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Influence of soil impoverishment on the interaction between *Glomus mosseae* and saprobe fungi

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Abstract The effect of the saprobe fungi *Wardomyces inflatus* (Marchal) Hennebert, *Paecilomyces farinosus* (Holm & Gray) A. H. S. Brown & G. Sm., *Gliocladium roseum* Bain., *Trichoderma pseudokoningii* Rifai and *T. harzianum* Rifai, isolated from sporocarps of *Glomus mosseae*, on arbuscular mycorrhizal (AM) colonisation and plant dry matter of soybean was studied in 2/3 and 1/5 diluted soils in a greenhouse trial. Soil dilution to 1/5 had no effect on shoot dry matter of soybean but decreased AM colonisation and root dry weight of plants. CFU of saprobe fungi, except *T. harzianum*, were higher in 1/5 than in 2/3 diluted soils. *W. inflatus* and *Gliocladium roseum* decreased the shoot dry weight of soybean plant when inoculated together with *Glomus mosseae.* The saprobe fungi *P. farinosus* and *T. pseudokoningii* increased the shoot dry weights of plants grown in 1/5 diluted soil. The shoot dry weight and AM colonisation in 1/5 diluted soil were also increased when *T. harzianum* was inoculated together with *Glomus mosseae*. Thus, saprobe fungi increased AM colonisation of soybean plants by indigenous endophytes. The AM colonisation of plants at both soil dilutions was increased by *Glomus mosseae*. The highest level of AM colonisation was observed when *P. farinosus* and *T. pseudokoningii* were inoculated together *Glomus mosseae*. The dilution of soils influenced the interaction between inoculated microorganisms and their effect on plant growth.

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Introduction

Intensive agricultural practices can degrade the ecosystem, especially the agrosystem (Bethlenfalvay and Schüepp 1994). These degradative processes include soil erosion and loss of available nutrients, organic matter and microbial propagules (Barea and Jeffries 1995). This leads to poor fungal growth due to low concentrations of suitable metabolites and to soil fungistasis (Wainwright 1992).

Arbuscular mycorrhizal (AM) fungi are a major component of the soil microbial biomass in most ecosystems (Allen 1991). These fungi, especially their mycelium, are important in the consolidation of unstable soils and in the recovery of degraded soils (Miller and Jastrow 1992; Jeffries and Dodds 1996). Various researchers have reported the reduction and/or loss of infectivity of AM fungi as a result of natural or man-induced disturbance of ecosystems (Harley and Smith 1983; Abbott and Gazey 1994).

Under unfavourable environmental conditions, the effects of AM symbiosis can be decisive for the successful establishment of plants in revegetation strategies (Jasper 1994; Estaun et al. 1997). Two strategies have been proposed for re-establishing microbial communities in disturbed ecosystems (Jeffries and Dodds 1996): 1) restoration of indigenous microorganisms to a level able to sustain the plant community and 2) increase in soil microbiota by re-introduction of effective exotic species from similar ecosystems. Both strategies can be applied either by the inoculation of selected AM fungi or by increasing the effectivity of the indigenous AM populations. The introduced AM fungi may or may not synergise with indigenous endophytes and this interaction may be affected by the environment (Ocampo 1980; Hayman 1983).

On the other hand, it is known that soil microorganisms affect AM symbiosis. Saprobe fungi are important and common components of the soil rhizosphere (Dix and Webster 1995). Their importance lies in the large microbial biomass they supply to soil. They can grow out from the sites of microbial activity. Hyphae extend out into the soil, producing very fine mycelial networks which facilitate substrate collection (Wainwright 1992). Some experimental results confirm the existence of synergistic effects of saprobe fungi on AM spore germination and plant root colonisation by AM fungi (Calvet et al. 1993; McAllister et al. 1996; Fracchia et al. 1998; García-Romera et al. 1998). Because soil degradation reduces the size of the microbial population, the role of AM and saprobe fungi in restoration of these soils may be important.

The influence of the inoculation of *Glomus mosseae* and several saprobe fungi on mycorrhizal colonisation and growth of soybean in two diluted soils was studied.

Materials and methods

Plants were grown in 300-ml open pots of soil from Pergamino in the Province of Buenos Aires, Argiudol type, pH of 7.1. Non-sterilised soil was mixed with sterilised quartz sand in proportions of 2:3 or 1:5 (v:v). Seeds of soybean (*Glycine max* cv. Nidera) were surface-sterilised with $HgCl_2$ for 10 min, thoroughly rinsed with sterilised water and sown in moistened sand. After germination, uniform seedlings were each planted into 300 g of the soil mixes and grown in a greenhouse with supplementary light from Sylvania incandescent, cool-white lamps (400 μE m^{-2} s^{-1}, 400–700 nm) with a 16/8-h day/night cycle at 25/19 °C and 50% relative humidity. Plants were watered from below and fed with a nutrient solution at 10 ml per week (Hewitt 1952).

The AM inoculum consisted of 5 g of rhizosphere soil from alfalfa plant pot cultures of *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe, isolated from Ciudad Universitaria soil in the province of Buenos Aires, Argentina (Fracchia et al. 1998), which contained spores, mycelium and colonised root fragments. The AM inoculum was mixed into the 300 ml soil in each pot. Soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of mycorrhizal plants was added to the AM noninoculated treatment. The filtrate contained common soil microorganisms but no propagules of *Glomus mosseae*.

The saprobe fungi *Wardomyces inflatus* (Marchal) Hennebert (BAFC Cult. no. F8992; Hennebert 1968), *Paecilomyces farinosus* (Holm & Gray) A. H. S. Brown & G. Sm., (BAFC Cult. no. F8846; Samsom 1974), *Gliocladium roseum* Bain. (BAFC Cult. no. F8845; Domsch et al. 1980), *Trichoderma pseudokoningii* Rifai (BAFC Cult. no. F8844; Rifai 1969) and *T. harzianum* Rifai (BAFC Cult. no. F8842; Rifai 1969) were isolated from *Glomus mosseae* sporocarps (Fracchia et al. 1998). The fungal isolates were transferred to tubes of potato dextrose agar (PDA) and 2% malt extract at 4 °C as stock cultures.

An aqueous suspension in sterile distilled water containing approximately 2×10^8 spores ml^{-1} of each saprobe fungus was prepared from cultures grown in PDA for 1 week at 27 °C.

To evaluate the population of inoculated saprobe fungi during the experiments, rhizosphere soils were sampled after 1, 15, 30 and 45 days, as described by McAllister et al. (1994). This soil was replaced by autoclaved soil mix. About 5 g of rhizosphere soil was taken from each of the experimental pots and tenfold aqueous dilution series (from 10^{-1} to 10^{-4}) were prepared for each sample. The number of saprobe colony forming units (CFU) in suitable dilutions of such samples taken from the five replicate pots of each treatment were counted on malt extract agar medium. Rhizosphere soil was quantified by removing soil from the dilutions of 10^{-1} and 10^{-2}, drying at 105 °C and weighing. CFU was expressed per g of dry rhizosphere soil.

Plants were harvested after 45 days and their dry weights determined. Approximately half of the root system was cleared and stained (Phillips and Hayman 1970) and the percentage root length colonized by AM fungi was measured (Giovannetti and Mosse 1980).

The experiment was designed as a $2 \times 2 \times 5$ factorial with soil dilution, *Glomus mosseae* inoculation and saprobe fungi inoculation as factors. The experiments were repeated three times and the means of five replicate pots from one representative experiment are given. The percent values were arcsine transformed and the data obtained for dry weight, percentage AM colonisation and CFU of saprobe fungi were subjected to ANOVA. The means were compared using standard errors of means and Duncan's multiple range test ($p = 0.05$).

Table 1 Shoot and root dry weight (mg) of soybean grown in 2/3 and 1/5 diluted soils in the presence or absence of *Glomus mosseae* and inoculated or not with saprobe fungi. The data are in each case the means of five replicates. Column means followed by the same letter are not significantly different ($p = 0.05$)

Saprobe fungi	Minus *Glomus mosseae*		Plus *Glomus mosseae*	
	Shoot	Root	Shoot	Root
2/3 diluted soil				
Control	1514 ab	977 bc	1547 d	1067 bc
Wardomyces inflatus	1797 b	1337 e	1243 b	1104 bc
Gliocladium roseum	1762 b	1496 ef	1371 bc	1278 c
Trichoderma harzianum	1678 b	1145 d	1649 d	981 ab
Paecilomyce farinosus	1526 ab	1271 de	1645 d	1273 c
Trichoderma pseudokoningii	1667 b	1500 f	1620 d	1003 b
1/5 diluted soil				
Control	1391 a	664 a	1421 c	871 a
W. inflatus	1566 ab	749 a	975 a	905 a
G. roseum	1426 a	952 bc	840 a	899 a
T. harzianum	1490 a	1222 de	1910 e	1237 c
P. farinosus	1701 b	847 b	2745 f	1558 d
T. pseudokoningii	1784 b	1039 c	2640 f	1375 c

Results

There was a significant overall effect of saprobe fungi ($p=0.0001$) and a significant interaction between dilution of soils and inoculation with *Glomus mosseae* ($p=0.006$) on the shoot dry weight of soybean plants. There was no difference in shoot dry weight between noninoculated plants grown in 2/3 and 1/5 soils (Table 1). The shoot dry weights of soybean plants grown in 1/5 diluted soil were increased by the presence of *P. farinosus* and *T. pseudokoningii*. *T. harzianum* increased shoot dry weights when inoculated together with *Glomus mosseae* in 1/5 diluted soil. When *W. inflatus* and *Gliocladium roseum* were inoculated together with *Glomus mosseae* a significant decrease in soybean shoot dry weight was observed in both soil dilutions.

Dilution of soil to 1/5 significantly decreased the root dry weight of soybean ($P=0.001$). Saprobe fungi increased root dry weight of plants grown in 2/3 and 1/5 soil dilutions, except *W. inflatus* inoculated in 1/5 diluted soil. In the presence of *Glomus mosseae*, saprobe fungi did not increase root dry weight at 2/3 soil dilution. In 1/5 diluted soil, only *T. harzianum*, *P. farinosus* and *T. pseudokoningii* increased root dry weight (Table 1).

Percentage root colonisation by AM fungi was significantly affected by the presence of saprobe fungi ($P=0.0001$) and by inoculation of *Glomus mosseae* ($P=0.001$) (Table 2). Soil dilution decreased mycorrhization of plants inoculated with *Glomus mosseae*. However, in the presence of saprobe fungi, no significant differences in percentage root colonisation between soil mixes were found. The saprobe fungi increased the percentage AM colonisation of soybean plants by indigenous endophytes. A higher AM colonisation of soybean roots was observed when soil mixes were inoculated with *Glomus mosseae*. In both soil mixes, inoculation of *Glomus mosseae* together with *P. farinosus* and *T. pseudokoningii* increased percentage AM root colonisation to higher levels than when the saprobes were inoculated in the absence of *Glomus mosseae*. However, *T. harzianum* increased the percentage AM colonisation of soybean plants when *Glomus mosseae* was added to 1/5 diluted soil.

At 24 h after inoculation, CFU of saprobe fungi ranged from 400 ± 8.8 to 411 ± 5.6 with the exception was *T. harzianum*, which decreased to 272 ± 8.2 per g soil. No significant differences in the number of saprobe fungi in the different treatments were found at this time. CFU of saprobe fungi per g rhizosphere soil decreased throughout the experiment (Table 3). There was a significant overall effect of *Glomus mosseae* ($P=0.0001$) and of soil mixes ($P=0.001$) on CFU of saprobe fungi during the experiment. CFU of saprobe

Table 2 Percentage AM colonisation of soybean grown in 2/3 and 1/5 diluted soils in the presence or absence of *Glomus mosseae* and inoculated or not with saprobe fungi. The data are in each case the means of five replicates. Column means followed by the same letter or row means followed by the same number are not significantly different ($p=0.05$)

Saprobe fungi	Minus *Glomus mosseae*	Plus *Glomus moseae*
2/3 diluted soil		
Control	2.8 a 1	22.1 bc 2
W. inflatus	20.4 cd 1	20.8 bc 1
G. roseum	21.6 c 1	22.4 bc 1
T. harzianum	10.4 b 1	15.2 b 1
P. farinosus	16.1 c 1	36.1 de 2
T. pseudokoningii	15.8 c 1	42.3 ef 2
1/5 diluted soil		
Control	1.1 a 1	9.6 a 2
W. inflatus	18.4 cd 1	19.6 b 1
G. roseum	22.3 d 1	24.1 c 1
T. harzianum	10.2 b 1	25.5 c 2
P. farinosus	15.6 c 1	34.6 d 2
T. pseudokoningii	13.3 bc 1	54.4 f 2

Table 3 Colony-forming units ($\times 10^3\ g^{-1}$ soil) of saprobe fungi from the rhizosphere of soybean grown in 2/3 and 1/5 diluted soils in the presence or absence of *Glomus mosseae* after 15,30 and 45 days. The data in each case are the means of five replicates. Column means followed by the same letter or row means followed by the same number are not significantly different ($p=0.05$)

Saprobe fungi	Minus *Glomus mosseae*			Plus *Glomus moseae*		
	15	30	45	15	30	45
2/3 diluted soil						
W. inflatus	174 b 5	101 a 3	84 b 2	282 d 6	132 c 4	70 c 1
G. roseum	192 c 4	155 b 3	74 a 1	218 b 5	163 e 3	94 e 2
T. harzianum	202 c 3	205 c 3	144 f 2	204 b 3	143 d 2	110 f 1
P. farinosus	159 a 4	103 a 2	98 c 2	315 e 5	131 c 3	54 a 1
T. pseudokoningii	194 c 3	101 a 3	82 b 1	210 b 4	93 a 2	77 d 1
1/5 diluted soil						
W. inflatus	205 c 4	139 b 3	112 d 2	232 c 6	196 f 5	91 e 1
G. roseum	216 d 5	162 c 4	152 g 3	230 c 6	135 c 2	100 f 1
T. harzianum	149 a 3	102 a 2	95 c 2	135 a 3	103 b 2	64 b 1
P. farinosus	254 e 4	188 d 3	145 cd 1	336 f 5	168 e 2	149 g 1
T. pseudokoningii	188 c 2	199 d 2	134 e 1	285 d 5	234 g 4	214 h 3

fungi was higher in 1/5 diluted than in 2/3 diluted soil, except for *T. harzianum*, which was presenting higher amounts in 2/3 than in 1/5 diluted soil. The presence of *Glomus mosseae* increased CFU of saprobe fungi, except for *T. harzianum*, in 2/3 soil dilution after 15 days. However, after 45 days CFU of saprobe fungi decreased, except for *Gliocladium roseum*, in presence of *Glomus mosseae* in the 2/3 soil dilution. In the 1/5 soil dilution, *Glomus mosseae* increased CFU of *T. pseudokoningii*. The presence of *Glomus mosseae* did not affect CFU of *T. harzianum* after 15 or 30 days, but a decrease was observed after 45 days. CFU of *W. inflatus*, *Gliocladium roseum* and *P. farinosus* inoculated together with *Glomus mosseae* in 1/5 diluted soil increased after 15 days, but the population of *W. inflatus* and *Gliocladium roseum* decreased after 45 days (Table 3).

Discussion

The combined application of saprobe fungi and *Glomus mosseae* to diluted soil contributed to recolonisation by AM fungi. The increased percentage AM colonisation of soybean roots observed when *Glomus mosseae* was inoculated to both soil mixes indicates a non-antagonistic interaction between *Glomus mosseae* and indigenous AM fungi. However, there were no differences in their effects on plant dry matter. No consistent relationship has been found between percentage colonisation and the effect of AM fungi on plant growth (Vierheilig and Ocampo 1991).

Antagonistic, synergistic and neutral interactions between *Glomus mosseae* and the saprobe fungi used in this experiment have been reported under sterilise conditions (Fracchia et al. 1998). However, interactions between AM and saprobe fungi vary depending on the soil and the microbial population where plants are growing (McAllister et al. 1996; García-Romera et al. 1998). Competition for metabolites between soil microorganisms and AM fungi may decrease the effect of AM fungi on plant growth (Bethlenfalvay et al. 1983; Ruiz-Lozano and Azcon 1993). We found that in more diluted soil, *W. inflatus* and *Gliocladium roseum* significantly decreased shoot dry weight of plants inoculated with *Glomus mosseae*, whereas these saprobe fungi did not affect shoot dry weight when inoculated with *Glomus mosseae* in less-diluted soil.

In more-diluted soil, the population of inoculated saprobe fungi was higher than in less-diluted soil. This suggests that indigenous soil microorganisms competed with inoculated saprobe fungi. Under the poorest soil conditions, there was a synergistic interaction between *Glomus mosseae* and *T. pseudokoningii* and the combined application of these microorganisms was more effective in restoring saprobe and AM fungal populations, and soybean growth in impoverished soil (restoration and revegetation). However, no generalisation can be made about the effects of application of microorganisms in soils of different fertility. Negative effects of *T. harzianum* on *Glomus mosseae* were found in the 2/3 diluted soil when the population of the saprobe fungi was high. When CFU of *T. harzianum* decreased in 1/5 diluted soil, an increase in mycorrhizal colonisation and plant dry matter was observed.

Our results show that dual inoculation of AM and some saprobe fungi can contribute to increased shoot dry weight of plants, and that the highest effect of this combined application occurs in impoverished soil. This could have practical significance in soils containing few or ineffective indigenous mycorrhizal fungi.

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