Symbiosis, 36 (2004) 169–182
Balaban, Philadelphia/Rehovot

Influence of Saprobe Fungi and Their Exudates on Arbuscular Mycorrhizal Symbioses

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Received August 18, 2003; Accepted October 27, 2003

Abstract

The effects of saprobe fungi *Aspergillus niger* (341), *Penicillium restrictum* (512) and *Trichoderma harzianum* (153) isolated from soil, or their exudates on the arbuscular mycorrhizal (AM) fungi *Glomus mosseae* and *Gigaspora rosea* were studied in greenhouse and *in vitro* experiments. *A. niger* and its exudates stimulated the germination and hyphal outgrowth of *G. mosseae* and *Gi. rosea* spores and AM colonization of roots by these and native fungi in an unsterilized soil. In contrast, *T. harzianum* and its exudates had no effects, and *P. restrictum* and its exudates had no effects on spore germination or hyphal outgrowth from spores of either *G. mosseae* or colonization by the native AM endophytes in an unsterilized soil. Effect of *A. niger* were dependent on the concentration of exudates and varied according to the AM endophyte. For example, the application of 1–2.5 µl/ml of exudates of *A. niger* increased the outgrowth of hyphae from spores of *G. mosseae*, but for *Gi. rosea* increases only occurred in the range 1–10 µl/ml. On the other hand, the colonization of plants by *Gi. rosea* increased with the application of 10 ml/pot of exudates of *A. niger*, but the colonization of soybean by *G. mosseae* or by native

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AM endophytes increased in the presence of 20 µl/pot of exudates. The similarity in trends observed in the effects of the saprobe fungi or their exudates on spore germination and hyphal length and effects on AM colonization of plants and number of propagules of AM fungi suggest that the effects of saprobe fungi are largely due to effects on the extraradical phase of the AM fungi.

Keywords: Arbuscular mycorrhizal fungi, *Gigaspora rosea*, *Glomus mosseae*, plant growth, saprobe fungi

1. Introduction

Numerous works have demonstrated the importance of studying the interactions that involve filamentous fungi and arbuscular mycorrhizal (AM) fungi and other organisms that occur in the soil (García Romera et al., 1998; Green et al., 1999; Fracchia et al., 2000) and effects on the formation and development of the AM symbiosis have been demostrated (Ames et al., 1984; Bagyaraj, 1984; Gryndler, 2000). Saprobe fungi are particulary important and common components of the soil rhizosphere (Dix and Webster, 1995), because of the large amount of microbial biomass they supply to the soil, and their role in litter decomposition (Wainwright, 1992). Saprobe fungi belonging to the genera *Aspergillus*, *Penicillium* and *Trichoderma* are involved in complex interactions such as antibiosis, fungistasis and mycoparasitism (Cook and Baker, 1983; Elad, 1986; Hattori, 1973; Krasilnikow and Korenyako, 1945; Pavlica et al., 1978). Consequently, the use of saprobe fungi as biocontrol agents of root pathogenic fungi has led to studies on the effect of these fungi on the development of the AM symbiosis (Calvet et al., 1993; Rouseau et al., 1996; Wyss et al., 1992).

Data for several experiments have demonstrated the existence of interactions between AM and saprobe fungi in the soil rhizosphere and in the colonization of plants roots (Gryndler, 2000). In particular the effect of species of fungi belonging to the genera *Aspergillus*, *Penicillium* and *Trichoderma* on AM symbiosis has been examined, and synergistic and antagonistic effects have been observed (Datnoff et al., 1995; Godeas et al., 1999; Green et al., 1999; Kucey, 1987; McAllister et al., 1994, 1995; Tarafdar and Marschner, 1995). The mechanisms involved in the effect of saprobe fungi on AM fungi are not understood yet, but, it is known that exudates produced by saprobe fungi influence the level of germination of AM fungal spores (Fracchia et al., 1998; Garcia-Romera et al., 1998; McAllister et al., 1994, 1995). Thus, it is possible that the exudates produced by saprobe fungi could interact with AM fungi and influence the development and function of the AM symbiosis.

AM associations are especially important in soils from degraded areas, because they typically have low fertility and when plants are under

environmental stress the effects of AM symbiosis can be decisive for the successful establishment of plants employed in revegetation efforts (Estaun et al., 1997; Jasper, 1994). Importantly these areas often have a low density of propagules of AM fungi and the restoration of AM indigenous endophytes to a level able to sustain plant communities has been proposed for disturbed ecosystems (Jeffries and Dodds, 1996). It is known that some saprobe fungi can increase the population of AM indigenous endophytes in soils (Fracchia et al., 2000; Godeas et al., 1999). It is thus hypothesized that if exudates of saprobe fungi are able to increase AM fungal population of soil, their direct application could assist in the restoration of AM populations of degraded soils.

