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Interactions between *Glomus mosseae* and arbuscular mycorrhizal sporocarp-associated saprophytic fungi

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Abstract

The saprophytic fungi *Wardomyces inflatus* (Marchal) Hennebert, *Paecilomyces farinosus* (Holm & Gray) A. H. S. Brown & G. Sm., *Gliocladium roseum* Bain., sterile dark mycelium (SDM-54), *Trichoderma pseudokoningii* Rifai and *Trichoderma harzianum* Rifai were isolated from sporocarps of *Glomus mosseae*. The effect of saprophytic fungi on *G. mosseae* spore germination was tested on water agar. *Wardomyces inflatus* decreased the percent germination of *G. mosseae* spores; *G. roseum, T. pseudokoningii* and *T. harzianum* had no effect on germination; and *P. farinosus* and SDM-54 increased the percentage of spore germination of *G. mosseae* after 4 d. *Wardomyces inflatus* significantly decreased hyphal length of spores which germinated, but no other saprophytic fungi affected hyphal growth. *Trichoderma pseudokoningii, T. harzianum, P. farinosus* and SDM-54 increased the number of auxiliary cells formed by *G. mosseae*. The effect of saprophytic fungi on arbuscular mycorrhizal colonization of soybean was studied in a greenhouse trial. The percentage of soybean root length colonized was decreased by *W. inflatus*, unaffected by SDM-54 and *T. harzianum*, and increased by *P. farinosus*. *Gliocladium roseum* decreased root length colonized when plants were 12 wk old, and *T. pseudokoningii* increased colonization of roots when plants were 4 wk old. Antagonistic, synergistic and neutral actions of *G. mosseae* upon the saprophytic fungi were observed. The population of *T. harzianum* decreased and the populations of *T. pseudokoningii* and SDM-54 increased in the presence of *G. mosseae*. Our results indicate a complex interaction between *G. mosseae* and associated saprophytic fungi.

Introduction

Saprophytic fungi live in the rhizoplane and mycorrhizosphere of plants, from which they obtain nutritional benefit in the form of inorganic compounds, exudates, and mucilages from living roots, as well as, from sloughed cells and dead fungi (Finlay and Söderström, 1992). Their metabolism may result in the production of substances that promote or inhibit the growth of other rhizosphere microorganisms (Dix and Webster, 1995). Fungi of the genus *Wardomyces* are involved in the decomposition of plant residues (Domsch et al., 1980). Saprophytic fungi of the genus *Trichoderma* are antagonistic to pathogenic fungi (Camporota, 1985); some of them produce substances that may have a fungistatic effect on other species (Chu and Wu, 1981; Claydon et al., 1987). Sterile dark mycelia (SDM) are very abundant in soils, but their activity and interactions are poorly understood (Domsch et al., 1980). *Gliocladium roseum* is a common soil fungus that colonizes rotting plants. It also parasitises sclerotia of various fungi, and its inhibitory effects have been observed in a number of cellulolytic, saprophytic fungi. *Paecilomyces farinosus*, a polyphagous saprophytic fungi, lives in dead insects, wood and soil (Samsom, 1974).

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Arbuscular mycorrhizal (AM) symbioses are widespread in the plant kingdom. Since AM fungi are partially outside the host root, external factors such as the presence of soil microorganisms influence the formation and function of the symbiosis (Barea and Jeffries, 1995). Spores of AM fungi are vulnerable to parasitism from soil microorganisms (Fitter and Garbaye, 1994). However, some spore-associated bacteria (Mayo et al., 1986) and fungi (Vidal-Dominguez, 1991) enhance spore germination and hyphal growth.

In spite of the increasing interest in the interaction between AM and saprophytic fungi, information about these interactions is scarce (Camprubi et al., 1995; McAllister et al., 1994; Tarafdar and Marschner, 1995). Synergistic and antagonistic interactions between *G. mosseae* and selected saprophytic fungi have been observed (McAllister et al., 1994). The purpose of this study was to determine the relationships between *G. mosseae* and some saprophytic fungi isolated from sporocarps of this AM fungus.

