INFLUENCE OF *PAECILOMYCES LILACINUS* STRAIN 251 ON THE BIOLOGICAL CONTROL OF THE BURROWING NEMATODE *RADOPHOLUS SIMILIS* IN BANANA

A. R. Mendoza,[1]* R. A. Sikora,[1] and S. Kiewnick[2]

[1]University of Bonn, Institute for Crop Sciences and Resource Conservation, INRES Dept. of Plant Pathology, Research Unit Nematology in Soil Ecosystems. Nussallee 9, D-53115 Bonn, Germany; [2]Agroscope Changins-Wädenswil ACW, Research Station ACW, Plant Protection, Ecotoxicology and Soil Zoology, Schloss P.O. Box 185, Ch-8820 Wädenswil, Switzerland. *Corresponding Author: mendozaal66@yahoo.com.

ABSTRACT

Mendoza A. R., R. A. Sikora, and S. Kiewnick. 2007. Influence of *Paecilomyces lilacinus* strain 251 on the biological control of the burrowing nematode *Radopholus similis* in banana. Nematropica 37:203-213.

The efficacy of the bionematicide *Paecilomyces lilacinus* strain 251 (Bioact® WG) toward the burrowing nematode *Radopholus similis* in banana was determined. Greenhouse experiments were conducted to evaluate application form and dose response on nematode penetration in banana plants. The potential of multiple applications to enhance the efficacy of the fungus was also examined. A significant correlation between dosage and the level of *R. similis* suppression was detected. A concentration of 6×10^6 cfu of *P. lilacinus*/g dry soil was considered the optimum dose. The highest level of suppression was obtained with 6×10^6 cfu/g dry soil of *P. lilacinus* applied to the soil three times; 6 days before planting, at-planting, and as a plantlet drench. The results demonstrated that *P. lilacinus* is an effective biocontrol agent against *R. similis* in banana and can be an important component of integrated pest management strategies.

Key words: biocontrol, Bioact® WG, *Musa*, *Paecilomyces lilacinus*, *Radopholus similis*.

RESUMEN

Mendoza A. R., R. A. Sikora, and S. Kiewnick. 2007. Influencia de la dosis y el tiempo de aplicación de *Paecilomyces lilacinus* cepa 251 sobre el control biológico del nematodo barrenador *Radopholus similis* en banano. Nematropica 37:203-213.

Se investigó la eficacia del biopesticida *Paecilomyces lilacinus* cepa 251 (Bioact® WG) contra el nematodo barrenador de las raíces en plantas de banano. Los experimentos llevados a cabo en invernaderos fueron realizados para evaluar la forma de aplicación y el efecto de la dosis sobre la penetración del nematodo en las raíces de plantas de banano. Adicionalmente, se evaluó el potencial de las multiples aplicaciones para reforzar la eficacia del hongo. Se determinó una correlación significativa entre la dosis y el nivel de biocontrol del nematodo *R. similis*. Una concentración de 6×10^6 cfu g^{-1} suelo seco fue considerada como la dosis óptima de aplicación. El mayor nivel de control se alcanzó cuando se aplicó una concentración de 6×10^6 cfu g^{-1} suelo seco 3 veces: 1) 6 días antes de la siembra, 2) durante la siembra y 3) una aplicación sobre el suelo de las plántulas. Los resultados demuestran que *P. lilacinus* es un agente efectivo para el control biológico de *R. similis* en el cultivo del banano y podría ser un importante componente en una estrategía de manejo integrado de plagas.

Palabras claves: biocontrol, Bioact® WG, *Musa*, *Paecilomyces lilacinus*, *Radopholus similis*.

INTRODUCTION

Commercial banana production requires nematode management strategies that are reliable, effective, economically and environmentally viable, and which can be integrated in overall banana production systems. Nematode parasitism of banana roots is usually characterized by simultaneous infestations by several species, including *Radopholus simi-*

lis, *Helicotylenchus multicinctus* and several species of *Pratylenchus* and *Meloidogyne*. The burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949, the causal agent of "Black-head toppling of banana", is a major pathogen of banana production worldwide (Gowen *et al.*, 2005; Moens *et al.*, 2004; Ferraz and Brown, 2002). Root invasion by *Radopholus similis* results in elongated dark lesions, as well as uprooting and premature toppling in fruit bearing plants (Ploetz *et al.*, 2003).

To manage nematodes in commercial banana plantations and reduce yield losses, soil treatments with nematicides are essential. In many instances, the banana crop cannot be grown economically without the use of nematicides (Gowen *et al.*, 2005). Repeated use of nematicides has led in some cases to a condition known as "enhanced biodegradation" where the active ingredient is rapidly metabolized by soil microflora (Gowen *et al.*, 2005; Moens *et al.*, 2004; Anderson and Lafuerza, 1992). Although many studies (zum Felde *et al.*, 2006; Vu *et al.*, 2006; Siddiqui *et al.*, 2000) have been conducted to identify alternatives to the use of nematicides in banana, biological control is not widely used.

The nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson 1974 is a facultative egg pathogen of many sedentary nematodes and the most extensively field tested biological control agent for nematode management (Holland *et al.*, 2003; Kiewnick and Sikora, 2003; Siddiqui *et al.*, 2000; Kerry *et al.*, 1996). *Paecilomyces lilacinus* strain 251 (PL251) has been commercialized and registered for sale as Bioact® WG for control of nematode pests in several countries. Moreover, PL251 has received EPA registration in the USA as a biological nematicide under the trade name Melocon® WG (Kiewnick and Sikora, 2006). This strain, originally isolated from a *Meloidogyne* egg mass in the Philippines, has been shown to be highly effective in controlling *Meloidogyne* spp. on tomato and *Globodera rostochiensis* on potato (Davide, 1985; Davide and Zorrila, 1983).

The use of *Paecilomyces lilacinus* 251 to control plant parasitic nematodes, in particular *Meloidogyne incognita* (Kiewnick and Sikora, 2006; Dube and Smart, 1987; Jatala *et al.*, 1980), *Meloidogyne javanica* (Holland, *et al.*, 1999), *Meloidogyne arenaria* (Gomes Carneiro *et al.*, 1991), and *Radopholus similis* (Khan *et al.*, 2006; Jonathan and Rajendran, 2000) has been studied. However, there is only limited information available on dosage and application time of the PL251 commercial product, Bioact® WG, for controlling the burrowing nematode, *Radopholus similis,* in banana.

The objectives of the current study were to examine the effect of application rates, timing of application and use of multiple applications of *Paecilomyces lilacinus* strain 251 on the suppression of *Radopholus similis* in banana.

MATERIALS AND METHODS

Bioact® WG containing *Paecilomyces lilacinus* strain 251 used in this study was supplied by Prophyta (GmbH, Malchow-Poel, Germany). The commercial product is formulated as a water dispersible granule (WG) containing at least 1×10^{10} viable conidia/g of product. One liter plastic pots filled with 630 g (dry weight) sterile field soil:sand mixture (1:1, v:v) passed through a 2 mm mesh sieve, were used in all experiments. The field soil was a silty loam (14.6% clay, 77.6% silt, 7.8% sand) with a pH of 5.2 and an organic matter content of 0.97%.

In vitro propagated plantlets of banana, *Musa* AAA cv. 'Grande Naine', were used in all experiments (VITROPIC S.A, Saint Mathieu de Tréviers, France). Plants were placed in seedling trays (24 cells, 35 cm ×

22 cm) acclimatized for 8 weeks in a climate chamber (MISA S.p.A., Roma, Italia) at 30 ± 1°C with 16 hours of light/day and fertilized with a commercial NPK-fertilizer (14-10-14, 2 g/l) (AGLUKON, Düsseldof, Germany) as required. *Radopholus similis* isolated from bananas in Uganda and reared on carrot disks were used as inoculum (Speijer and De Waele, 1997).

Efficacy and Dose-response

Four rates of PL251 containing 1.5, 3.0, 6.0 or 9.0 × 10^6 conidia/g dry soil were applied and incorporated into the soil 6 days before planting. Mixed stages (males, females and juveniles) of 1000 *Radopholus similis*/630 g soil were inoculated directly thereafter into five holes, each 1 cm in diameter and 2 cm deep and 2 cm from the plant base or from the pot center in the absence of a plant. Pots containing soil treated only with water, and soil inoculated only with nematodes were included as controls.