The aim of the research described here was to examine the role of the exudates produced by saprobe fungi on the AM symbiosis and to explore the possibility of applying these exudates directly to the increase the indigenous AM fungal population of a degraded soil occurring naturally.

2. Materials and Methods

Experiment 1. Isolation of saprobe fungi

The saprobe fungi *A. niger* (Raper and Fennell, 1965), *Penicillium restrictum* (Domsch et al., 1980) and *T. harzianum* (Samuels, 1996) present as active fungi with high frequency, in rhizosphere soil of clover (*Trifolium repens*) from Boulogne (Buenos Aires province, Argentina), were isolated by the particle washing method using a multichamber washing apparatus (Widden and Bisset, 1973). Thirty washings were necessary to remove propagules (fungal sclerotia, spores, etc.) from the surface of soil particles. Twenty soil particles (2 mm diam.) were dried on sterilized filter paper and placed at the surface of 2% malt extract agar (MEA) containing an antibiotic (5 μg l^{-1} Streptomycin and 10 μg l^{-1} Tetracycline). Strains isolated from the resulting colonies (*A. niger* 341, *Penicillium restrictum* 512 and *T. harzianum* 153) were tested for interactions with *G. mosseae* and *Gi. rosea*. Strains were maintained in tubes of 2% potato dextrose agar (PDA) and 2% malt extract and maintained at 4°C. These strains of filamentous fungi, were identified using morphological and cultural characteristics with specific keys (Raper and Fennell, 1965; Domsch et al., 1980; Samuels, 1996).

Experiment 2. Effect of saprobe fungi on spore germination of G. mosseae and Gi. rosea

The effect of saprobe fungi on spore germination of *G. mosseae* (BEG 12) and *Gi. rosea* (BEG 9) was tested in two different assays conducted in 9-cm diameter

plastic Petri dishes. In the first assay the effect of saprobe fungi on spore germination *in vitro* was tested on a substrate consisting of 10 ml of 10 mM 2-(N-morpholin) ethane sulphonic acid (MES) buffer (pH 7) plus 0.04 g of Gel-Gro (ICN Biochemicals, Aurora, Ohio, USA). Spores of *Gi. rosea* and sporocarps of *G. mosseae* were isolated by wet sieving (Gerdemann, 1955) soil from clover pot cultures, stored in water at 4°C and used within 1 month. The spores of *G. mosseae* were obtained by dissecting the sporocarps. All spores were surface-sterilized as described by Mosse (1962). Five surface-sterilized spores of AM fungi were placed near (1 cm from) the edge of a Petri dish, and a thin streak of saprobe fungi was inoculated opposite and at least 7 cm away from them.

The second assay tested the effect of exudates from saprobe fungi on germination of AM fungi *in vitro*. Exudates were obtained by growing the fungus in 250 ml flasks containing 125 ml of sterile AG liquid medium (Galvagno, 1976) in a shaker at 28°C. The AG medium consisted in 1 g glucose, 0.4 g asparagine, 0.05 g $MgSO_2$, 0.05 K PO_2 and 100 ml distilled water. After 72 h the culture medium was filtered through a filter paper and sterilized twice, by filtration through a 0.45 and then 0.2 µm Millipore® membrane. These exudates were added 10, 25, 50, 100 and 300 µl to 10 ml of 10 mM 2-(N-morpholin) ethane sulphonic acid (MES) buffer (pH 7) plus 0.04 g of Gel-Gro to a Petri dish. Five spores of *G. mosseae* or *Gi. rosea* were placed at the vertices of an imaginary pentagon inside the dish. In the control treatment the same volume of AG liquid medium was substituted for the exudates.

In both assays ten replications and 10 controls of each treatment were prepared, with germinating *G. mosseae* or *Gi. rosea* spores, which were placed onto the surface of each culture medium. The plates were incubated at 25°C in the dark, and sealed with Parafilm (to reduce dehydration and contamination). Spore germination was periodically examined under a light microscope for 12 days. The assays were then ended and hyphal growth from germinated spores of *G. mosseae* or *Gi. rosea* were assessed using the gridline intersect method (Marsh, 1971).