Materials and methods

Isolation of G. mosseae *sporocarps and spores*

Sporocarps of *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe were isolated by wet sieving soils (Gerdemann, 1955) from Boulogne and Ciudad Universitaria in the province of Buenos Aires in Argentina. These soils were of the Argiudol type, with a pH of 6.8 and 7.1, respectively. The sporocarps and spores were identified as *G. mosseae* according to Gerdemann and Trappe (1974). Alfalfa (*Medicago sativa* L.) pot cultures of these isolates of *G. mosseae* were obtained after 4 months of plant growth.

Isolation of saprophytic fungi

The active fungi present in the sporocarps of *G. mosseae* from field soils were isolated by the particle washing method (Haley and Waid, 1955) modified by using a Millipore portafilter, in which the sporocarps were placed in a sandwich of filter papers (Millipore 0.2 μm pore diameter above and Whatman no. 4 below). Fifteen sporocarps from each of the two soils were used. Thirty washings were necessary to remove fungal sclerotia, spores, etc., from the sporocarps. The washed sporocarps were dried on sterilized filter paper and plated on 2% malt extract agar containing antibiotics (5 μg L^{-1} streptomycin and 10 μg L^{-1} tetracyclin) and incubated at 28 °C. Colonies from each sporocarp were observed periodically under light microscope for 3 wk. Only distinctly different saprophytic fungi colonies from the same sporocarp were considered. From the resulting colonies *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), sterile dark mycelium (SDM-54) (BAFC Cult. no. F8868; Domsch et al., 1980), *Trichoderma pseudokoningii* Rifai (BAFC Cult. no. F8844; Rifai, 1969) and *T. harzianum* Rifai (BAFC Cult. no. F8842; Rifai, 1969) were selected as the most frequent saprophytic fungi present in the different sporocarps. The fungal isolates were transferred to tubes of potato dextrose agar (PDA) and 2% malt extract at 4 °C as stock cultures.

Effect of saprophytic fungi on the development of G. mosseae

The effect of each saprophytic fungus on spore germination and hyphal length of *G. mosseae* was tested *in vitro* on 1% sterile water agar. Prior to autoclaving media we added 10 m*M* 2-(N-morpholino) ethane sulfonic acid (MES) to 1% water agar to maintain the medium at pH 7 throughout the experiment. This buffer was previously shown to have no effect on germination of AM fungal spores *in vitro* (Calvet et al., 1992; McAllister et al., 1994). Sporocarps of *G. mosseae* were isolated by wet sieving (Gerdemann, 1955) soil from alfalfa pot cultures and were stored in water at 4 °C and used within 1 month. The spores of *G. mosseae* were obtained by dissecting the sporocarps and spores were surface-sterilized as described by Mosse (1962). Five surface-sterilized spores per plate were placed 1 cm from the edge of a (10 cm dia.) Petri dish, and a thin slice of PDA with spores and mycelium of one of the 6 saprophytic fungi was placed opposite and at least 7 cm away from them. Ten replicate plates of each saprophytic fungus treatment and 10 controls (plates with spores of *G. mosseae* without saprophytic fungi) were used. The plates were incubated at 25 °C in the dark, and were sealed to reduce the risks of dehydration and contamination. Spore germination of *G. mosseae* was determined periodically under a light microscope for 11 d. After 11 d mycelia of some saprophytic fungi contacted *G. mosseae* hypha. At the end of the experiment (11 d) hyphal length of the germinated *G. mosseae* spores was assessed using the gridline intersect method (Olson, 1950).

