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Control of plant-parasitic nematodes by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* in pot trials

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Abstract. The common soil inhabiting nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson and the nematode trapping fungus *Monacrosporium lysipagum* (Drechsler) Subram were assayed for their ability to reduce the populations of three economically important plant-parasitic nematodes in pot trials. The fungi were tested individually and in combination against the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood, cereal cyst nematode *Heterodera avenae* Wollenweber, or burrowing nematode *Radopholus similis* (Cobb) Thorne on tomato, barley and tissue cultured banana plants, respectively. In all cases, nematode populations were controlled substantially by both individual and combined applications of the fungi. Combined application of *P. lilacinus* and *M. lysipagum* reduced 62% of galls and 94% of *M. javanica* juveniles on tomato when compared to the experiment with no fungi added. Sixty five percent of *H. avenae* cysts were reduced on barley by combined application of fungi. Control of *R. similis* on banana, both in the roots and in the soil, was greatest when *M. lysipagum* was applied alone (86%) or in combination with *P. lilacinus* (96%), using a strategy where the fungi were inoculated twice in 18 weeks growth period. Overall, combined application of *P. lilacinus* and *M. lysipagum* was the most effective treatment in controlling nematode populations, although in some cases *M. lysipagum* alone was as effective as the combined application of fungi, particularly against *M. javanica*.

Key words: banana, barley, biological control, *Heterodera*, *Meloidogyne*, *Monacrosporium*, *Paecilomyces*, pot trial, *Radopholus*, tomato

Abbreviations: ANOVA – analysis of variance; CRD – completely randomized design; DNMRT – Duncan's New Multiple Range Test; PCA – potato carrot agar; PDA – potato dextrose agar

Introduction

Plant-parasitic nematodes cause diseases thereby interfering with crop production. The cosmopolitan root-knot nematode *Meloidogyne* spp. has been considered the most damaging of the ten genera of plant-parasitic nematodes (Sasser and Freckman, 1987). Common hosts for *Meloidogyne* spp. are food crops, vegetables, fruit and ornamental plants (Netscher and Sikora, 1990). The cereal cyst nematode *Heterodera avenae* Wollenweber is of worldwide concern as a parasite of cereal crops that constitute the most important source of food globally. Wheat, barley and oats are suitable hosts for *H. avenae* (Swarup and Sosa-Moss, 1990; Al-Hazmi et al., 1994). The burrowing nematode *Radopholus similis* (Cobb) Thorne occurs in tropical and sub-tropical areas of the world (Loof, 1991). More than 250 plant species including banana, citrus, sugarcane, tea, coffee and maize are known to be the hosts for *Radopholus* spp. (Gowen and Quénéhervé, 1990; Loof, 1991). These nematodes were selected as targets for biological control because of the deleterious effects of *Meloidogyne*, *Heterodera* and *Radopholus* spp. on several economically important crops.

The fungus *Paecilomyces lilacinus* (Thom) Samson, a nematode egg parasite is currently used as a biological control agent against various plant-parasitic nematodes, particularly the *P. lilacinus* strain 251 for which a commercial formulation is available (Kiewnick et al., 2002; Brand et al., 2004; http://www.prophyta.com). *P. lilacinus* successfully controlled the nematode *M. incognita* on potato (Jatala et al., 1980) and on tomato (Villanueva and Davide, 1984; Lara Martez et al., 1996) in field conditions, and on banana (Jonathan and Rajendran, 2000) in greenhouse conditions. In contrast to successful control of *M. incognita* in various crops, it has been reported that *P. lilacinus* was not effective against *M. javanica* on tobacco in microplots (Hewlett et al., 1988). It should be noted that *P. lilacinus* was applied as a sole biocontrol agent against *Meloidogyne* spp. in both the successful and unsuccessful experiments discussed above.

Paecilomyces lilacinus is generally specialized in parasitizing stationary stages of nematode, particularly nematode eggs. However, it has also been reported that *P. lilacinus* was able to control the mobile nematode *R. similis* on banana (Davide and Zorilla, 1985) and on betel vine when introduced into the soil prior to nematode inoculation (Sosamma et al., 1994). Infection of the cyst nematode *Heterodera* spp. particularly *H. schachtii* (Nigh et al., 1980) and *H. glycines* (Chen and Dickson, 1996) by *P. lilacinus* in laboratory conditions

have been reported. However, there is no published information on the infection of *H. avenae* in the laboratory experiments or control of *H. avenae* by *P. lilacinus* in field conditions.

Migratory stage juveniles are the infective stage of most plant-parasitic nematode genera and the size of their initial population in the soil essentially determines the degree of damage to the plants. If the level of migratory stages of nematodes is reduced during or prior to crop growing, it is likely that the initial number of nematodes establishing in crops would be minimized. *Monacrosporium* spp. captures migratory stages of nematodes during their random migration in the soil using adhesive knobs (Rubner, 1996), thereby reducing nematode populations (Santos et al., 1992; Jaffee and Muldoon, 1995a; Dalla-Pria and Ferraz, 1996). The nematode trapping fungus *Monacrosporium lysipagum* (Drechsler) Subram, isolated from the egg masses of *Meloidogyne javanica* (Treub) Chitwood on tomato and used in this work, has been found to be efficient in catching and rapid killing of mobile nematodes in the laboratory (Khan et al., 2006).

Success in the control of root-knot nematodes by *Monacrosporium* spp. in soil has been variable when the fungus has been applied as a sole bicontrol agent. For example, *M. ellipsosporium* efficiently controlled *M. incognita* (Mankau and Wu, 1985; Dalla Pria and Ferraz, 1996). Control of *M. javanica* by *M. cionopagum* in a soil microcosm experiment was nearly 100% (Jaffee and Muldoon, 1995b). Contrary to this, it has been reported that three species of *Monacrosporium* (*M. sinense*, *M. thaumasium* and *M. ellipsosporum*) did not significantly control *M. javanica* population in a greenhouse test (Ribeiro and Ferraz, 2000). Reports on the control of *H. avenae* or *R. similis* by *Monacrosporium* spp. either in pot or in field conditions are currently not available.

In contrast to the inconsistent effects of a sole biocontrol agent (either *P. lilacinus* or *M. lysipagum*), discussed above, more consistent control of nematodes by simultaneous application of more than one biocontrol agents into the soil has been reported. *P. lilacinus* together with *Verticillium lecanii* (Zimmermann) Viegas controlled *M. incognita* on crossandra (*Crossandra undulaefolia* L.) significantly better than either fungus individually (Nagesh and Reddy, 1997). Furthermore, *P. lilacinus* applied together with the obligate bacterial antagonist *Pasteuria penetrans* controlled *M. incognita* on Okra (Zaki and Maqbool, 1991) and winter vetch (Dube and Smart, 1987) better than either antagonist alone. To our knowledge, there has been no report on the combined application of *P. lilacinus* and *M. lysipagum* for the control of nematode populations.

In this work, we describe the effects of the individual and combined application of *P. lilacinus* and *M. lysipagum* on the control of *M. javanica*, *H. avenae* and *R. similis* on tomato, barley and banana plantlets in controlled pot experiments.

Materials and methods

Cultures of fungi

Paecilomyces lilacinus strain 251 culture, deposited at National Measurement Institute (NMI) formerly known as Australian Government Analytical Laboratory (AGAL), accession number 89/030550 was maintained on potato dextrose agar plates (PDA, Difco, MI, USA). The fungus was grown at 26 ± 1 °C for 7–10 d and conidia were harvested in 8–10 ml of sterile salt solution as described by Holland et al. (1999). *Monacrosporium lysipagum* (IMI 375301), isolated from the eggs mass of *M. javanica* on tomato in a glasshouse at Macquarie University, Sydney, Australia, was grown on potato carrot agar (PCA) plates. PCA was prepared by boiling chopped potato and carrot slices (20 g/l each) and adding 15 g agar (Calbiochem, CA, USA) to the extract after making the volume up to 1 l with double distilled water. The medium was autoclaved and poured on 100 mm $\times$ 15 mm Petri dishes. *M. lysipagum* was grown on PCA plates at 21 ± 1 °C in a prolonged daylight (16 h/d) for 15 d to enhance sporulation before harvesting the spores in a sterile salt solution.