Effect of saprobe fungi on AM fungi in the rhizosphere of soybean

The experiments were carried out in 0.3 l pots filled with a grey loam soil obtained from the field of the Estación Experimental del Zaidín (Granada, Spain). The soil had a pH of 8.1 (1:1 soil:water). The levels of P, N, K, Fe, Mn, Cu and Zn were determined by methods of Lachica et al. (1965). $NaHCO_3$-extractable P was 6.2 mg kg^{-1}, N was 2.5 mg kg^{-1}, K was 132 mg kg^{-1}, Fe was 10 g kg^{-1}, Mn was 110 mg kg^{-1}, Cu was 5.8 mg kg^{-1} and Zn was 5.7 mg kg^{-1}. The soil texture was 35.8% sand, 43.6% silt, 20.5% clay and 1.8% organic matter. The soil was used either unsterilised or steam-sterilized and mixed with sterilised

perlite (Terrafertil®) at a proportion of 1:1 (V:V). Seeds of soybean (*Glycine max*) were surface-sterilised with $HgCl_2$ for 10 min and rinsed thoroughly with sterilised water and sown in moistened sterile sand. After germination, uniform seedlings were planted into the two soils and grown in a greenhouse with supplementary light provided by Sylvania incandescent and cool-white lamps (400 E 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 1/5 strength Long Ashton nutrient solution (Hewitt, 1952).

The AM inoculum consisted of 5 g of rhizosphere soil from clover plants pot culture of *G. mosseae* and *Gi. rosea*, which contained spores, mycelium and colonised root fragments. Soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of mycorrhizal plants was added to the AM non-inoculated treatment. The filtrate contained common soil microorganisms, but no propagules of AM fungi. Two different greenhouse assays were carried out. In the first assay, an aqueous suspension in sterile distilled water containing approximately 10^8 spores ml^{-1} of each saprobe fungus was prepared from cultures grown in potato dextrose agar (PDA, DIFCO®) for 1 wk at 27°C, and 2.5 ml of this suspension were used to inoculate each pot (0.3 l). In the second assay 10 and 20 ml of exudates from each saprobe fungus obtained as describe above, were added to pots.

The following treatments were tested: (1) noninoculated controls, (2) unsterilized or sterilized soils inoculated with saprobe fungi or its exudate, (3) sterilized soil inoculated with *G. mosseae* or *Gi. rosea*, and (4) sterilized soil inoculated with both saprobe fungi or its exudates and either *G. mosseae* or *Gi. rosea*. Plants were inoculated at the time of transplanting (after 2 weeks of growth). Inoculation with the saprobe fungi took place one week after inoculation with *G. mosseae* and *Gi. rosea*.

Plants were harvested after 8 weeks and dry matter weight was determined. Part of the root system was cleared and stained (Phillips and Hayman, 1970), and the percentage of root colonisation was measured (Giovannetti and Mosse, 1980). The number of effective AM fungal propagules of the pots with nonsterilized soil or sterilized soil inoculated with *G. mosseae* or *Gi. rosea* was estimated at the beginning and at the end of the experiment by the most probable number (MPN) method (Porter, 1979) using *Medicago sativa* as the test plant. To determine the MPN, five, 10 ml samples, from each pot (with spores of saprobe fungi or with 20 ml exudates) were used. From each sample, five dilutions (10^{-1}–10^{-5}) were prepared diluting 10 ml of the sample in 99 ml of sterilized soil. Each dilution was tested in five 100 ml pots. The *M. sativa* plants were harvested after 6 weeks to record the presence or absence of mycorrhizal colonization. The experiments were repeated three times, and the data are the averages of five replicate pots from one representative experiment.

Table 1. Effect of saprobe fungi on percentage of spore germination and hyphal length of *Glomus mosseae* and *Gigaspora rosea* after 15 days of growth on Gel-Gro.

Saprobe fungi	% Spore germination		Hyphal length (mm)	
	G. mosseae	*Gi. rosea*	*G. mosseae*	*Gi. rosea*
Control	87a	95a	1.9a	14.3b
A. niger	96a	100a	3.9b	26.6c
P. restrictum	94a	98a	1.9a	6.5a
T. harzianum	89a	100a	2.4a	13.6b

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

Table 2. Effect of exudates of saprobe fungi on percentage of spore germination and hyphal length of *Glomus mosseae* and *Gigaspora rosea* after 15 days of growth on Gel-Gro.

Saprobe fungi	Exudates doses (µl/ml)	% Spore germination		Hyphal length (mm)	
		G. mosseae	*Gi. rosea*	*G. mosseae*	*Gi. rosea*
Control	0	77a	98a	1.8a	24.6a
A. niger	1	92b	95a	5.3c	38.2d
A. niger	2.5	84ab	99a	3.4b	51.1e
A. niger	5	72a	97a	2.3a	48.7e
A. niger	10	69a	97a	2.8a	40.1de
A. niger	30	70a	95a	2.5a	22.7a
P. restrictum	1	78a	94a	1.7a	15.4bc
P. restrictum	2.5	80a	96a	1.9a	8.7c
P. restrictum	5	80a	95a	2.1a	6.7c
P. restrictum	10	71a	98a	2.4a	7.6c
P. restrictum	30	75a	95a	2.3a	8.6c
T. harzianum	1	74a	99a	1.9a	26.2a
T. harzianum	2.5	85ab	99a	1.8a	27.4a
T. harzianum	5	85ab	97a	2.1a	28.1a
T. harzianum	10	84ab	98a	2.1a	24.5a
T. harzianum	30	66a	96a	2.2a	25.6a

Each figure is the mean of ten replicate plates each containing 5 spores of *G. mosseae* or *Gi. rosea*. Values in the same column followed by the same letter are not significantly according to Duncan's multiple range test (P = 0.05).

Statistical analysis

The percent values were arcsine transformed to aid statistical analysis. The data obtained for percentage of spore germination, hyphal length, plant dry weight parameters, percentage of AM colonisation and the most probable number of propagules of AM fungi were subjected to ANOVA. Comparisons of means were made by the Duncan's Multiple Range Test (P = 0.05).

3. Results

The percentage of *in vitro* germination of *G. mosseae* and *Gi. rosea* spores were not affected by the presence of the saprobe fungi tested (Table 1). However, the hyphal length of *G. mosseae* and *Gi. rosea* was increased by the presence of *A. niger*. *G. mosseae* was unaffected by the presence of *T. harzianum*. *P. restrictum* decreased the hyphal length of *Gi. rosea* but had no effect on the hyphal length of *G. mosseae* (Table 1).

Table 2 shows that there were measurable effects of exudates from the saprobe fungi on the germination, *in vitro*, of *G. mosseae* spores. Effects on spores of *Gi. rosea* were largely absent, with an effect on germination only evident when exudates of *A. niger* were incorporated at 1 µl/ml, the lowest concentration. There were no effects observed when higher levels were incorporated. Influences on the growth of the hyphae were more marked. The exudate of *A. niger* applied at the dose of 1 and 2.5 µl/ml increased the length of hyphae growing from *G. mosseae* spores, but the exudates of *P. restrictum* and *T. harzianum* had no effect. The length of hyphae growing from spores of *Gi. rosea* increased in presence of 1, 2.5, 5 and 10 µl/ml of exudates of *A. niger*, and the 2.5 µl/ml level of exudates produced the highest hyphal growth. No increase of the hyphal length of *Gi. rosea* was measured in presence of 30 µl/ml of exudates of *A. niger*. The doses of 2.5, 5, 10 and 30 µl/ml of exudates of *P. restrictum* decreased the hyphal length of *Gi. rosea* spores. Exudates of *T. harzianum* did not influence the growth of hyphae from *Gi. rosea* spores.

In growth experiments in soil the presence of *A. niger* increased the shoot and root dry weight of soybean and the percentage of root length colonization by AM when grown in non-sterilized or sterilized soil inoculated with *G. mosseae* or *Gi. rosea* (Table 3). The shoot and root dry weight of soybean and the percentage of root length colonised by AM when inoculated with *Gi. rosea* decreased in the presence of *P. restrictum*.

However, the presence of *P. restrictum* did not affect the shoot and root dry weight and the percentage of root length colonised by AM when grown in non-sterilized soil or in sterilized soil inoculated with *G. mosseae*.

Table 3. Effect of saprobe fungi on shoot and root dry weights and percentage of AM root length of soybean grown in nonsterilized (native) or sterilized soil inoculated or not (control) with *G. mosseae* or *Gigaspora rosea*.

Saprobe fungi	Shoot dry weight (mg)				Root dry weight (mg)				% Root length colonization		
	Control	*G. mosseae*	*Gi. rosea*	Native	Control	*G. mosseae*	*Gi. rosea*	Native	*G. mosseae*	*Gi. rosea*	Native
Control	70a	130a	187a	100a	62a	76a	112a	50a	34a	38a	15a
A. niger	71a	192b	256c	150b	57a	142b	163c	60a	58b	62c	25b
P. restrictum	80a	131a	100b	101a	61a	82a	62b	51a	36a	22b	12a
T. harzianum	82a	143a	180a	110a	54a	102ab	133a	53a	35a	36a	13a

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P = 0.05). Native = Indigenous AM fungi in nonsterilized soil.

Table 4. Effect exudates of saprobe fungi on shoot and root dry weights of soybean grown in nonsterilized (native) or sterilized soil inoculated or not (control) with *Glomus mosseae* or *Gigaspora rosea*.

Saprobe fungi	Exudate doses (ml/pot)	Shoot dry weight (mg)				Root dry weight (mg)				% Root length colonization		
		Control	*G. mosseae*	*Gi. rosea*	Native	Control	*G. mosseae*	*Gi. rosea*	Native	*G. mosseae*	*Gi. rosea*	Native
Control	0	101a	140a	160a	130a	63a	68a	70ab	63a	32a	34a	17a
A. niger	10	102a	142a	243c	135a	51a	75ab	89 b	68a	35a	56c	20ab
A. niger	20	105a	195b	250c	150b	61a	89b	95 b	70a	53b	56c	28b
P. restrictum	10	104a	140a	100b	138a	60a	65a	52 a	60a	41a	15b	16a
P. restrictum	20	107a	146a	100b	127a	61a	67a	51 a	62a	43a	17b	12a
T. harzianum	10	101a	135a	160a	120a	70a	66a	72 ab	58a	39a	30a	15a
T. harzianum	20	104a	143a	158a	122a	75a	65a	68 ab	61a	47a	32a	18a

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P = 0.05). Native = Indigenous AM fungi in nonsterilized soil.

The presence of *T. harzianum* did not affect the dry matter and AM colonization of soybean plants grown in non-sterilized or in sterilized soil inoculated with either of the two AM fungi.

In assays with exudates of the saprobe fungi different trends were observed. Here, the shoot and root dry weight of soybean and the percentage of root length colonised by AM fungi when grown in nonsterilized or in sterilized soil inoculated with *G. mosseae* increased, but only when 20 ml/pot of exudates of *A. niger* were applied (Table 4). However, in soybean inoculated with *Gi. rosea* dry matter and AM root colonization was increased when 10 and 20 ml/pot of exudates of *A. niger* were applied. The different doses of exudates of *P. restrictum* and *T. harzianum* tested had no effect on the shoot and root dry weight or the percentage of root length colonised by AM when grown in nonsterilized or in sterilized soil inoculated with *G. mosseae*. The dry matter and the AM root length colonization by *Gi. rosea* was decreased by the application of 10 and 20 ml/pot of exudates of *P. restrictum*. Application of exudates from *T. harzianum* had no effect on the dry matter and mycorrhization of soybean inoculated with *Gi. rosea*.

Data of most probable number showed that the number of AM fungal propagules in the soil pots at the beginning of the experiment was 12 + 3 in nonsterilized soil, 28 + 6 in sterilized soil inoculated with *G. mosseae* and 22 + 8 in sterilized soil inoculated with *Gi. rosea*.

Table 5. The most probable number of propagules of AM fungi in nonsterilized or sterilized soil inoculated with *Glomus mosseae* or *Gigaspora rosea* in presence of saprobe fungi or their exudates (20 ml/pot) after soybean (*Glycine max*) culture.

Saprobe fungi	Number of propagules × 10 g^{-1} soil With saprobe fungi			With exudates of saprobe fungi		
	Nonsterilized soil	*G. mosseae*	*Gi. rosea*	Nonsterilized soil	*G. mosseae*	*Gi. rosea*
Control	8a	16a	14a	7a	16a	14a
A. niger	25b	39b	50c	22b	35b	40c
P. restrictum	8a	17a	7b	6a	17a	8b
T. harzianum	9a	17a	14a	6a	18a	15a

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P = 0.05).

At the end of the experiment the most probable number of propagules in nonsterilized or sterilized soil inoculated with *G. mosseae* or *Gi. rosea* were significantly higher than the control when *A. niger* or its exudates were applied to soil (Table 5). The application of *P. restrictum* decreased the most probable number of propagules in soil inoculated with *Gi. rosea*, but had no effect on the most probable number of propagules in nonsterilized or sterilized soil inoculated with *G. mosseae*. The most probable number of propagules in nonsterilized or sterilized soil inoculated with *G. mosseae* or *Gi. rosea* was not affected by the application of *T. harzianum* or its exudates (Table 5).