Interaction between saprophytic fungi and G. mosseae*, inoculated as spores, in the rhizosphere of soybean*

This experiment was performed in 300-mL pots of soil collected from Buenos Aires, Argentina. The soil (Argiudol type, pH 7.1, 9.5 ppm P) was steam-sterilized and mixed 1:1 (V/V) with sterilized quartz sand. In each pot, 100 surface-sterilized spores of *G. mosseae* were placed under the seedlings. Seeds of soybean (*Glycine max* cv. Nidera) were surface-sterilized with $HgCl_2$ for 10 min and thoroughly rinsed with sterilized water and sown in moistened sand. After germination, uniform seedlings were planted and grown in a greenhouse with supplementary light provided by Sylvania incandescent and 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 (Hewitt, 1952).

An aqueous suspension of each of the saprophytic fungi containing approximately 2×10^5 spores mL^{-1}, was prepared from cultures grown in PDA for 1 week at 27 °C. For the sterile fungus SDM-54, thin slice of PDA (1 × 1 cm) with mycelia was placed beneath the seedlings.

Four treatments were used in all experiments: (1) uninoculated controls, (2) inoculated with (one of the 6) the saprophytic fungi, (3) inoculated with *G. mosseae*, and (4) inoculated with both *G. mosseae* and each of the saprophytic fungi. Plants were inoculated with both microorganisms at the time of transplanting.

To evaluate the length of mycelia of the saprophytic fungi, 1 g of rhizosphere soil from each of five replicate pots was sampled after 2, 4, 6 and 12 wk. The quantity of mycelium was determined by the agar film method as described by Parkinson (1982), only septate hyphae with less than 3 μm dia. were considered and was expressed as m of mycelium per g of dry soil.

Plants were harvested after 4 and 12 wk; and shoot and root dry matter were weighed, and foliar area measured. After the plants were harvested, the root system in each of the 5 replicates per treatment was cleared and stained (Phillips and Hayman, 1970), and percent root colonization was determined (Giovannetti and Mosse, 1980).

The experimental design was completely randomized with two factors (AM fungus, saprophytic fungus inoculation), each with two levels (presence or absence).

Statistical treatments

The percent values were arcsine transformed for statistical analysis. The data were analyzed by one-way analysis of variance. Comparisons of means were made by the Duncan's multiple range test (P = 0.05). Regression lines for the effect of saprophytic fungi on the percentage of germination (g) of *G. mosseae* against time were fitted by least-squares regression analysis ($g = a+bt+ct^2$).

Results

Effect of saprophytic fungi on G. mosseae *development*

Of the 27 fungal isolates from the sporocarps of *G. mosseae*, more than 75% belonged to the species examined in this experiment. The frequency of recovery of the other 7 species observed from the isolations was very low.

Percent germination of *G. mosseae* spores (Figure 1) decreased significantly in the presence of *W. inflatus*, but germination was not affected by *G. roseum*, *T. pseudokoningii* and *T. harzianum*. However, improved spore germination resulted when *G. mosseae* was exposed to *P. farinosus* and SDM-54. Hyphal length of *G. mosseae*, measured as the number of intersections, was not affected by the saprophytic fungi, except for *W. inflatus* which significantly decreased hyphal length of the AM fungus (Table 1). *Wardomyces inflatus* also significantly decreased the number of auxiliary cells formed by *G. mosseae* (Table 1). The presence of *P. farinosus*, *T. pseudokoningii*, SDM-54 or *T. harzianum* increased the number and shortened the time to appearance of auxiliary cells of *G. mosseae* as compared to the control.

Rhizosphere interactions between saprophytic fungi and G. mosseae

Soybean plants inoculated with saprophytic fungi were healthy after 12 wk and no root disease symptoms were observed. No AM fungi were evident in uninoculated controls and in plants inoculated only with the saprophytic fungi.

The dry weights and leaf area of soybean plants were not significantly different among any treatments. Shoot and root dry weight averaged 780 and 235 mg, respectively, at 4 wk and 1653 and 432 mg at 12 wk.

Table 1. Effect of saprophytic fungi on hyphal length and production of auxiliary cells of *Glomus mosseae* after 11 d of growth on water-agar*

Saprophytic fungi	Hyphal length (number of intersections per spore)	Number of auxiliary cells per spore	Time of appearance of auxiliary cells (days)
Control	2.28b	2.6b	11
W. inflatus	0.89a	1.4a	11
G. roseum	2.26b	3.2b	11
P. farinosus	2.14b	6.1c	7
SDM-54	2.31b	6.2c	7
T. harzianum	2.52b	6.0c	7
T. pseudokoningii	2.61b	6.2c	7

*Each figure is the mean of 10 replicates each with 5 spores of *G. mosseae*. Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

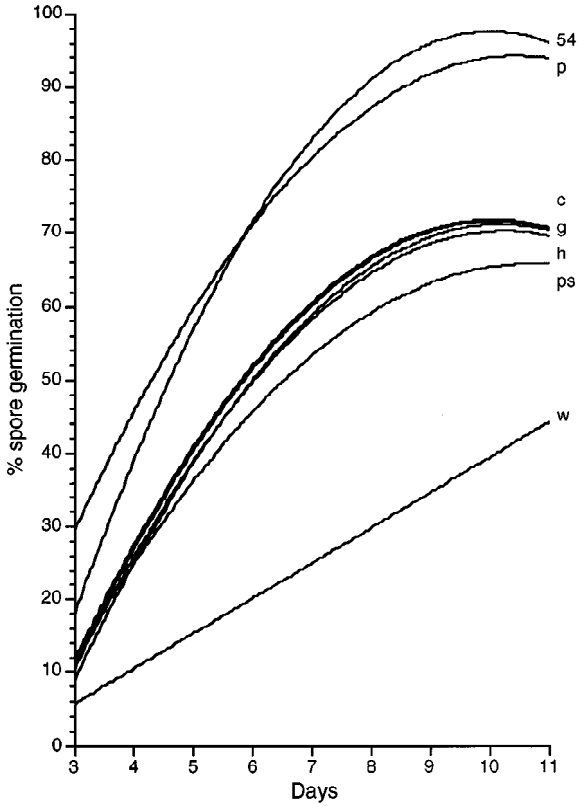

Figure 1. Regression lines for the effect of saprophytic fungi on the percentage of germination (g) of *Glomus mosseae* on water-agar. c = Control ($g = -38.273 + 19.455t - 0.909t^2$); w = *W. inflatus* ($g = -34.654 + 13.432t - 0.604t^2$); g = *G. roseum* ($g = -49.226 + 23.341t - 1.140t^2$); p = *P. farinosus* ($g = -91.273 + 42.849t - 2.427t^2$); 54 = SDM-54 ($g = -49.015 + 30.303t - 1.606t^2$); h = *T. harzianum* ($g = -53.810 + 24.528t - 1.204t^2$); ps = *T. pseudokoningii* ($g = -51.416 + 24.461t - 1.217t^2$).

Leaf areas averaged 123 and 117 at 4 and 12 wk, respectively.

Inoculation with *W. inflatus* significantly decreased the percent root length of soybean plants colonized by *G. mosseae* (Figure 2). *Gliocladium roseum* decreased root length colonization in 12-week-old soybean plants. The presence of SDM-54 and *T. harzianum* did not affect percent root length colonized by the AM fungus, whereas, *T. pseudokoningii* and *P. farinosus* increased root colonization by *G. mosseae* in 4-week-old soybean plants.

The quantity of saprophytic fungal mycelium (Table 2) increased for 6 wk when *G. mosseae* or saprophytic fungi were inoculated onto soybeans; thereafter the length of hyphae remained almost constant. No significant increase in the quantity of hyphae of saprophytic fungi was found when *W. inflatus*, *G. roseum* or *P. farinosus* were inoculated alone compared to when *G. mosseae* was also present. Joint inoculation of *T. harzianum* and *G. mosseae* decreased the quantity of fungal mycelia of saprophytic fungi in soil. However, significant increases in the amount of mycelium were found when SDM-54 and *T. pseudokoningii* were co-inoculated together with *G. mosseae*.

