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Colonisation of seminal roots of wheat and barley by egg-parasitic nematophagous fungi and their effects on *Gaeumannomyces graminis* var. *tritici* and development of root-rot

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Abstract

This study provides evidence that egg-parasitic nematophagous fungi, *Pochonia chlamydosporia*, *Pochonia rubescens* and *Lecanicillium lecanii*, can also reduce root colonisation and root damage by a fungal pathogen. Interactions of nematophagous fungi with the take-all fungus, *Gaeumannomyces graminis* var. *tritici* (Ggt), and their influence on severity of the root disease it causes were studied in laboratory and pot experiments. In Petri dish experiments the three nematophagous fungi reduced colonisation of barley roots by Ggt and also reduced necrotic symptoms. On the contrary, root colonisation by nematophagous fungi was unaffected by Ggt. In growth tube experiments, the three nematophagous fungi again reduced Ggt root colonisation and increased effective root length of barley seedlings. This was true for both simultaneous and sequential inoculation of nematophagous fungi versus Ggt. In the pot experiments the inoculum of the tested fungi in soil was applied in the same pot, as a mixture or in layers, or in coupled pots used for wheat grown with a split-root system. The nematophagous fungi *P. chlamydosporia* (isolate 4624) and *L. lecanii* (isolate 4629), mixed with Ggt or in split root systems with the pathogen, promoted growth of wheat (i.e. increased shoot weight), although no disease reduction was found. In split root systems, lower levels of peroxidase activity were found in seedlings inoculated with Ggt in combination with the nematophagous isolates 4624 and 4629 than when the take-all fungus was applied alone.

Our results show that nematophagous fungi reduce root colonisation by Ggt, root damage and stress induced senescence in Ggt-inoculated plants.

Keywords: Biocontrol; Take-all; Nematophagous fungi; *Gaeumannomyces graminis* var. *tritici*; *P. chlamydosporia*; *L. lecanii*

1. Introduction

Pochonia chlamydosporia (=*Verticillium chlamydosporium*) is widespread in cyst and root-knot nematode infested soils around the world (Kerry, 1993). However, *P.chlamydosporia* has also been described as a mycoparasite of plant parasitic fungi, e.g. rust fungi (Leinhos and Buchenauer, 1992). Recent studies have demonstrated its antagonism towards certain phytopathogenic fungi (i.e. *Fusarium oxysporum and Rhizoctonia solani*) (Ehteshamul et al., 1994; Jacobs et al., 2003; Monfort et al., 2003 unpublished data) and its capability to colonise barley and tomato roots (Lopez-Llorca et al., 2002; Bordallo et al., 2002). These properties make *P. chlamydosporia* a potential biocontrol agent for both disease-causing fungi and plant parasitic nematodes. Dual purpose biocontrol organisms have a great potential in agriculture, and need to be better documented and understood.

Take-all of wheat (*Triticum aestivum* L.) caused by *Gaeumannomyces graminis* var. *tritici* (take-all fungus, Ggt), is a major root disease of wheat, worldwide. Cultural strategies, such as crop rotation and monoculture, are available for managing take-all; however, these measures are not suitable strategies for all wheat growing regions.

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Although many saprophytic and non-pathogenic root-infecting fungi have been recorded to suppress the take-all fungus (Wong and Southwell, 1980; Dewan and Sivasithamparam, 1988, 1989; Wong, 1994; Duffy and Weller, 1992; Duffy et al., 1996), little is known about the potential of root invading nematophagous fungi to suppress the root-rot caused by the take-all fungus.

The aim of this study was to determine whether egg-parasitic nematophagous fungi could reduce colonisation of roots and severity of the root-rot caused by the take-all fungus, and whether such reduction of disease is associated with induced resistance of the host plant.

2. Materials and methods

2.1. Fungal cultures

To investigate the multitrophic interaction between nematophagous fungi, the plant pathogen *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Walker (Ggt) and its hosts (wheat and barley), laboratory experiments on plates, culture tubes and in pots were carried out. The following nematophagous fungi were used: *Pochonia chlamydosporia* (Goddard) Zare and W. Gams, *P. rubescens* Zare et al., and *Lecanicillium lecanii* (Zimmerm.) Zare and W.Gams. The latter species was included only in the pot experiments. Details of isolates used are given in the appropriate sections below. Fungal cultures were maintained at 4 °C in the dark on corn meal agar (CMA, Difco). For Petri dish and growth tube experiments, the fungi were multiplied on CMA at 25 °C.