Cultures of nematodes

A culture of *Meloidogyne javanica* was routinely maintained on tomato plants in a glasshouse (Holland and Williams, 1996). Approximately 100 *M. javanica* egg masses were collected with fine forceps and placed in a vial with 10–15 ml tap water to induce hatching of juveniles at 26 ± 1 °C. One to 8 d old juveniles were used as inoculum in all experiments. Dry soil containing *Heterodera avenae* cysts was collected from a cyst-infested wheat field in Murray Mallee, South Australia. Cysts were extracted from soil using wet sieve flotation technique.

These cysts were used as an inoculum of *H. avenae* for experimental purposes or inoculated in barley pots to maintain a culture in the glasshouse. A pure culture of *Radopholus similis* was kindly supplied by Dr. Julie Stanton (Department of Primary Industries, Meiers Road, Indooroopilly, Queensland). An axenic culture of *R. similis* was

maintained on carrot discs (Fallas and Sarah, 1994) at 26 ± 1 °C. Nematodes were harvested from a 6- to 8-week carrot culture with sterile water for experimental use or to set up a fresh culture.

Micropropagation (tissue culture) of banana

Banana flowers (an unknown commercial variety) were obtained from a grower from Coffs Harbour, NSW. Tissue culture was established in three stages according to Israeli et al. (1995). The plantlets grown in the MS medium were transplanted into pots containing about 1500 g of nematode free non-sterile loamy soil. The absence of nematodes was verified by extracting nematodes from the soil and conducting bioassays. Young plants were adapted to the external environment by keeping them covered with polyethylene bags under a shade. Plants were exposed to sunshine for 2 h every second day for two weeks. Plants established in the pot soil two months after transplanting were used for experimental purposes.

Control of Meloidogyne javanica *by* Paecilomyces lilacinus *and* Monacrosprium lysipagum *on tomato*

Three weeks old tomato seedlings were transplanted in plastic pots containing 1000 g nematode free non-sterile loamy soil. Ten days later, 1200 *M. javanica* juveniles (1–8 d old) were inoculated per pot by pipetting 4 ml of juvenile suspension into 4 holes made around the plant base especially for fungal inoculation. *P. lilacinus* and *M. lysipagum* were applied two weeks after nematode inoculation by pipetting 10 ml of a conidial suspension of each fungus into another 4 holes, separate to the holes made for nematode inoculation. Final concentration of conidia per pot was 2.7×10^8 for *P. lilacinus* and 1.4×10^6 for *M. lysipagum*. Plants in the control treatment received an equal amount of water. Viability of *P. lilacinus* conidia obtained from PDA plates was 80% and viability of the *M. lysipagum* conidia obtained from PCA plates was 75%. Viability of spores was determined by counting the number of germinated conidia on respective agar plates after incubation for one day (*P. lilacinus*) and 3 days (*M. lysipagum*) for all experiments in this work. Treatments were assigned as follows: T1 = *M. javanica* only (control), T2 = *M. javanica* and *P. lilacinus*, T3 = *M. javanica* and *M. lysipagum* and T4 = *M. javanica*, *P. lilacinus* and *M. lysipagum*. Each treatment had six replications. The plants were harvested 10 weeks after nematode inoculation.

Control of Heterodera avenae *by* Paecilomyces lilacinus *and* Monacrosporium lysipagum *on barley*

The pots were filled with 700 g of cyst free non-sterile loamy soil and barley seeds (var. Schooner) were sown. Pots were watered once to allow seed germination. Each pot was inoculated with six cysts (215 J2 containing eggs per cyst, averaged from 20 cysts) of *H. avenae* two weeks after seed sowing. *P. lilacinus* and *M. lysipagum* conidia were obtained from PDA and PCA plates, respectively, and inoculated into each pot at the concentration of 3.6×10^{8} (*P. lilacinus*, 80% viable) and 1.4×10^{6} (*M. lysipagum*, 60% viable) two weeks after cyst inoculation. The treatments were assigned as follows: T1 = nematode and fungus free, T2 = *H. avenae* only (control), T3 = *H. avenae* and *P. lilacinus*, T4 = *H. avenae* and *M. lysipagum* and T5 = *H. avenae*, *P. lilacinus* and *M. lysipagum*. Each treatment had six replications. The plants were harvested for data collection about six months after seed sowing when barley seeds had ripened.