Discussion

The present findings indicate that saprophytic fungi accompanying sporocarps of the AM fungus, *G. mosseae*, can affect spore germination and hyphal growth and root colonization by *G. mosseae*. Some saprophytic fungi (e.g., *W. inflatus*) inhibited spore

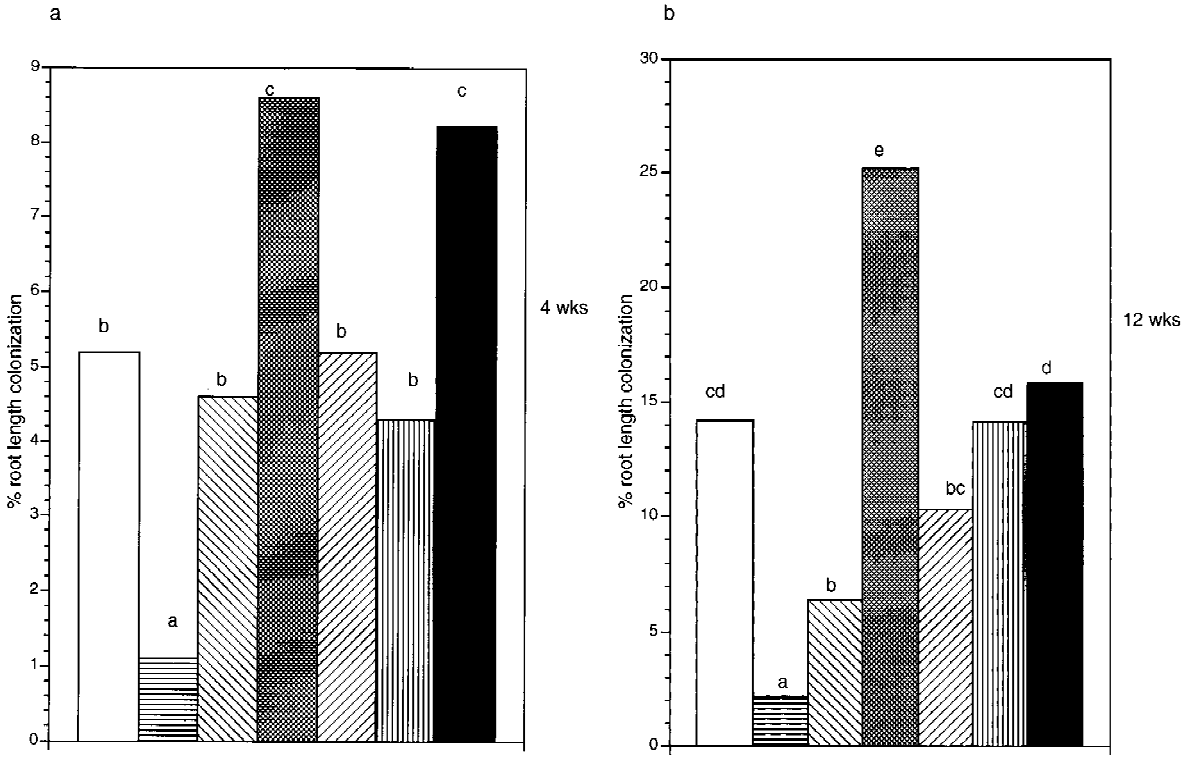

Figure 2. Percentage of AM root length colonized for 4-week-old (Figure 2a) and 12-week-old (Figure 2b) soybean (*Glycine max)* inoculated with *Glomus mosseae* in presence or in absence of saprophytic fungi. □ = Control; ▤ = *W. inflatus*; ▧ = *G. roseum*; ▩ = *P. farinosus*; ▨ = SDM-54; ▥ = *T. harzianum;* ■ = *T. pseudokoningii.*

Table 2. Mycelium of fungi from the rhizosphere (m g^{-1} dry weight of soil) of soybean *(Glycine max)* inoculated or not with *Glomus mosseae**

Saprophytic fungi	Mycelium (m g^{-1} soil) after (wk)							
	2		4		6		12	
	–M	+M	–M	+M	–M	+M	–M	+M
Control	12a	16bc	12a	19c	15ab	22d	24d	28e
W. inflatus	26ab	24a	29ab	29ab	33c	32bc	36c	32bc
G. roseum	21a	26a	34b	26a	41b	40b	39b	43b
P. farinosus	28a	29a	30ab	31abc	37bcd	36bcd	38cd	39d
SDM-54	25a	30b	27ab	36c	31b	40c	36c	45d
T. harzianum	25ab	23a	35d	29bc	44e	33cd	42e	34cd
T. pseudokoningii	24a	40c	34b	48d	38bc	50de	41c	54e

* Each figure is the mean for 5 pots. Row values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
–M = minus spores of *G. mosseae*; +M = plus spores of *G. mosseae*.

germination, the number of auxiliary cells, the time to appearance of auxiliary cells, and percentage of root colonization. These results suggest a direct interaction between the mycorrhizal fungus and the resident saprophytic fungi in the extramatrical phase of the former. Similar interactions have been proposed for other saprophytic fungi (McAllister et al., 1994) and other microorganisms (Caron et al., 1985; García-Garrido and Ocampo, 1988). Our *in vitro* assays suggest that these saprophytic fungi affect AM colonization partly by inhibiting the germination of *G. mosseae* chlamydospores. However, *G. roseum* did not affect the development of AM spores *in vitro* or AM colonization of soybean plant roots after 4 wk of growth, but decreased the percentage of AM root length colonization in 12-week-old plants. These results suggest that *G. roseum* influenced the AM fungus capacity to colonize roots.

Spore germination was increased in the presence of the saprophytic fungus SDM-54, but was not affected by *T. harzianum* or *T. pseudokoningii*. Although *T. harzianum*, *T. pseudokoningii* and SDM-54 did not affect the hyphal growth of spores which did germinate, they increased the production of auxiliary cells and shortened the period of formation relative to controls. Germination and hyphal growth are two separate processes that may be stimulated or inhibited by different compounds (Becard and Piche, 1989; Le Tacon et al., 1983; Vidal-Dominguez, 1991). However, these beneficial effects of SDM-54 and *T. harzianum* were insufficient to increase root length colonization of soybean plants. The growth of the propagule toward the root can be favoured by some rhizosphere microorganisms, even though, this influence may not be reflected in greater AM root colonization (Fitter and Garbaye, 1994). Moreover, *P. farinosus* increased percent spore germination, the number of auxiliary cells, the time to appearance of auxiliary cells, and percent root length colonized, but not hyphal length or plant growth.

Antagonistic, synergistic and neutral actions of *G. mosseae* on the saprophytic fungi were observed in the rhizosphere. In fact, the population of *T. harzianum* decreased and the population of *T. pseudokoningii* and SDM-54 increased in the presence of *G. mosseae*. The effect of AM fungi on the population of a saprophytic fungus through the former's effect on the plant has been reported in an earlier study (McAllister et al., 1995). Modifications in root exudates caused by the AM fungus have been proposed to explain this finding (McAllister et al., 1995; Schwab et al., 1983).

Although mycoparasitism has often been described as the mechanism of antagonistic action of *Trichoderma* species (Camporota, 1985), this was not observed in our experiments. Competition has been observed between *G. mosseae* and *T. koningii* (McAllister et al., 1994) but not *T. aureoviride* (Calvet et al., 1993). Therefore, the interaction between AM and saprophytic fungi may differ between species of the same genus. *Trichoderma pseudokoningii* increased the AM root length colonization of 4-week-old soybean plants while *T. harzianum* had no effect in this experiment. However, *G. mosseae* increased the population of *T. pseudokoningii* but decreased the population of *T. harzianum*.

Interestingly, *T. pseudokoningii* and *P. farinosus* increased root length colonization in soybean plants inoculated with spores of *G. mosseae*. Further studies will investigate these effects with different AM inocula, soils and plants.

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