Experimental pots were then incubated at 28 ± 2°C with 16 hour/day supplemental artificial light for 6 days in a climate chamber before transplanting of banana. Six-week-old banana plantlets were transplanted into the pots, and later arranged in a completely randomized design on a glasshouse bench with a daily mean temperature of 28 ± 2°C and 16 hours supplemental artificial light/day. All treatments were replicated a minimum of 6 times. Plants were harvested 20 days after planting. Fresh root and shoot weight were determined and root length was measured using a scanner (AGFA. Model SNAPSCAN 1236; Agfa Gevaert, Motsel, Belgium) and the WinRhizo software (Ver. 5.1; Regent Instruments, Canada). Afterwards, the roots were stained with acid fuchsin (Ferris, 1985) and macerated in water using an Ultra-Turrax (IKA-WERKE, Staufen, Germany) at 11.000 U/min to quantify numbers of nematodes that had penetrated the root system. Nematodes recovered per root system were counted under a stereomicroscope. Nematode suppressiveness was calculated using the following equation: % *R. similis* suppressed = [100 – (# *R. similis* in treatment * 100%/# *R. similis* in inoculated control)].

Application Timing—Single Application

Glasshouse experiments were carried out to determine the effect of PL251 application time on its efficacy against *R. similis* infecting banana roots. PL251 was applied as described above, with variations as follows: 1) pre-planting application: an aqueous suspension of PL251 conidia was incorporated into the soil at a concentration of 6 × 10^6 conidia/g dry soil and inoculated with 1000 mixed stages (males, females and juveniles) of *R. similis* into five holes; 2) at-planting application: six days after inoculation of the soil with nematodes as described previously, PL251 was applied and incorporated into the soil just before transplanting, at a dose of 6 × 10^6 conidia/g dry soil; 3) post-planting application: PL251 conidia were applied into five holes, placed 2 cm from the plant, six days after planting and 12 days after the soil was inoculated with mixed stages of nematodes. Plants treated with water and plants inoculated only with nematodes served as controls.

Experimental pots were incubated at 28 ± 2°C for 6 days in a climate chamber before transplanting. After transplanting one plant into each pot, the pots were arranged in a completely randomized design on a glasshouse bench with a daily temperature of 28 ± 2°C and 16 hours supplement artificial light/day. Plants were watered daily and fertilized as required. All treatments were replicated six times and the experiment was conducted twice.

Banana plants were harvested 14 days after transplanting. Fresh root and shoot weight, root length as well as nematode penetration were determined.

Application Timing—Multiple Applications

Two experiments were conducted to improve the nematode suppressive effect of PL251 attained in the previous experiments. PL251 was applied at a rate of 6×10^6 conidia/g dry soil in three treatment combinations: 1) pre-planting: PL251 was incorporated into soil 6 days before transplanting six-week-old banana plantlets; 2) pre-planting and at-planting: PL251 was incorporated into soil six days before and at the time of transplanting; 3) pre-planting, at-planting plus a seedling tray drench 24 hours prior to transplanting.

One thousand *R. similis* mixed stages (males, females and juveniles) per 630 g soil were inoculated simultaneously with the pre-planting application of PL251 as described previously. Plants treated with water and plants inoculated only with nematodes were included as controls. Prior to transplanting bananas, all pots were incubated at 28 ± 2°C for 6 days in a climate chamber. Banana plantlets were then transplanted into pots, which were arranged in a completely randomized design on a glasshouse bench with a daily mean temperature of 28 ± 2°C and 16 hour supplemental artificial light/day. All treatments were replicated six times and the experiment was conducted twice. Banana plants were harvested and nematode infection and plant growth parameters were recorded as previously described.

Effect of PL251 on the Second Generation of R. similis

PL251 at a rate of 6×10^6 conidia/g dry soil was applied pre-planting, at-planting and as a plantlet drench. One thousand mixed stages of *R. similis* were inoculated. Pots were acclimatized for 6 days in the climate chamber, and after transplanting banana plantlets, pots were maintained on a glasshouse bench described for the previous experiments. Plants treated with water and plants inoculated only with nematodes were included as controls. Treatments were arranged in a completely randomized design and replicated six times. The experiment was conducted twice.

Plants were harvested after the required number of degree-days had been reached for *R. similis* to reproduce a second generation (Wilson and Barnett, 1983). The hatch of juveniles from the eggs of the first generation occurred approximately 414 accumulated degree-days after transplanting, with a developmental threshold or base temperature of 20°C. Accumulated degree-days were calculated by subtracting the *R. similis* developmental threshold temperature of 20°C from the average mean glasshouse daily temperature [Degree-days = (daily high + daily low)/2 – developmental threshold]. After harvesting, shoot and root weight was determined. Nematodes were extracted and counted as described previously. Treatments were replicated six times and the experiment was conducted twice.

Statistical Analysis

The analysis of variance procedure (ANOVA) was used to determine significant differences. When the overall F test was significant, means were compared using the Least Significant Difference (LSD) method. Differences were considered significant at the 95% confidence level ($P = 0.05$). After verifying homogeneity of variances, data from two experiments were pooled for statistical analysis. In addition, for the dose-response experiment, regression analysis, between application

rate and the number of *R. similis* recovered per root system was performed by using STATGRAPHICS Plus for Windows Version 3.1 (Statistical Graphics Corp., Rockville, MD, USA). The reproductive index (RI) of *R. similis* was calculated by dividing the final number of *R. similis* by the number inoculated (Nf/Ni).

RESULTS

Efficacy and Dose-response

Regression analysis revealed a negative relationship (P = 0.0001) between nematode penetration per gram of root and dosage of *P. lilacinus* (r^2 = 0.732*** and 0.897*** in experiment I and II, respectively) (Fig. 1). Nematode suppression ranged from 32% at 3×10^6 cfu/g dry soil to 77% at 9×10^6 cfu/g dry soil in experiment I, and from 11% at 1.5×10^6 cfu/g soil to 86% at 9×10^6 cfu/g soil in experiment II (calculated from the data in Fig. 1). The number of nematodes per root system decreased as PL251 spore concentration increased. In both experiments, the lowest dose did not affect ($P < 0.05$) overall nematode root penetration (Fig. 1). In both experiments, no differences in root and shoot fresh weight were observed among the treatments when compared to the controls (data not shown). In experiment II, the highest dose of PL251 significantly increased root length compared to the nematode-inoculated control ($P <$ 0.05; data not shown).

Application Timing

When PL251 was applied prior to planting, the total number of nematodes per g of root was lowest ($P < 0.05$) (Table 1). Treatments with applications at other times did not suppress the number of *R. similis* as compared to the nematode-inoculated control. Pre-plant application of PL251 reduced the number of nematodes in the root by 56%, whereas at-planting and post-planting applications only reduced nematode penetration by 17 and 8%, respectively. Only PL251 applied prior to planting increased root and shoot fresh weight as well as root length compared to the untreated-nematode-inoculated control ($P < 0.05$; Table 1). Applying PL251 at planting increased root length ($P < 0.05$), whereas root length in the post-plant treatment was not different from the nematode-inoculated control (Table 1).

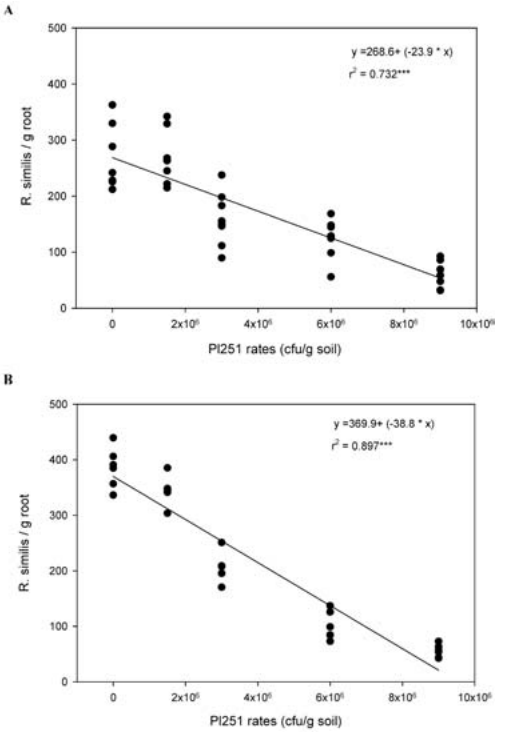

Fig. 1. Effect of four single application doses of *Paecilomyces lilacinus* strain 251 applied 6 days before planting on *Radopholus similis* penetration into banana roots under glasshouse conditions. Plants were harvested after 20 days at 28°C. A and B are repeat tests ($P < 0.0001$) (n = 6).

The results of the first two experiments indicated that the populations of *R. similis*

Table 1. Effect of application time (pre-, at- and post-planting) of a single dose of 6×10^6 cfu/g soil of *Paecilomyces lilacinus* strain 251 on root and shoot weight, root length and *Radopholus similis* penetration in banana roots under greenhouse conditions. Plants were harvested after 20 days at 28 ± 2°C.

Treatment	*R. similis*/ g of root	Reduction (%)[x]	Root fresh weight (g)	Shoot fresh weight (g)	Root length (m)
Control[y]	—	—	3.16 b	10.81 a	8.71 ab
Inoculated control[z]	89 a	—	5.63 a	8.22 b	8.20 bc
Pre-planting	39 b	56	3.21 b	11.14 a	10.97 a
At-planting	74 ab	17	2.82 b	10.00 ab	5.76 c
Post-planting	82 a	8	3.54 ab	11.25 a	6.71 bc
LSD ($P \leq 0.05$)	31.46	—	1.52	1.75	1.78

Means (n = 12) in the same column followed by a different letter are significantly different according to LSD test ($P \leq 0.05$).

[x]The percentage reduction was calculated using the number of *R. similis*/g root in the nematode-inoculated control as 100% penetration.

[y]Control treatment did not receive nematodes or PL251.

[z]Inoculated control treatments received nematodes but not PL251.

in the root system were reduced when PL251 was applied prior to planting. In addition, it was also demonstrated that increasing the concentration of PL251 in the soil further decreased *R. similis* infection of banana roots. The number of burrowing nematodes recovered per g of root in all PL251 treatments showed differences compared to the untreated-nematode-inoculated control in both experiments (Table 2). Multiple applications of PL251 performed better than the single application in both experiments (Table 2). Nematode suppression by PL251 in experiment I ranged from 52% to 92% for treatment 1 and 3, respectively, whereas in experiment II, nematode suppression in the same treatments ranged from 41% to 85%, respectively (Table 2).

The results in table 3 show that all treatments tested led to an increase in root and shoot weight compared to the nematode-inoculated control. However, only PL251 applied pre- and at-planting (treatment 2) in experiment I and PL251 applied pre-planting, at-planting and as a drench to the plantlets (treatment 3, both experiments) were different compared to the nematode-inoculated control (Table 3). In experiment I, root length slightly increased in all the treatments but was not significantly different compared to the untreated-nematode-inoculated control in experiment I. In experiment II, treatments 2 and 3 increased root length when compared to the nematode-inoculated control. However, there were no differences between treatments 2 and 3 for any of the parameters determined (Table 3).

Effect of PL251 on the Second Generation of R. similis

When comparing the effect of multiple applications of PL251 on the population dynamics of *R. similis*, differences (P = 0.0001) were detected in the number of nematode eggs per gram of root in all treatments compared to the nematode-inoculated control (Table 4). In addition, differences in numbers of eggs/g root found between treatments 1 and 3 were sig-

Table 2. Effect of multiple applications of 6×10^6 cfu/g soil of *Paecilomyces lilacinus* strain 251 on *Radopholus similis* penetration in banana root under greenhouse conditions. Plants were harvested after 20 days at 28 ± 2°C.

	Experiment I		Experiment II	
Treatment	*R. similis*/g root	Reduction (%)[u]	*R. similis*/g root	Reduction (%)
Control[v]	—	—	—	
Inoculated control[w]	402 a	—	148 a	—
Treatment 1[x]	193 b	52	88 b	41
Treatment 2[y]	74 c	82	51 bc	66
Treatment 3[z]	33 c	92	23 c	85
LSD ($P \leq 0.05$)	86.53		41.58	

Means (n = 6) in the same column followed by a different letter are significantly different according to LSD test ($P \leq 0.05$).
[u]The percentage reduction was calculated using the number of *R. similis*/g root in the nematode-inoculated control as 100% penetration.
[v]Control treatment did not receive nematodes or PL251.
[w]Inoculated control treatment received nematodes but not PL251.
[x]Treatment 1: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting soil treatment.
[y]Treatment 2: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment.
[z]Treatment 3: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment plus a plantlets drench using the seedling tray, 24 hours prior to transplanting.

Table 3. Effect of multiple applications of 6×10^6 cfu/g soil of *Paecilomyces lilacinus* strain 25 on root weight, shoot weight and root length of banana plants under greenhouse conditions. Plants were harvested after 20 days at 28 ± 2°C.

	Experiment I			Experiment II		
Treatment	Root fresh weight (g)	Shoot fresh weight (g)	Root length (m)	Root fresh weight (g)	Shoot fresh weight (g)	Root length (m)
Control[v]	2.10 bc	3.80 ab	10.48 a	3.24 ab	6.83 a	8.64 a
Inoculated control[w]	1.34 d	3.09 c	4.20 b	2.61 c	4.92 b	5.66 b
Treatment 1[x]	1.63 cd	3.26 bc	6.36 b	2.72 bc	5.12 b	5.69 b
Treatment 2[y]	2.34 ab	3.90 a	6.48 b	2.99 abc	6.36 a	8.61 a
Treatment 3[z]	2.67 a	4.16 a	7.03 b	3.40 a	6.60 a	10.13 a
LSD ($P \leq 0.05$)	0.55	0.58	3.37	0.58	0.79	2.79

Means (n = 6) in the same column followed by a different letter are significantly different according to LSD test ($P \leq 0.05$).
[v]Control treatment did not receive nematodes or PL251.
[w]Inoculated control treatment received nematodes but not PL251.
[x]Treatment 1: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting soil treatment.
[y]Treatment 2: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment.
[z]Treatment 3: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment plus a plantlets drench using the seedling tray, 24 hours prior to transplanting.

Table 4. Effect of multiple applications of 6×10^6 cfu/g soil of *Paecilomyces lilacinus* strain 251 on the reproduction and penetration of *Radopholus similis* in banana root under greenhouse conditions. Plants were harvested after 414 degree-days at 28 ± 2°C.

Treatment	Eggs/g of root	*R. similis*/g of root	Nematode reduction (%)[T]	Reproductive index[U]
Control[v]	—	—	—	—
Inoculated control[w]	3295.0 a	6201.8 a	—	105.2 ± 44.0 a
Treatment 1[x]	2237.1 b	5962.2 a	3.9 a	86.0 ± 33.5 b
Treatment 2[y]	1534.5 bc	3776.1 b	39.1 b	63.9 ± 27.5 c
Treatment 3[z]	943.6 c	2970.0 b	52.1 c	5.8 ± 26.7 d
LSD ($P \leq 0.05$)	951.0	1752.9		

Means (n = 12) in the same column followed by a different letter are significantly different according to LSD test ($P \leq 0.05$).

[t]The percentage reduction was calculated using the number of *R. similis*/g root in the nematode-inoculated control as 100% penetration.

[u]Final number of *R. similis* (Nf) divided by the number inoculated (Ni).

[v]Control treatment did not receive nematodes or PL 251.

[w]Inoculated control treatment received nematodes but not PL251.

[x]Treatment 1: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting soil treatment.

[y]Treatment 2: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment.

[z]Treatment 3: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment plus a plantlets drench using the seedling tray, 24 hours prior to transplanting.

nificant. The number of nematodes recovered per g of root for treatments 2 and 3 were significantly different (P = 0.0007) compared to treatment 1 and the nematode-inoculated control. Between treatment 1 and the untreated-nematode-inoculated control, as well as between treatments 2 and 3, no differences were observed.

The reduction of *R. similis*/g root was significantly different between treatments and ranged from 3.9% for treatment 1 to 52.1% for treatment 3 (Table 4). Lowest numbers of *R. similis*/g root were found for the two multiple application treatments. All PL251 treatments resulted in reproduction rates significantly different from the inoculated control (Table 4).

Data presented in Table 5 shows no differences in root and shoot weight among all the treatments inoculated with *R. similis*. Except treatment 3, all PL251 applications resulted in significantly lower root fresh weight, compared to the untreated, non inoculated control.

DISCUSSION

The present study demonstrated that *P. lilacinus* strain 251, a known nematode egg pathogen, controlled the burrowing nematode, *R. similis* efficiently in banana in greenhouse pot trials. A clear dose response relationship was observed between the concentration of PL251 conidia applied to the soil 6 days before transplanting and the level of nematode suppression. This result is similar to the effect of PL251 on *Meloidogyne incognita* infecting tomato (Kiewnick and Sikora, 2006).

Soil densities of PL251 higher than 3×10^6 cfu/g of dry soil added before transplanting were need to reduce *R. similis* root infection. The number of nematodes penetrating the roots decreased consider-

Table 5. Effect of multiple applications of 6×10^6 cfu/g soil of *Paecilomyces lilacinus* strain 251 on root and shoot weight of banana plantlets under greenhouse conditions. Plants were harvested after 414 degree-days at 28 ± 2°C.

Treatment	Root fresh weight (g)	Shoot fresh weight (g)
Control[v]	19.0 a	44.7
Inoculated control[w]	16.9 bc	45.4
Treatment 1[x]	15.4 c	39.6
Treatment 2[y]	16.9 bc	41.6
Treatment 3[z]	18.6 ab	45.4
LSD ($P \leq 0.05$)	2.0	n.s.

Means (n = 12) in the same column followed by a different letter are significantly different according to LSD test ($P \leq 0.05$).

[v]Control treatment did not receive nematodes or PL 251.

[w]Inoculated control treatment received nematodes but not PL251.

[x]Treatment 1: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting soil treatment.

[y]Treatment 2: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment.

[z]Treatment 3: 6×10^6 cfu/g soil of *P. lilacinus* applied as a pre-planting and at-planting soil treatment plus a plantlets drench using the seedling tray, 24 hours prior to transplanting.

ably as the cfu of PL251 in soil increased. Kiewnick and Sikora (2003; 2004) and Gomes and Cayrol (1991) demonstrated that a PL251 threshold density of 10^6 cfu/g of soil was needed to suppress of *M. incognita* and *M. arenaria* on tomato.

The results of a single pre-plant, at-plant or post-plant application demonstrated that PL251 reduced *R. similis* penetration in all treatments. The highest reduction in nematode penetration was achieved when 6×10^6 cfu/g soil was applied 6 days before transplanting. Kiewnick and Sikora (2003) demonstrated that the application of PL251 before transplanting reduced the number of nematodes surviving in the soil at planting and resulted in reductions in damage caused by *M. incognita* in tomatoes. At-plant and post-plant applications of PL251 also reduced *R. similis* penetration in the roots of banana. However, the level of nematode suppressiveness was lower than with pre-plant applications of the fungus. Khan *et al.* (2006) demonstrated the capability of PL251 to control *R. similis* in pot trials when it was applied two weeks after nematode inoculation. In their experiment, *P. lilacinus* reduced *R. similis* by 21% and 86%, ten and eighteen weeks after nematode inoculation, respectively.

The results of multiple applications clearly demonstrated that increasing the dosage and number of applications of PL251 increased protection of the banana plant from *R. similis*. The multiple applications caused significant reductions in nematode penetration. Furthermore, nematode density in the second generation was reduced by PL251.

This study clearly demonstrated the effectiveness of PL251 in protecting banana plants from *R. similis*, with no associated phytotoxicity. These findings demonstrate that *P. lilacinus* strain 251 has significant biocontrol effect toward the burrowing nematode *R. similis* on young plants when applied during the pre-planting process.

At present, the use of tissue-culture plants is an attractive alternative for rapid replacement of planting material on commercial banana farms. Tissue-culture plantlets are left to acclimatize and grow in a nursery before being planted in the field. During this time, PL251 can be applied without significantly increasing labor costs. PL251 has the opportunity to colonize the soil in which the plants are growing and when the plant is transplanted into the field, the fungi has the opportunity to kill nematodes prior to their penetration into

the roots. Further research is needed to determine long-term field efficacy of PL251 in banana production, and field trials would be beneficial to verify greenhouse results.

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LITERATURE CITED

Anderson, J. and A. Lafuerza. 1992. Microbiological aspects of accelerated pesticide degradation. Proceeding of International Symposium of Environmental Aspects of Pesticide Microbiology. Swedish University of Agriculture Science, Uppsala. 184-192.

Davide, R. G. 1985. Summary report on the current status, progress and needs for *Meloidogyne* research in region VI. Pp. 369-378 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne*. Vol. I. Biology and Control.