4. Discussion

The interactions between saprobe fungi and AM fungi may be important for plant growth. However, these interactions are complex, and synergistic, antagonistic or neutral relationships between microorganisms have all been found (Gryndler, 2000).

Different effects of saprobe fungi on AM fungi and colonisation have been observed in our experiments. *A. niger* stimulated the spore germination and hyphal length of AM fungi and AM colonization of plants. In contrast, *T. harzianum* had no effect on the different AM fungi tested. However, *P. restrictum* inhibited spore germination, hyphal length and AM colonization of soybean by *Gi. rosea* but, to by *G. mosseae* or the indigenous soil endophytes. Effects of these saprobe fungi on AM symbiosis have been reported previously. However, effects of these fungi have varied. McAllister et al. (1995) for example, reported antagonistic action of other *A. niger* strains on *G. mosseae* and other authors reported both synergistic and antagonistic action of other *T. harzianum* strains on AM fungi (Fracchia et al., 1998; Rousseau et al., 1996).

In contrast to our data beneficial effects of other *Penicillium* strains on AM colonization of plants (Kucey, 1987; Omar, 1998) have been reported. The reasons for these differences are not understood but one mechanism that could be implicated in this variability is differences in sensitivities of the different AM fungi used in the studies. Evidence to support this possibility has been presented here. In our experiments *G. mosseae* and *Gi. rosea* had similar sensitivity either to *A. niger* or to *T. harzianum*. However, *P. restrictum* inhibited the development of *Gi. rosea* but had no effect on *G. mosseae* or indigenous soil endophytes. Other important factors implicated in the effect of saprobe fungi on AM fungi are the exudates produced by the saprobe fungi (McAllister et al., 1994; 1995).

In our experiments we observed that exudates of *P. restrictum* and *T. harzianum* behaved similarly with regard to their action on spore germination and hyphal length of *G. mosseae* or *Gi. rosea* and on AM colonization of plants.

Nevertheless, the effect of the exudates of *A. niger* changed according to the concentration and the species of AM fungi. In fact, the application of 1–2.5 µl/ml of exudates of *A. niger* increased the hyphal length from *G. mosseae* spores but 1–10 µl/ml of exudates of this saprobe fungus were able to increase the hyphal length of *Gi. rosea*. Differences in sensitivity of AM colonization of soybean by *G. mosseae*, *Gi. rosea* or the AM indigenous endophytes to the application of exudates of *A. niger* were also found.

These results suggest that differences in the sensitivity of AM fungi to the exudates of saprobe fungi exist and are probably important. Moreover, the quantity of exudates produced by saprobe fungi may be also important. The similarity in trends observed in the effects of the saprobe fungi or their exudates on spore germination and growth of hyphae and effects of on AM colonization of plants and number of propagules of AM fungi reinforce the suggestion that the effects of saprobe fungi are on the external soil phase of the AM fungi (Fracchia et al., 1998; García-Romera et al., 1998).

In this study, *A. niger* or its exudates increased the infectivity and effectiveness of either indigenous AM endophytes or *G. mosseae* or *Gi. rosea* on the growth of soybeans plants. These data suggest that *A. niger* might be exploited to improve the colonization of some AM inoculated fungi, especially in plant nurseries or in degraded soils. However, one of the most important limitations in the use of AM fungi for field crops in agriculture is the impossibility of culturing the AM fungus in the absence of plant roots. The beneficial effect of application of *A. niger* or its exudates on root colonization by indigenous endophytes, on the number of its propagules and its effect on plant growth indicates the possibility of using this microorganism to increase the population and effectiveness of indigenous AM fungi. This could be of special importance for the restoration of degraded soils, which are often of low fertility and where the population of AM fungi is low (Godeas et al., 1999).

The role of saprobe fungi on AM symbiosis is complex and further investigation of the mechanisms through witch saprobe fungi act on AM fungi is necessary. Further studies should investigate the mechanisms of the nature and role of exudates produced by saprobe fungi on AM fungi. They should also consider the effects of exudates produced by or induced in the presence of AM fungi. These have been shown to have effects on the development of plant pathogens in the rhizosphere and could also affect the development of the saprobe fungi (Norman and Hooker, 2000).

Acknowledgements

Financial support of this study was provided by the Comision Interministerial de Ciencia y Tecnologia, Spain and by the University of Buenos Aires (Grant X028) Argentina.

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