia of *Aspergillus flavus*. Applied Microbiology and Biotechnology 39:99-103.

Hillocks, R. J. 2001. The implication for plant health of nematode–fungal interactions in the root zone. Pp. 269-283 *in* M. J. Jeger and N. C. Spence, eds. Biotic interactions in plant-pathogen associations. CAB International, Wallingford, UK.

Kegley, S., B. Hill, and S. Orme. 2007. PAN pesticide database. San Francisco, USA: Pesticide Action Network. Online: http://www.pesticideinfo.org.

Kerry, B. R., 2000. Rhizosphere interaction and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. Annual Review of Phytopathology 38:423-441.

Khan, A., K. Williams, and H. Nevalainen. 2003. Testing the nematophagous control isolate *Paecilomyces lilacinus* 251 for paecilotoxin production. FEMS Microbiology Letters 227:107-111.

Loos, C. A., and S. Loos. 1960. The blackhead disease of bananas (*Musa acuminata*). Proceedings of the Helminthological Society of Washington 27:189-193.

Luc, M., D. J. Hunt, and J. E. Machon. 1990. Chapter 1. Morphology, anatomy and biology of plant parasitic nematodes—a synopsis. Pp. 1-44 *in* M. Luc, R. A. Sikora and J. Bridge, eds. Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, UK.

Marban, M. N., E. R. Garcia, M. B. Dicklow, and B. M. Zuckerman. 1992. Studies on *Paecilomyces marquandii* from nematode suppressive chinampa soils. Journal of Chemical Ecology 18:775-783.

Mateille, T. 1994. Comparative host tissue reactions of *Musa acuminata* (AAA group) cvs Poyo and Gros Michel roots to three banana-parasitic nematodes. Annals of Applied Biology 124:65-73.

Mendoza, A., R. A. Sikora, and S. Kiewnick. 2004. Efficacy of *Paecilomyces lilacinus* (strain 251) for the control of *Radopholus similis* in banana. Communications in Agricultural and Applied Biological Sciences 69:365-372.

Nikolay, R., and R. A. Sikora. 1991. Interrelationships between fungal egg parasitism in *Heterodera schactii* Schmidt and nematode population density. Revue de Nématologie 14:231-249.

Nirenberg, H. I. 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. Canadian Journal of Botany 59:1599-1609.

Park, J. O., J. R. Hargreaves, E. J. McConville, G. R. Stirling, E. L. Ghisalberti and K. Sivasithamparam. 2004. Production of leucinostatins and nematicidal activity of Australian isolates of *Paecilomyces lilacinus* (Thom) Samson. Letters in Applied Microbiology 38:271-276.

Rubaihayo, P. R., and C. S. Gold. 1993. Rapid rural appraisal of highland banana production in Uganda. InfoMusa 2:15-16.

Samson, R. A. 1974. *Paecilomyces* and some allied Hyphomycetes. Centraalbureau voor Schimmelculturen, Baarn, The Netherlands.

Sikora, R. A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant-parasitic nematodes. Annual Review of Phytopathology 30:245-270.

Speijer, P. R., and D. Dewaele. 1997. Screening of *Musa* germplasm for resistance and tolerance to nematodes. International Plant Genetic Resources Institute, Rome, Italy.

Speijer, P. R., C. S. Gold, E. B. Karamura, and I. N. Kashaija. 1994. Banana weevil and nematode distribution pattern in highland banana systems in Uganda: preliminary results of a diagnostic survey. Pp. 285-289 *in* E. Adipala, J. S. Tenywa, and M. W. Ogenga-Latigo, eds. Proceedings of African crop science conference, Kampala, Uganda, June 14-18, 1993. Makerere University, Kampala, Uganda.

Stirling, G. R. 1991. Biological control of plant parasitic nematodes: progress, problems and prospects. CAB International, Wallingford, UK.

Van Weerdt, L. G. 1960. Studies on the biology of *Radopholus similis* (Cobb, 1893) Thorne 1949. Nematologica 5:43-51.

Viaene, N., D. L. Coyne, and B. R. Kerry. 2006. Biological and cultural control. Pp. 346-369 *in* Moens, M., and R. Perry, eds. Plant Nematology. CAB International, Wallingford, UK.

Von Arx, J. A. 1974. The genera of fungi sporulating in pure culture. J. Cramer, Voduz, Germany.

Received: 8/I/2007

Recibido:

Accepted for publication: 20/III/2007

Aceptado para publicación: