

ORIGINAL PAPER

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Interaction between *Alternaria alternata* or *Fusarium equiseti* and *Glomus mosseae* and its effects on plant growth

Received: 16 January 1996

Abstract The effect of inoculation with the saprophytic fungi *Alternaria alternata* or *Fusarium equiseti* on maize (*Zea mays*) and lettuce (*Lactuca sativa*) with or without arbuscular mycorrhizal (AM) colonization by *Glomus mosseae* was studied in a greenhouse trial. Plant dry weights of non-AM-inoculated maize and lettuce were unaffected by the presence of *A. alternata* and *F. equiseti*. In contrast, *A. alternata* and *F. equiseti* decreased plant dry weights and mycorrhization when inoculated to the rhizosphere before *G. mosseae*. The saprophytic fungi inoculated 2 weeks after *G. mosseae* did not affect the percentage of root length colonized by the AM endophyte, but did affect its metabolic activity assessed as succinate dehydrogenase activity. Although *F. equiseti* inoculated at the same time as *G. mosseae* did not affect mycorrhization of maize roots, its effect on AM colonization of lettuce roots was similar to that with *A. alternata*. In the rhizosphere of both plants, the population of saprophytic fungi decreased significantly, but was not affected by the presence of *G. mosseae*. Our results suggest that there may have been a direct effect of the saprophytic fungi on the mycorrhizal fungi in the extramatrical phase of the latter, and when the AM fungus was established in the root the AM fungus was less affected by the saprophytic fungi.

Key words *Alternaria alternata* · Arbuscular mycorrhizas · *Fusarium equiseti* · *Glomus mosseae* · *Lactuca sativa* · Lettuce · Maize · Saprophytic fungi · *Zea mays* ·

Introduction

The beneficial effects of arbuscular mycorrhizal (AM) fungi on plant growth depend in part on the members of the symbiosis and their interactions with other organisms present in the rhizosphere (Ocampo 1993). Previous studies showed that *Alternaria alternata* and *Fusarium equiseti* produced soluble and volatile substances which were able to inhibit the germination of spores of *Glomus mosseae*, although these saprophytic fungi did not reduce hyphal length of the AM fungus (McAllister et al. 1996). However, inoculation of *A. alternata* and *F. equiseti* at the same time as spores of *G. mosseae* significantly decreased the percentage of the root length of maize plants colonized by AM, but no effect on AM colonization was observed when these saprophytic fungi were inoculated 2 weeks after *G. mosseae* (McAllister et al. 1996). The inhibition of root mycorrhization by saprophytic fungi may be due not only to a direct interaction between the two fungi, but also to induction of plant defense mechanisms, which can differ widely depending on the type of plant (Wyss et al. 1992). The results of research on the interaction between soil microorganisms and AM also differ widely depending on the substrate (Caron et al. 1985) and on the time of inoculation of one microorganism with respect to the other (McAllister et al. 1994).

Our study was designed to obtain more detailed knowledge of the interactions between *A. alternata* or *F. equiseti* and *G. mosseae* inoculated at different times in soil pots with lettuce and maize plants.

Materials and methods

Plants were grown in 300-ml-capacity open pots of soil collected from the Province of Granada, Spain. The soil was a calcixerollic xerochrept type, pH 7.6 (for full details see Garcia-Romera and Ocampo 1988). It was steam sterilized and mixed with sterilized quartz sand in the proportions of 1:1 (V:V). Maize (*Zea mays* cv. Calderon) and lettuce (*Lactuca sativa* cv. Romana) were used as test plants. Seeds were sown in moistened sand, and after 2 weeks seedlings were transplanted to the pots and grown under greenhouse con-

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Table 1 Plant dry weight (shoot and root) of maize (*Z. mays*) and lettuce (*L. sativa*) in the presence or in the absence of *G. mosseae*, and inoculated or non-inoculated with *A. alternata* or *F. equiseti*

Inoculation time with saprophytes	Treatments	Dry weight (mg)			
		Maize		Lettuce	
		Shoot	Root	Shoot	Root
Two weeks before <i>G. mosseae</i>	C	690 a	570 a	381 a	314 a
	Aa	698 a	546 a	483 a	312 a
	Fe	650 a	520 a	482 a	502 a
	M	1095 b	690 a	782 b	493 a
	M+Aa	730 a	635 a	464 a	313 a
	M+Fe	720 a	590 a	343 a	395 a
At same time as <i>G. mosseae</i>	C	690 a	570 a	382 a	312 a
	Aa	695 a	587 a	427 a	423 a
	Fs	713 a	637 a	426 a	421 a
	M	1095 b	690 a	783 b	490 a
	M+Aa	870 c	580 a	509 a	307 a
	M+Fe	890 c	620 a	483 a	466 a
Two weeks after <i>G. mosseae</i>	C	658 a	592 a	462 a	416 a
	Aa	710 a	578 a	422 a	377 a
	Fe	730 a	640 a	403 a	382 a
	M	1116 b	705 a	866 b	480 a
	M+Aa	980 b	620 a	745 b	501 a
	M+Fe	1010 b	740 a	682 b	553 a

C control, Aa plants inoculated with *A. alternata*, Fe plants inoculated with *F. equiseti*, M plants inoculated with *G. mosseae*. Each value is the mean for five pots. Column values followed by the same letter are not significantly different according to orthogonal contrast comparison ($P=0.05$)

ditions. Natural light was supplemented by Sylvania incandescent and cool-white lamps, $400 \text{ nmol m}^{-2} \text{ s}^{-1}$, 400–700 nm; with a 16–8 h light-dark cycle at 25–19°C and around 50% relative humidity. Plants were watered from below by capillarity, and fed with a nutrient solution (Hewitt 1952) lacking phosphate for AM-inoculated plants.

The AM inoculum consisted of 5 g rhizosphere soil from alfalfa plant pot culture of a Rothamsted isolate of *G. mosseae* (Nicol. & Gerd.) Gerd. and Trappe (BEG No. 12), which contained spores ($15 \text{ sporocarps g}^{-1}$ with 1–5 spores sporocarp⁻¹), mycelium and colonized root fragments. 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 *G. mosseae*.

The saprophytic fungi *Alternaria alternata* (Joly 1964) and *Fusarium equiseti* (Booth 1977) were obtained from the rhizosphere and rhizoplane of maize plants by the particle-washing method (Widden and Bisset 1973). An aqueous suspension in sterile distilled water containing approximately 2×10^3 spores ml⁻¹ of each saprophytic fungus was prepared from cultures grown in potato dextrose agar (PDA, Difco) for 1 week at 27°C.

Six treatments were used in all experiments: (1) noninoculated controls, (2) inoculated with *A. alternata* or *F. equiseti*, (3) inoculated with *G. mosseae*, and (4) inoculated with both *G. mosseae* and either *A. alternata* or *F. equiseti*. Plants were inoculated with AM fungus at the time of transplanting or after 2 weeks of growth. The saprophytic fungi were inoculated 2 weeks before, at the same time as, or 2 weeks after *G. mosseae*.

To evaluate the population of inoculated *A. alternata* or *F. equiseti* during the experiments, rhizosphere soils were sampled after 0, 3, 7 and 12 weeks, as described by Garcia-Garrido and Ocampo (1988). This soil was replaced by additional autoclaved soil. About 1.5 g rhizosphere soil was taken from each of the experimental pots, then ten fold aqueous dilution series (from 10^{-1} to 10^{-4}) were prepared for each sample. The number of saprophytic colony-forming units (CFUs) in suitable dilutions of such samples, taken from the five replicate pots of each treatment, were counted on PDA medium. Rhizosphere soil was quantified as follows: soil from dilutions of 10^{-1} and 10^{-2} was recovered, dried at 105°C and weighed. The number of CFUs was expressed per gram of dry rhizosphere soil.

Plants were harvested after 12 weeks and dry matter weight was determined. After the plants were harvested, the roots were carefully washed free from soil and the root system in each of the five replicates per treatment was divided into two portions to record the following: (1) mycorrhizal root length: Part of the root system was cleared and stained (Phillips and Hayman 1970), and the percentage of root colonization was measured as described by Ocampo et al. (1980); (2) mycorrhizal fungus with succinate dehydrogenase (SDH) activity (E.C. 1.3.99.1) was detected in the fungal mycelium by the reduction of tetrazolium salts at the expense of added succinate (MacDonald and Lewis 1978), and the percentage of AM mycelium in root with SDH activity was measured under a compound microscope (Ocampo and Barea 1985).