Davide, R. G. and R. A. Zorilla. 1983. Evaluation of a fungus *Paecilomyces lilacinus* (Thom.) Samson, for the biological control of potato cyst nematode *Globodera rostochiensis* Woll. as compared with some nematicides. Phillipine Agriculturist 66:397-404.

Dube, B. and G. C. Smart. 1987. Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. Journal of Nematology 19:222-227.

Ferraz, L. C. C. B. and D. J. F. Brown. 2002. An introduction to nematodes: Plant Nematology, A student's text book. Pensoft Publishers, Sofia, Bulgaria.

Ferris, J. M. 1985. Crop loss prediction and modelling for management decisions. Pp. 27-33 *in* B. N. Zuckermann, W. F. Mai, and M. B. Harrison, eds. Plant nematology laboratory manual. University of Massachusetts, Agric. Exp. Station, Amherst, MA.

Gomes Carniero, R. M. D., and J. C. Cayrol. 1991. Relationship between inoculum density of the nematophagous fungus *Paecilomyces lilacinus* and control of *Meloidogyne arenaria* on tomato. Revue Nématologie 14:629-634.

Gowen, S. R., P. Quénéherve, 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. CAB International, Wallingford, UK.

Holland, R. J., K. L. Williams, and A. Khan. 1999. Infection of *Meloidogyne javanica* by *Paecilomyces lilacinus*. Nematology 1:131-139.

Holland, R. J., K. L. Williams, and H. K. M. Nevalainen. 2003. *Paecilomyces lilacinus* strain Bioact251 is not a plant endophyte. Australasian Plant Pathology 32:473-478.

Jatala, P., R. Kaltenback, M. Bocangel, A. J. Devaus, and R. Campos. 1980. Field application of *Paecilomyces lilacinus* for controlling *Meloidogyne incognita* on potatoes. Journal of Nematology 12:226-227.

Khan A., K. L. Williams, and H. K. M. Nevalainen. 2006. Control of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* in pot trials. BioControl 51:643-658.

Kerry, B. R. and K. Evans. 1996. New strategies for the management of plant parasitic nematodes. Pp. 134-152 *in* Hall, ed. Principles and practice of managing soilborne plant pathogens. APS, St. Paul, MN.

Kiewnick, S. and R. A. Sikora. 2003. Efficacy of *Paecilomyces lilacinus* (Strain 251) for the control of root-knot nematodes. Communications in Applied Biological Science 68:123-128.

Kiewnick, S. and R. A. Sikora. 2004. Optimizing the efficacy of *Paecilomyces lilacinus* (strain 251) for the control of root-knot nematodes. Communications in Applied Biological Science 69:373-380.

Kiewnick, S. and R. A. Sikora. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. Biological Control 38:179-187.

Moens, T., M. Araya, R. Swennen, and D. De Waele. 2004. Enhanced biodegradation of nematicides after repetitive applications and its effect on root and yield parameters in commercial banana plantations. Biology and Fertility of Soils 39:407-414.

Ploetz, R. C., J. E. Thomas, and W. Slabaugh. 2003. Diseases of Banana and Plantain. Pp. 73-134 *in* Ploetz, R. C. ed. Diseases of Tropical Fruit Crops. CABI Publishing, Wallingford, UK.

Siddiqui, I. A., S. A. Qureshi, V. Sultana, S. Ehtesham-ul-Haque, and A. Ghaffar. 2000. Biological control of root rot-root knot disease complex of tomato. Plant and Soil 227:163-169.

Speijer, P. R. and D. De Waele. 1997. INIBAP Technical Guidelines. 1. Screening of *Musa* Germplasm for Resistance and Tolerance to Nematodes. INIBAP, Montpellier, France.

Vu Tam, R. Hauschild, and R. A. Sikora. 2006. *Fusarium oxysporum* endophytes induced systemic resistance against *Radopholus similis* on banana. Nematology 8:847-852.

Wilson, L. T. and W. W. Barnett. 1983. Degree-Days: An Aid in Crop and Pest Management. California Agriculture 37:4-7.

zum Felde, A., L. E. Pocasangre, C. A. Carñizares Monteros, R. A. Sikora, F. E. Rosales, and A. S. Rivero. 2006. Effect of combined inoculations of endophytic fungi on the biocontrol of *Radopholus similis*. Info*Musa* 15:12-18.

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