For pot experiments the following procedures were adopted. Inocula of the two nematophagous fungi *P. chlamydosporia* (isolate 4624) and *L. lecani* (isolates 4625 and 4629) and that of Ggt were prepared as described in Seah et al. (1996). The inoculum was prepared by incubating ten 5 mm (diam.) plugs of the fungi with sterilised ryegrass (*Lolium rigidum* L.) seeds for 8–11 days at 25 °C. Before incubation, 50 g of ryegrass seeds were soaked overnight in deionised water in 500 ml conical flasks. After removing excess water, seeds were autoclaved on three consecutive days at 121 °C for 30 min. After the autoclaved ryegrass had been uniformly colonised by each of the three fungi, the inoculum was air-dried overnight in a laminar-flow cabinet and stored at room temperature until required. Inoculum, autoclaved three times at 121 °C for 30 min, served as the control.

2.2. Petri dish experiment (Experiment 1):

Although wheat is the main host for Ggt, barley also acts as a host (Gutteridge et al., 1993 and the barley variety (*Hordeum vulgare* L. var. *disticum*) used in this study was found to be susceptible to Ggt. Barley seeds were surface sterilized using 5% sodium hypochlorite for 1 h and then washed 3 times (5 min each) in sterile distilled water. The seeds were pre-germinated on germination medium (agar 12 g/l, glucose 10 g/l, peptone 0.1 g/l and yeast extract 0.1 g/l) for 2 days at 25 °C.

To investigate colonization by Ggt, four germinated barley seeds were placed on Petri dishes with either corn meal agar (CMA), potato dextrose agar (PDA) or malt extract agar (MEA) colonised by *G. graminis* var. *tritici*. After 1, 2, 5 and 7 days incubation in the dark at 25 °C, the roots were harvested and treated with 1% NaClO for 1 min, washed and thereafter cut into 1-cm pieces. The root pieces were placed on CMA plates and examined for Ggt colonization.

To study root colonization of Ggt after pre-colonisation by nematophagous fungi, barley seedlings were placed onto CMA plates precolonized by *P. chlamydosporia* (isolate 123), *P. chlamydosporia* (isolate 144) or *P. rubescens* (CBS 464.88). After 2 days' incubation at 25 °C in the dark, the seedlings were cleaned of adhering agar and transferred to plates colonized by Ggt. These samples were incubated for 3 and 5 days at 25 °C in the dark, and the roots were then examined as above. After incubation, the percentage of fragments colonized by Ggt, the percentage of fragments with necrotic symptoms and percentage of fragments colonized by the nematophagous fungi were recorded. The experiment consisted of 9 replicates per treatment.

2.3. Growth tube experiment (Experiment 2):

Two treatments were tested: (a) nematophagous fungus and Ggt inoculated simultaneously, and (b) nematophagous fungus preinoculated ahead of Ggt. In the first experiment, barley seedlings were grown in autoclaved growth tubes (25×150 mm, Sigma C-5916) filled with 30 ml vermiculite and 20 ml distilled water. Each tube with barley seedlings was inoculated with 4 plugs (5 mm diam) taken from the margins of actively growing colonies of *P. chlamydosporia* or *P. rubescens* (placed 2 cm below the surface of vermiculite), and 2 pieces of Ggt (4.5 cm deep). Each experiment consisted of 9 replicates per treatment. In the second experiment, seedlings (pre-inoculated on colonies of the nematophagous fungi for 2 days) were then placed in tubes with 4 (5 mm diam) plugs of Ggt colonies 2 cm deep. Each experiment included 6 replicates per treatment. In both experiments, the tubes were placed in a growth chamber with a photoperiod of 16/8 h (light/dark) at 25 °C for 2 weeks. The percentage of fragments colonised by Ggt and the percentage of fragments colonised by *P. chlamydosporia* or *P. rubescens* were scored. The percentage effective root length (ERL, estimated distance from stem base to the proximal necrotic lesion) and percentage necrotic fragments were also recorded.

ERL was calculated according to Seah et al. (1996); Aberra et al. (1998) where it provides a measure of the uninfected root above the take-all lesion most proximal to the crown.

2.4. Statistical analysis

Non-parametric Kruskal–Wallis analysis was used to determine the possible differences between the treatments. The level of significance was 95%. For comparison, two by two *U* Mann–Whitney test was performed.

2.5. Pot experiments

2.5.1. Inoculum introduced as a mixture in soil (Experiment 3)

For experiments dealing with the interaction of the nematophagous fungi and the take-all fungus in pots, the inoculum of the test fungi was introduced in the soil as a mixture.

A pot experiment was set up using 75×135 mm plastic pots containing 600 g of Lancelin soil (Brennan et al., 1980). The ryegrass (*Lolium rigidum* L.) seed-based inoculum of nematophagous fungi was applied at a rate of 0.5 g of precolonised seeds per 100 g soil and the ryegrass seed inoculum of Ggt at a rate of 0.1 g of precolonised seeds per 100 g soil (a ratio 5:1, nematophagous fungus: Ggt). The treatments were as follows: (1) Control, no Ggt and no nematophagous fungal inoculum; (2) Ggt inoculum only; (3) *P. chlamydosporia* 4624 only; (4) *L. lecanii* 4625 only; (5) *L. lecanii* 4629 only; (6) *P. chlamydosporia* 4624+Ggt; (7) *L. lecanii* 4625+Ggt and (8) *L. lecanii* 4629+Ggt. Wheat (*T. aestivum* cv. Machete) seeds (10 per pot) were then sown approx. 1 cm deep. Pots were incubated at 20–15 °C (14/10 h day/night period) in a phytotron and watered with tap water every other day to field capacity. Wheat plants were harvested after 21 d. Fresh shoot weight (g) and effective root length (cm) (Aberra et al., 1998) were measured. The experiment included 8 replicate units of each of the 8 treatments. Means of parameters scored were compared by analysis of variance (ANOVA) F-test (F-protected) using the GENSTAT package.

2.5.2. Inocula introduced as layers (Experiment 4)

The second experiment was also set up using the same sized pots, soil and ratio of nematophagous fungi: Ggt inoculum (5:1) as in Experiment 3. In experiment 4, the pathogen and the antagonist were added to soil as separate layers as follows: 300 g of soil were added to each pot. Over it, 0.3 g rye grass seed based Ggt inoculum per pot was spread. A further 200 g of soil was added to each pot and 0.5 g of nematophagous fungus was spread over it. Pots were then topped-up to 600 g of soil. The treatments were as follows: (1) Control, no Ggt or nematophagous fungal inoculum, (2) Ggt inoculum only; (3) *P. chlamydosporia* 4624 only; (4) *L. lecanii* 4625 only; (5) *L. lecanii* 4629 only; (6) *P. chlamydosporia* 4624+Ggt; (7) *L. lecanii* 4625+Ggt and (8) L. *lecanii* 4629+Ggt. All treatments had 4 replicates. Wheat seeds were sown and pots maintained as described in Experiment 3. Plants were harvested 18 d after sowing and fresh shoot weight (g), fresh root weight (g), total root length (cm) and effective root length (cm) were measured.

2.5.3. Induced resistance assay, using split roots (Experiment 5)

Wheat seeds were pre-germinated in water overnight. They were then planted in a container with a mixture of soil-vermiculite (50:50) and incubated for 4 days at 20–15 °C. To prepare the seedlings for the split-root test, the middle seminal root was excised and seedlings transplanted into a Y-shaped plastic tube which was inserted into a pair of 70×160 mm plastic pots (A and B) taped together. Each half of the root system was inserted into one of the pots containing 640 g of Lancelin soil using the Y-shaped tube. Plants were grown in a glasshouse maintaining 15–20 °C.

The rye-grass inoculum of the nematophagous fungi (0.5%) was applied mixed in soil within one pot of the split root system. In the other pot two Ggt infested rye-grass seeds were point-inoculated at the opening of the Y-tube, where applicable. The treatments were as follows: (1) Control, sterilized rye-grass seeds only; (2) Ggt inoculum only (in pot A); (3) *P. chlamydosporia* isolate 4624 inoculum only (in pot B); (4) *L. lecanii* isolate 4629 inoculum only (in pot B); (5) Ggt (pot A)+isolate 4624 (pot B); (6) Ggt (pot A)+isolate 4629 (pot B). Ggt inoculum was added 7 d after the introduction of the nematophagous fungi. Plants were harvested 19 d after inoculation with the appropriate nematophagous fungi (and 12 days after Ggt infestation, where appropriate). Fresh shoot weight (g), fresh root weight (g), total root length (m), effective root length (%, as previously described) and leaf guaiacol peroxidase activity (∇Abs/min×g) were measured (Modified after Moerschbacher et al., 1988; Bergmeyer et al., 1983). Six replicates were used for each treatment.

3. Results

3.1. Petri dish experiment (Experiment 1):

All treatments pre-treated with either of the nematophagous fungi significantly reduced the percentage of fragments colonised by Ggt (Table 1).

The colonisation of the barley roots was approximately the same for the three isolates of nematophagous fungi which colonized the roots either with or, especially, without the presence of Ggt.

3.2. Growth tube experiment (Experiment 2):

3.2.1. Simultaneous inoculation

There was a clear reduction in the percentage of root fragments colonised by Ggt with the treatments with the *Pochonia* spp. at 2 weeks, but not at 1 week (Table 2). Similarly, there were increases in percentage effective root

Table 1
Reduction in Ggt colonisation and necrotic symptoms in barley roots in the presence of nematophagous fungi (Experiment 1, Petri dish test)

Incubation time (days)	Treatments	% Ggt ± SD	% Necrosis ± SD	% *Pochonia* spp. ± SD	% Ggt + *Pochonia* spp ± SD
3	Control (Ggt)	76.48 ± 1.71[a]	54.93 ± 13.67[a]	0	0
	P. chlamydosporia 123-Ggt	26.95 ± 9.78[b]	25.76 ± 14.47[b]	37.71 ± 22.09[a]	10.07 ± 13.23[a]
	P. chlamydosporia 144-Ggt	31.21 ± 2.24[b]	20.75 ± 18.24[b]	23.84 ± 16.58[a]	3.77 ± 3.97[a]
	P. rubescens-Ggt	22.24 ± 1.29[b]	19.40 ± 15.10[b]	34.84 ± 17.72[a]	5.07 ± 4.36[a]
5	Control (Ggt)	82.44 ± 0.97[a]	69.49 ± 15.93[a]	0	0
	P. chlamydosporia 123-Ggt	18.07 ± 2.21[b]	32.1 ± 13.72[b]	46.76 ± 26.68[a]	14.826 ± 9[a]
	P. chlamydosporia 144-Ggt	32.94 ± 8.02[b]	34.62 ± 25.20[b]	30.17 ± 17.04[a]	11.78 ± 13.18[a]
	P. rubescens-Ggt	20.75 ± 9.66[b]	28.31 ± 12.62[b]	37.32 ± 19.88[a]	19.38 ± 16.28[a]

% Ggt, percentage of fragments colonised by Ggt; % Necrosis, percentage of fragments with necrotic symptoms; % *Pochonia* spp., percentage of fragments colonised by nematophagous fungi; % Ggt + *Pochonia* spp., percentage of fragments colonised by nematophagous fungi with the presence of *Gaeumannomyces graminis* var *tritici* (Ggt). SD, Standard Deviation. Figures followed by different letters (in columns, within groups) are significantly different at the 95% level: $P < 0.05$. Means are based on 9 measurements.

length (ERL) in the *Pochonia* treated plants compared to Ggt controls at 2 weeks. However, there were no significant effects from *Pochonia* spp. in relation to the percentage fragments with necrotic symptoms nor the percentage of fragments colonised by the different *Pochonia* isolates.

3.2.2. Pre-inoculation with nematophagous fungi

In this experiment we observed a slight non-significant reduction in the percentage of fragments colonised by Ggt after pre-treatment with the *Pochonia* spp., as well as a non-significant reduction in the percentage of root fragments with necrotic symptoms at two weeks (Table 3). The effective root length increased significantly after *Pochonia* pre-treatment at 2 weeks. The percentage of root fragments colonised by the nematophagous fungi were not different between the *Pochonia* species, but was, as expected, much higher than in the previous experiment involving simultaneous inoculation (Tables 2 and 3).

Table 2
Reduction in Ggt colonisation and increase in effective root length when barley roots are inoculated simultaneosly with Ggt and nematophagous fungi (Experiment 2)

Incubation time (weeks)	Treatments	% Ggt ± SD	% Necrosis ± SD	% *Pochonia* spp. ± SD	% ERL ± SD
1	Control (Ggt alone)	11.75 ± 8.95[a]	13.80 ± 9.56[a]	0	62.77 ± 28.77[a]
	P. chlamydosporia 123 + Ggt	9.22 ± 4.45[a]	10.68 ± 3.99[a]	2.27 ± 3.19[a]	66.92 ± 21.28[a]
	P. chlamydosporia 144 + Ggt	13.43 ± 10.04[a]	14.83 ± 11.25[a]	0.96 ± 2.09[a]	60.52 ± 29.13[a]
	P. rubescens + Ggt	18.17 ± 6.87[a]	19.21 ± 9.02[a]	2.27 ± 3.59[a]	57.73 ± 21.06[a]
2	Control (Ggt alone)	75.1 ± 14.16[a]	59.55 ± 14.82[a]	0	12.11 ± 16.39[a]
	P. chlamydosporia 123 + Ggt	59.50 ± 14.09[b]	46.95 ± 14.66[a]	5.91 ± 6.87[a]	23.74 ± 18.20[b]
	P. chlamydosporia 144 + Ggt	58.24 ± 18.52[a]	47.48 ± 17.52[a]	2.33 ± 4.93[a]	28.74 ± 26.02[b]
	P. rubescens + Ggt	52.68 ± 10.20[b]	41.65 ± 14.94[a]	6.48 ± 5.21[a]	26.77 ± 20.38[b]

SD, Standard deviation; ERL, Effective root length (estimated distance from the stem base to the most proximal necrotic lesion). See legend for Table 1 for details of other assessments. Figures followed by different letters (in columns, within groups) are significantly different at the 95% level: $P < 0.05$. Means are based on 9 measurements.

Table 3
Increase in effective root length when barley roots are pre-inoculated with nematophagous fungi (Experiment 2)

Incubation time (weeks)	Treatments	% Ggt ± SD	% Necrosis ± SD	% *Pochonia* spp. ± SD	% ERL ± SD
1	Control (Ggt alone)	3.36 ± 4.74[a]	13.74 ± 0.63[a]	0	89.33 ± 6.85[a]
	P. chlamydosporia 123 + Ggt	1.63 ± 2.52[a]	20.26 ± 8.86[a]	29.29 ± 6.51[a]	92 ± 15.47[a]
	P. chlamydosporia 144 + Ggt	1.97 ± 3.11[a]	25.71 ± 6.36[a]	21.28 ± 5.36[a]	95.72 ± 1.93[a]
	P. rubescens + Ggt	1.80 ± 2.08[a]	25.44 ± 5.88[a]	25.86 ± 3.96[a]	93.77 ± 4.50[a]
2	Control (Ggt alone)	23.11 ± 6.94[a]	25.43 ± 9.22[a]	0	47.11 ± 29.52[a]
	P. chlamydosporia 123 + Ggt	15.51 ± 7.97[a]	17.45 ± 5.21[a]	35.66 ± 1.99[a]	73.61 ± 2.00[b]
	P. chlamydosporia 144 + Ggt	11.39 ± 1.18[a]	16.04 ± 5.37[a]	31.40 ± 8.34[a]	77.88 ± 6.77[b]
	P. rubescens + Ggt	17.87 ± 1.01[a]	16.64 ± 9.22[a]	27.90 ± 3.22[a]	77.66 ± 4.68[b]

SD, Standard Deviation; ERL, Effective root length. See legends for Tables 1 and 2 for details of assessments. Figures followed by different letters (in columns, within groups) are significantly different at the 95% level: $P < 0.05$. Means are based on 6 measurements.

Table 4
Increase in fresh shoot weight of wheat grown in the presence of nematophagous fungi alone or in combination with Ggt in the dispersed inoculum experiment (Experiment 3)

Treatments	Fresh shoot weight ± SD (g)	ERL ± SD (cm)
Control	1.194 ± 0.117[a]	ND
P. chlamydosporia 4624	1.609 ± 0.238[b]	ND
L. lecanii 4625	1.441 ± 0.352[b]	ND
L. lecanii 4629	1.383 ± 0.151[a]	ND
Ggt alone	1.166 ± 0.191[a]	0.723 ± 1.490[a]
P. chlamydosporia 4624 + Ggt	1.720 ± 0.126[b]	0.486 ± 1.049[a]
L. lecanii 4625 + Ggt	1.672 ± 0.254[b]	0.950 ± 1.971[a]
L. lecanii 4629 + Ggt	1.253 ± 0.218[a]	0.653 ± 1.167[a]

ND, not determined. See legend of Table 2 for details of assessment of Effective Root Length (ERL). Figures followed by different letters (in columns) are significantly different at the 95% level: $P < 0.05$. Means are based on 8 measurements.

3.3. Inoculum introduced as a mixture in soil (Experiment 3)

Fresh shoot weight was not significantly reduced by Ggt infection alone (Table 4). Plants exposed to *P. chlamydosporia* isolate 4624 or to *L. lecanii* isolate 4625 produced greater shoot weight than uninoculated control plants. Fresh shoot weight of seedlings inoculated with Ggt alone was significantly less than in those inoculated with *P. chlamydosporia* 4624 or *L. lecanii* 4625 in addition to Ggt.

The percentage effective root length of take-all fungus infected plants was not affected by the inclusion of nematophagous fungi.

3.4. Inoculum introduced as layers (Experiment 4)

Fresh shoot weight was not significantly reduced by Ggt colonisation (Table 5), despite the chlorosis of leaves of Ggt-inoculated plants. Exposure to the nematophagous fungi did not affect shoot weight of plants inoculated with *P. chlamydosporia* 4624 or *L. lecanii* (4625 or 4629) alone or in addition to Ggt.

Fresh root weight was not significantly reduced by Ggt colonisation, nor was it affected by inoculation with *P. chlamydosporia* 4624 or *L. lecanii* (4625 or 4629) in addition to Ggt.

Total root length was not significantly reduced by Ggt colonisation, nor was it affected by inoculation with *P. chlamydosporia* 4624 or *L. lecanii* (4625 or 4629) in addition to Ggt.

Effective root length was not affected by inoculation with Ggt alone nor by inoculation with *P. chlamydosporia* 4624 or *L. lecanii* 4629 in addition to Ggt. However, it was significantly affected in the presence of *L. lecanii* isolate 4625.

3.5. Induced resistance assay using split roots (Experiment 5)

Fresh shoot weight was not significantly reduced by Ggt colonisation alone (Table 6). However, plants exposed to either of the nematophagous fungi *P. chlamydosporia* or *L. lecanii*, in the presence or absence of Ggt, had significantly higher shoot weights than either the uninoculated control or the Ggt alone plants.

Neither the fresh root weight nor the total root length was significantly reduced by Ggt colonisation (Table 6). However, both *P. chlamydosporia* 4624 and *L. lecanii* 4629, in the presence or absence of Ggt, had significantly higher fresh root weights and total root lengths than either the inoculated control or the Ggt alone plants.

Effective root length of take-all fungus infected plants was not affected by any of the treatments, including those with nematophagous fungi.

Peroxidase activity was not modified by Ggt colonisation alone. Peroxidase activity, however was significantly lower in seedlings inoculated with Ggt combined with either of the nematophagous fungi than in seedlings inoculated with Ggt alone.

4. Discussion

In the Petri dish experiment (Experiment 1) we demonstrated that co-noculation of Ggt and the *Pochonia* species reduced colonisation of the barley roots by the plant

Table 5
Fresh shoot and root weights, total root length and effective root length of wheat grown in pots when fungal inoculum was introduced as layers (Experiment 4)

Treatments	Fresh shoot weight ± SD (g)	Fresh root weight ± SD (g)	TRL ± SD (m)	ERL ± SD (%)
Control	1.056 ± 0.168[a]	2.066 ± 0.246[a]	11.483 ± 2.192[a]	100[a]
P. chlamydosporia 4624	1.096 ± 0.046[a]	2.001 ± 0.117[a]	12.371 ± 0.53[a]	100[a]
L. lecanii 4625	0.962 ± 0.123[a]	1.649 ± 0.250[a]	9.297 ± 2.487[a]	100[a]
L. lecanii 4629	1.054 ± 0.146[a]	1.904 ± 0.251[a]	11.271 ± 2.551[a]	100[a]
Ggt alone	1.058 ± 0.090[a]	1.905 ± 0.204[a]	10.548 ± 0.553[a]	5.255 ± 1.876[a]
P. chlamydosporia 4624 + Ggt	0.988 ± 0.129[a]	1.683 ± 0.305[a]	9.294 ± 2.136[a]	5.589 ± 4.528[a]
L. lecanii 4625 + Ggt	0.993 ± 0.170[a]	1.671 ± 0.243[a]	10.106 ± 2.418[a]	3.039 ± 1.240[b]
L. lecanii 4629 + Ggt	1.069 ± 0.148[a]	1.870 ± 0.337[a]	9.861 ± 2.192[a]	4.649 ± 2.690[a]

SD, Standard Deviation; TRL, Total root length; ERL, Effective root length. Figures followed by different letters (in columns) are significantly different at the 95% level: $P < 0.05$. Means are based on 4 measurements.

Table 6
Increase in fresh shoot and root weights and in total root length and decrease in leaf guaiacol peroxidase activity in wheat grown in the presence of nematophagous fungi alone or in combination with Ggt, using the split root system (Experiment 5)

Treatments	Fresh shoot weight ± SD (g)	Fresh root weight ± SD (g)	TRL ± SD (m)	ERL ± SD (%)	PER activity ± SD (∇ Abs min^{-1} g^{-1})
Control	0.111 ± 0.022[a]	0.219 ± 0.086[a]	1.748 ± 0.692[a]	100[a]	9.567 ± 4.420[a]
P. chlamydosporia 4624	0.188 ± .047[b]	0.424 ± 0.129[b]	3.223 ± 0.900[b]	100[a]	ND
L. lecanii 4629	0.172 ± .020[b]	0.432 ± 0.074[b]	3.526 ± 0.480[b]	100[a]	ND
Ggt	0.129 ± 0.028[a]	0.279 ± 0.099[a]	1.827 ± 0.534[a]	2.1 ± 5.144[a]	9.031 ± 1.119[a]
P. chlamydosporia 4624 + Ggt	0.185 ± .023[b]	0.451 ± 0.103[b]	3.067 ± 0.536[b]	1.950 ± .141[a]	6.544 ± 1.094[b]
L. lecanii 4629 + Ggt	0.192 ± .046[b]	0.401 ± 0.055[b]	2.976 ± 0.498[b]	3.485 ± .871[a]	5.707 ± 0.827[b]

SD, Standard Deviation; ND, Not determined; TRL, Total root length; ERL, Effective root length; PER, Guaiacol peroxidase. Figures followed by different letters (in columns, within groups) are significantly different at the 95% level: $P < 0.05$. Means are based on 6 measurements.

pathogen Ggt at both 3 and 5 days. Similarly, there was a reduction in the percentage necrosis in roots treated with the nematophagous fungi compared to Ggt alone. These results suggest that the *Pochonia* spp. can protect the roots from severe infection by *G. graminis*.

In the growth tube experiment (Experiment 2), we showed that pre-inoculation of the nematophagous fungi offered no advantage in protecting the roots from Ggt. On the other hand, at 2 weeks, we found a reduction in Ggt colonization from simultaneous inoculation of Ggt and the *Pochonia* spp. This was accompanied by a significant increase in effective root length.

In the mixed inoculum experiment (Experiment 3), where the inoculum was dispersed in the soil, there was increased growth of wheat plants when *P. chlamydosporia* or *L. lecanii* (isolate 4625) were applied to soil. In Experiment 4, when the inoculum was introduced as layers, there were no beneficial effects from the three nematophagous fungi upon shoot or root growth. However, yellowing of the leaves in plants treated with Ggt, however, clearly demonstrated that the seedlings were affected by root-rot. While the lack of treatment effects in this experiment could be related to the dispersion of Ggt inoculum in the soil, we did, however, obtain similar results when the inoculum was presented as a layer in the soil well below the seed. This lack of response could be due to the presence of the nematophagous fungi being restricted to only around a limited portion of the roots, as it is known that some soil microorganisms only promote plant growth when there is a widespread colonisation of roots (Weller, 1988).

In experiment 5, involving the split-root system, both *P. chlamydosporia* and *L. lecanii* (isolate 4629) showed shoot and root growth promotion of wheat seedlings but, again, with no visual effects on root-rot caused by Ggt. In this case, the growth-promotion effect was related to the greater total root length when *P. chlamydosporia* or *L. lecanii* were applied to soil (alone or in combination with Ggt) than in the presence of Ggt only.

Our laboratory study clearly indicated that the *Pochonia* spp. has the capacity to reduce Ggt colonization of the plant roots. *P. chlamydosporia* has been previously demonstrated (Lopez-Llorca et al., 2002) to be a good rhizosphere and cortical colonizer. The ability to colonise rhizosphere has also been demonstrated for different nematophagous fungi (Bourne et al., 1996; Persmark and Jansson, 1997; Meyer et al., 1998; Lopez-Llorca et al., 2002; Bordallo et al., 2002). In the pot experiments it is possible that the soil saprophytic competence (Garrett, 1970) of the nematophagous fungal strains tested was inadequate to overcome Ggt's growth and pathogenicity. It is also possible that inoculum rate of the antagonists may need to be higher, as in the case of a sterile red fungus which showed suppression of Ggt when applied at a rate of 1 g of rye-grass colonised seeds per 100 g soil (Aberra et al., 1998). In our studies we used only 0.5 g of nematophagous fungi inoculum per 100 g soil.

Guaiacol peroxidase (GPO) activity in wheat leaf extracts, was significantly lower in seedlings inoculated with Ggt combined with *P. chlamydosporia* or *L. lecanii* (isolate 4629) than in seedlings inoculated with Ggt alone. These results suggest that, these two nematophagous fungi have a growth promotion effect on wheat through production of growth regulators. This enhanced vigour of the plants inoculated with the nematophagous fungi could have helped to reduce the stress imposed by the split-root system and the pathogen and could explain the associated decrease of peroxidase activity.

Although Schweizer et al. (1989) found that peroxidases were involved in the mechanisms of induced resistance in wheat to *Erysiphe graminis* f.sp. *tritici*, in our studies we found no differences between peroxidase activity in the control treatment (non-infested rye-grass seeds applied as inoculum) and the treatment with Ggt. Evidence from Aberra et al. (1998), indicated that wheat plants do have inducible mechanisms to resist Ggt so the lack of peroxidase activity induction could be due to repression of resistance mechanisms by Ggt or to the non-activation of these mechanisms. As, in our study, the inoculum was applied to the soil and the enzymatic activity measurement was done on the leaves, it is possible that peroxidase activity was enhanced in the roots (the site of infection) but not in the shoots.

There was sufficient evidence in our study to conclude that the nematophagous fungi *P. chlamydosporia* (isolate 4624) and *L. lecanii* (isolate 4629) can have a growth-promoting effect on wheat seedlings. The increase in fresh

shoot weight however does not appear to result from a reduction in root disease severity. The reduction in peroxidase production we found in nematophagous fungus inoculated wheat plants could be related to a decrease of stress following promotion of plant growth. It is known (Gazaryan and Lagrimini, 1996) that some peroxidases oxidize (and thus inactivate) the plant hormone IAA that promotes growth. Hence, lowering of peroxidase activity could also be related to increased plant growth. Peroxidases are also known to be associated with the integrity of the cell wall (Fry, 1986) and could therefore probably provide defence against the pathogen.

Whether induction of plant defence mechanisms takes place or not in roots inoculated with nematophagous fungi remains unknown and will require further investigations.

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