Control of Radopholus similis *by* Paecilomyces lilacinus *and* Monacrosporium lysipagum *on banana plantlets*

Two-month-old banana plantlets were inoculated with a mixed population of various life stages of *R. similis* at 3500 nematodes per pot. The soil was inoculated with 5.6×10^{8} *P. lilacinus* conidia, harvested from PDA plates (78% viable) and 6×10^{5} *M. lysipagum* conidia per plant, harvested from PCA plates (70% viable) two weeks after nematode inoculation. The treatments were assigned as follows: T1 = nematode and fungus free, T2 = *R. similis* only (control), T3 = *R. similis* and *P. lilacinus*, T4 = *R. similis* and *M. lysipagum* and T5 = *R. similis*, *P. lilacinus* and *M. lysipagum*, with eight replications of each treatment. Four plants from each treatment were harvested 10 weeks after nematode inoculation (first harvest). The remaining plants were inoculated with an additional dose of *P. lilacinus* and *M. lysipagum* conidia at the concentration of 2.5×10^{8} (viability 82%) and 4×10^{5} (viability 75%) per pot respectively. These plants were harvested 18 weeks after the nematode inoculation (second harvest).

Extraction of nematodes from the pot soil and root samples

Juveniles of *M. javanica* and migratory stages (juveniles and adults) of *R. similis* were extracted from 100 g of pot soil from all the experiments on tomato and banana using a modified Baermann funnel

method (Hooper, 1990). The cysts of *H. avenae* were extracted from the pot soil using the wet sieve floatation technique. Juveniles and adults of *R. similis* were extracted at 28 ± 1 °C from banana roots using the method described by Stemerding (1964). The juveniles and adults were collected every second day over 6 d and counted using a dissecting microscope (4× magnification).

Microscopic examination of infected Meloidogyne javanica *eggs and* Heterodera avenae *cysts*

Six egg masses of *M. javanica* (tomato) and 20 cysts of *H. avenae* (barley) from each treatment were placed on a *P. lilacinus* semi-selective medium (Cabanillas and Barker, 1989) at plant harvest. We have observed that also *M. lysipagum* grows on this medium (unpublished). However, the growth of *M. lysipagum* was weaker on the semi-selective medium compared to the PCA. The plates were incubated at 26 ± 1 °C for 7 d for egg masses and 10 d for cysts. Colonized egg masses were examined with a stereomicroscope (16× magnification). The eggs, either infected by direct hyphal penetration or of which the contents were disintegrated were counted as infected. *M. javanica* eggs from the nematode-inoculated control treatment showed no hyphal penetration but disintegration only. Colonized cysts were inspected using a dissecting microscope (4× magnification). These experiments were carried out to find out whether fungal conidia were associated with the egg masses and cysts at crop harvest.

Statistical analysis

Effects of *P. lilacinus* and *M. lysipagum* on the control of *M. javanica* on tomato, *H. avenae* on barley and *R. similis* on banana were carried out as a completely randomized design (CRD) and analysis of variance (ANOVA) was carried out on original data. When the overall *F* test was significant, the treatment mean values were compared with Duncan's New Multiple Range Test (DNMRT) at the 5% level of significance.

Results

Control of Meloidogyne javanica *by* Paecilomyces lilacinus *and* Monacrosporium lysipagum *on tomato*

Ten weeks after nematode inoculation, the number of galls on tomato roots and *M. javanica* juveniles in the pot soil were significantly

Table 1. Control of *Meloidogyne javanica* by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* on tomato in a pot trial

Treatment	Juveniles/100 g soil (±SE)	Galls/root systems (±SE)	% Infected eggs (±SE)
M. javanica (Mj)	216 (±18.91)a	108 (±18.37)a	2 (±0.93)d
Mj + *P. lilacinus* (Pl)	51 (±12.34)b	51 (±2.62)b	17 (±1.10)b
Mj + *M. lysipagum* (Ml)	15 (±0.48)c	46 (±2.34)b	12 (±1.15)c
Ha + Pl + Ml	13 (±1.49)c	41 (±2.24)b	33 (±2.21)a

Significant at 1% level; treatment means were calculated from six replications and separated by DNMRT ($p = 0.05$); same letters in a column are not significantly different. SE, standard error.

($p = 0.01$) decreased by either fungus alone or by their combined application (Table 1). The combined application of *P. lilacinus* and *M. lysipagum* reduced 62% of galls and 94% of juveniles, compared to the nematode-inoculated control. *P. lilacinus* and *M. lysipagum* alone reduced 53% and 57% of galls, and 76% and 93% of juveniles in the soil, respectively, compared to the nematode-inoculated control (Table 1). The fewest number of juveniles in the soil was observed in the combined treatment containing both fungi ($p = 0.01$). There was no difference between the effect of *M. lysipagum*, applied alone or in combination with *P. lilacinus*, on *M. javanica*.

The reduction of galls by either fungus alone was not significantly different to that obtained by their combination. Infected eggs (infected by hyphal penetration in the case of *P. lilacinus* or with disintegrated egg contents) were found at higher numbers ($p = 0.01$) among plants treated with the combined application of *P. lilacinus* and *M. lysipagum* (Table 1).

Control of Heterodera avenae *by* Paecilomyces lilacinus *and* Monacrosporium lysipagum *on barley*

The greatest reduction of *H. avenae* on barley ($p = 0.01$) was achieved when the *M. lysipagum* conidia were applied in the pot soil alone (Table 2). *P. lilacinus* and *M. lysipagum* caused significant reductions in number of cysts per root of 46% and 71%, respectively. Only the *P. lilacinus* treatment resulted in a significantly higher seed yield compared to the nematode-inoculated control. All treatments showed significantly lower seed weights ($p = 0.01$) compared to the untreated, not inoculated control. Association of *P. lilacinus* and *M. lysipagum* with

Table 2. Control of *Heterodera avenae* by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* on barley and the yield of barley seeds in a pot trail

Treatment	Seed wt (g) (±SE)	Cysts/root systems (±SE)	% Colonized cysts
Nematode and fungus free	12.50 (±0.52)a	0	0
H. avenae (Ha)	6.48 (±0.76)c	217 (±23.71)a	0
Ha + *P. lilacinus* (Pl)	8.80 (±0.66)b	118 (±8.24)b	75
Ha + *M. lysipagum* (Ml)	8.55 (±0.64)bc	64 (±11.81)c	0
Ha + Pl + Ml	8.25 (±0.76)bc	76 (±7.44)bc	80

Significant at 1% level; treatment means are from six replications separated by DNMRT ($p = 0.05$); same letters in a column are not significantly different. SE, standard error.

H. avenae cysts was examined at plant harvesting. *P. lilacinus* grew from about 80% of cysts on a semi-selective medium (Table 2). In contrast, *M. lysipagum* was not detected from any cyst.

Control of Radopholus similis *by* Paecilomyces lilacinus *and* Monacrosporium lysipagum *on banana plantlets*

Overall, the number of nematodes in the soil was significantly lower than in the nematode-inoculated control ($p = 0.01$) at both harvests in the fungus-treated banana plantlets. Reduction of nematodes in the root was not significant at the first harvest, however, nematodes were reduced significantly at the second harvest ($p = 0.01$). Individual effects of *P. lilacinus* and *M. lysipagum* on the reduction of *R. similis* population were significant compared to the untreated control (Figure 1). At the first harvest, reduction of *R. similis* by *P. lilacinus* was 57% in the soil ($p = 0.01$) and 21% in the roots ($p = 0.05$) (calculated from the numbers in Figure 1). At the second harvest, reduction of *R. similis* by *P. lilacinus* was 86% in the soil ($p = 0.01$) and 76% in the roots ($p = 0.01$). The *R. similis* population was also effectively reduced by *M. lysipagum* at both harvesting times. At the first harvest, reduction of the *R. similis* by *M. lysipagum* alone was 83% in the soil ($p = 0.01$) and 41% in the roots ($p = 0.05$). At the second (final) harvesting time, reduction of the *R. similis* by *M. lysipagum* alone was 98% in the soil ($p = 0.01$) and 77% in the roots ($p = 0.01$) (calculated from the numbers in Figure 1).

Radopholus similis populations were reduced by 92% in the soil and 54% in the roots at the first harvest through a combined effect of *P. lilacinus* and *M. lysipagum* (calculated from the numbers in Figure 1). Corresponding numbers for the second harvest were 99%

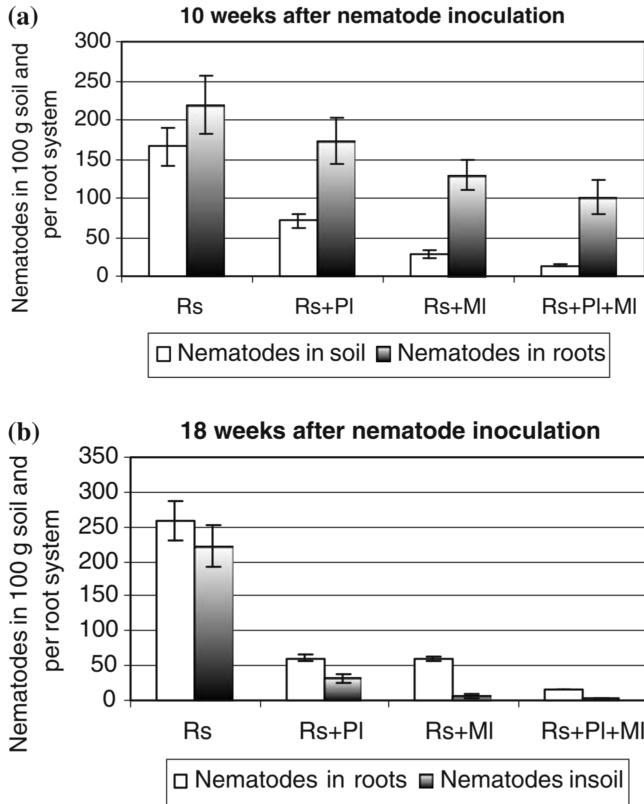

Figure 1. Control of *Radopholus similis* in the roots and in the pot soil by *Paecilomyces lilacinus* and *Monacrosporium lysipagum* on banana plantlets (a) 10 weeks and (b) 18 weeks after nematode inoculation. Results are the means of four replications. T1 = *R. similis* only (control), T2 = *R. similis* and *P. lilacinus*, T3 = *R. similis* and *M. lysipagum* and T4 = *R. similis*, *P. lilacinus* and *M. lysipagum*. Vertical bars represent standard error of the mean values.

in the soil and 94% in the roots (Figure 1). Nematode reduction in the roots was not significantly different between the different fungal treatments at the first harvest. However, reduction in the roots at the second harvest by either fungus alone or by their combination was significant. Overall reduction of *R. similis* was greatest when *M. lysipagum* was inoculated either alone (59%) or in combination with *P. lilacinus* (70%) ($p = 0.01$) at the first harvest (10 weeks after nematode inoculation), and 86% for *M. lysipagum* alone and 96% for the combined inoculation of *M. lysipagum* and *P. lilacinus* ($p = 0.01$) at the second harvest (18 weeks after nematode inoculation) (calculated from the numbers in Figure 1).

Fresh root weights were severely affected by *R. similis*, but a healthy weight was maintained in treatments involving individual application of *P. lilacinus* or combined application of *P. lilacinus* and *M. lysipagum* to banana pots (Figure 2 and Table 3). Root weights were significantly better in *R. similis*-containing pots treated with *M. lysipagum* than in plants inoculated with *R. similis* only, however, less than in plants with no fungus or nematodes. Fresh shoot weight was also severely affected by *R. similis*. Shoot weights after individual application of *P. lilacinus* or combined application of *P. lilacinus* and *M. lysipagum* were significantly better in plants inoculated with *R. similis* alone or *R. similis* and *M. lysipagum*, however, less than with no fungus or nematodes. The number and length of roots were increased in plants grown in pots with fungi and decreased in the presence of *R. similis* only (Table 3).

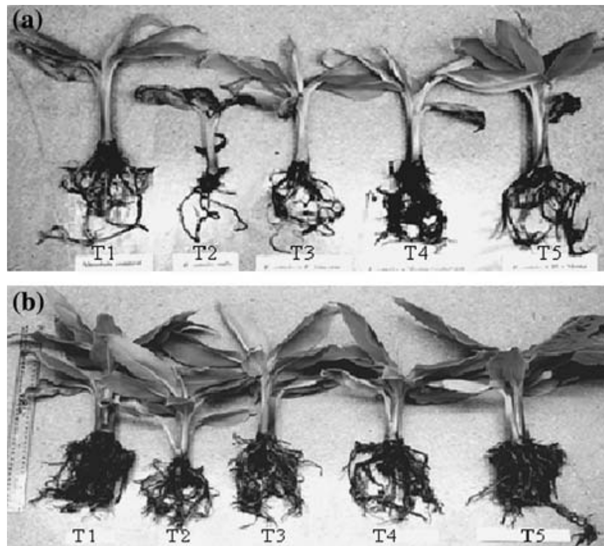

Figure 2. Effects of *Paecilomyces lilacinus* and *Monacrosporium lysipagum* on the vegetative growth of *Radopholus similis* infected banana plantlets 10 weeks after nematode inoculation (a) and 18 weeks after nematode inoculation (b). Banana plantlet in T2 (a) has grown very little within two months after the nematode inoculation took place. Plantlets in the pots with a combined application of *P. lilacinus* and *M. lysipagum* were healthier as in pots housing nematode and fungi free banana. The plants were arranged as follows (left to right): T1 = nematode and fungus free (control), T2 = *R. similis* only, T3 = *R. similis* and *P. lilacinus*, T4 = *R. similis* and *M. lysipagum* and T5 = *R. similis*, *P. lilacinus* and *M. lysipagum*.

Table 3. Effects of *Paecilomyces lilacinus* and *Monacrosporium lysipagum* on the vegetative growth of *R. similis*-inoculated banana plantlets 18 weeks after nematode inoculation

Treatment	Fresh root wt (g) (±SE)	Fresh shoot wt (g) (±SE)	Roots/plant (±SE)	Root length (cm) (±SE)
Nematode and fungus free	37.05 (±0.77)a	93.13 (±6.31)a	17 (±0.85)a	16.50 (±0.65)b
R. similis (Rs)	12.80 (±3.43)c	30.83 (±6.96)c	10 (±2.33)b	12.75 (±1.03)c
Rs + *P. lilacinus* (Pl)	33.98 (±3.67)a	63.30 (±7.75)b	18 (±1.32)a	19.50 (±0.65)a
Rs + *M. lysipagum* (Ml)	22.45 (±2.73)b	41.23 (±4.61)c	17 (±0.65)a	17.25 (±0.63)ab
Rs + Pl + Ml	39.43 (±3.73)a	70.75 (±6.71)b	21 (±0.75)a	19.75 (±1.03)a

Significant at 1% level; results are the treatment means of four replications and were separated by DNMRT ($p = 0.05$); the same letters in column are not significantly different. SE, standard error.

Discussion

In this work, the *P. lilacinus* and *M. lysipagum* fungi were inoculated two weeks after nematode inoculation in all pot trials. This was done to mimic the situation in the natural environment where a crop may be grown/seed sown in a nematode infested soil. If the nematodes and fungi had been inoculated at the same time, it is likely that the reduction of nematode population would have been higher as the fungi would have had the opportunity to kill nematodes prior to their penetration to the roots.

Paecilomyces lilacinus, which specializes in infecting stationary stages, and *M. lysipagum* that traps mobile stages of plant-parasitic nematodes were applied to potted plants in order to control nematode populations. Combined application of *P. lilacinus* and *M. lysipagum* controlled most effectively the number of nematodes in soil, particularly *R. similis* in banana. However, in some other cases the result of a combined application of the two fungi was not significantly different to the effect of *M. lysipagum* alone. Variations are to be expected as *P. lilacinus* and *M. lysipagum* target different life stages of the nematodes. Our experiments confirmed that *P. lilacinus*, a known nematode egg parasite was also capable of efficient control of *R. similis*, a migratory nematode.

The numbers of galls and *M. javanica* juveniles on tomato in the pot soil were reduced effectively by *P. lilacinus* and *M. lysipagum*. In this experiment, the fungi were applied in the same pots two weeks after nematode inoculation. It is possible that some juveniles in the soil had not yet penetrated the roots at the time of fungal inoculation (Khan et al., 1995) and hence were likely to be killed by *M. lysipagum*. Consequently, reduced number of *M. javanica* juveniles infected the tomato roots in the beginning, which in term reduced the number of galls and juveniles (following generation) in the soil. At the completion of experiment after 10 weeks, the final nematode population in soil was reduced by fungi. This is expected since after a 10 week growth period, *M. javanica* must have completed at least one generation and produced egg masses (Eisenback and Triantaphyllou, 1991) that provided new targets for fungal infection.

Reduction of *H. avenae* populations on barley by *P. lilacinus* and *M. lysipagum* was also observed. *H. avenae* juveniles penetrated the roots and juveniles gradually become premature females within 2–3 weeks. At this stage, the immature females burst through the root epidermis and the posterior end of the nematode body extrudes from the roots but the head remains attached (Wollenweber, 1924). These immature females are easy targets for *P. lilacinus* resulting in higher numbers being killed. The highest reduction of *H. avenae* was obtained with *M. lysipagum* only, which was able to kill about 50% of the juveniles in a laboratory study (Khan et al. elsewhere in this issue).

The *R. similis* population was drastically reduced by *P. lilacinus* and *M. lysipagum* on banana plantlets. This is to be expected since *R. similis* is a mobile nematode and the fungus *M. lysipagum* is specialized in capturing mobile nematodes (Rubner, 1996; Khan et al., 2006). The juveniles and adults of *R. similis* wandering out into the soil to find a fresh root are caught by *M. lysipagum*. This facilitated a continuous attack of nematodes by *M. lysipagum* over the entire experimental period, which would reduce the nematode population more effectively than when using *P. lilacinus* that will infect stationary stage eggs only. Interestingly, *P. lilacinus* also decreased the *R. similis* population in the soil. In this case, *R. similis* may have laid eggs in the root and/or in the soil, which are likely to be infected by *P. lilacinus*. In this work, no adult *R. similis* was found infected by direct penetration of fungal hyphae even though we have found that *P. lilacinus* can penetrate the nematode body in laboratory tests (Khan et al., 2006).

We have shown in this study that *P. lilacinus* and *M. lysipagum* controlled *M. javanica*, *H. avenae* and *R. similis* efficiently in pot trials, therefore showing promise for the continuing development of *M. lysipagum* as a biocontrol agent. Further studies into the biocontrol capability of *M. lysipagum* will involve the testing of potential pathogenicity and infectivity of the fungus to non-target organisms to ensure that there will be no adverse effects on other organisms in the environment. Towards this end, we have already shown that *M. lysipagum* does not attack the free-living nematode *Caenorhabditis elegans* in laboratory conditions (unpublished). Mass production protocol will also be required.

At present, *P. lilacinus* 251 is registered by prophyta (http://www.prophyta.de) as a biocontrol agent of nematodes in USA, Europe and South Africa. *P. lilacinus* strain 251 has already been tested against a wide range of non-target invertebrates and it was found that the strain 251 did not interfere with soil insects, earthworms and free-living nematodes (Holland, 2000).

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