The 2 (plants) x 2 (AM inoculation) x 3 (saprophytic inoculation time) factorial experiment design was completely randomized. The data were analyzed by one-way analysis of variance. The orthogonal contrast comparisons of means are given.

Results

Plant dry weights of maize and lettuce were not affected by the presence of *A. alternata* or *F. equiseti* inoculated alone; however, *G. mosseae* increased shoot dry weight of both plants (Table 1). When *A. alternata* and *F. equiseti* were inoculated 2 weeks after *G. mosseae*, shoot dry weights of maize and lettuce plants were similar to those in plants inoculated with *G. mosseae* alone. However, when *A. alternata* and *F. equiseti* were inoculated 2 weeks before *G. mosseae*, shoot dry weights of maize and lettuce plants were similar to those in non-AM-inoculated controls. When *A. alternata* and *F. equiseti* were inoculated at the same time as *G. mosseae*, shoot dry weights of maize plants were significantly higher than in plants inoculated with the saprophytic fungi alone. When *F. equiseti* was in-

Table 2 Percentage of AM root length and percentage of AM fungus-mycelium (in root) with SDH in maize (*Z. mays*) and lettuce (*L. sativa*) plants in the presence of *G. mosseae* and inoculated or not with *A. alternata* or *F. equiseti* at different times

Inoculation time with saprophytes	Treatments	Root length colonization (%)		% AMF mycelium with SDH activity	
		Maize	Lettuce	Maize	Lettuce
Two weeks before <i>G. mosseae</i>	M	40 a	84 a	78 a	86 a
	M+Aa	4 c	31 b	29 b	26 c
	M+Fe	17 b	29 b	36 b	29 c
At same time as <i>G. mosseae</i>	M	40 a	84 a	77 a	86 a
	M+Aa	25 b	43 b	35 b	29 c
	M+Fe	39 a	21 b	64 a	50 b
Two weeks after <i>G. mosseae</i>	M	38 a	76 a	72 a	80 a
	M+Aa	33 a	80 a	33 b	45 b
	M+Fe	30 a	69 a	66 a	48 b

See note to Table 1 for explanation of abbreviations and significance values

oculated before or at the same time as *G. mosseae*, shoot dry weight of lettuce plants was significantly lower than in plants inoculated with *G. mosseae* alone.

No significant effects of *A. alternata* and *F. equiseti* on the level of AM colonization were observed when the saprophytic fungi were inoculated 2 weeks after *G. mosseae*. *A. alternata* decreased the percentage of AM root length colonization of maize and lettuce plants when the saprophytic fungus was inoculated before or at the same time as *G. mosseae*. *A. alternata* decreased the percentage of AM fungal mycelium with SDH activity in all the treatments tested (Table 2).

The percentage of root length of maize plants colonized by *G. mosseae* and the percentage of AM fungal mycelium with SDH activity were unaffected by the presence of *F. equiseti* inoculated at the same time as *G. mosseae*, but in lettuce there was a decrease in both parameters. However, *F. equiseti* decreased both the percentage of AM root colonization in maize and lettuce plants and the percentage of AM fungal mycelium with SDH activity when this saprophytic fungus was inoculated before *G. mosseae*. *F. equiseti* decreased the percentage of AM fungal mycelium with SDH activity in lettuce plants when this saprophytic fungus was inoculated 2 weeks after *G. mosseae* (Table 2).

The number of CFUs of saprophytic fungi g^{-1} rhizosphere soil decreased throughout the experiments. The populations of *A. alternata* and *F. equiseti* in the rhizosphere of maize and lettuce were not affected by the presence of *G. mosseae* in any of the treatments tested (Table 3).

Discussion

Studies of the interaction between AM and rhizosphere microorganisms have shown the qualitative effect of AM fungi on the population of these microorganisms (Meyer and Linderman 1986). Synergistic and antagonistic interactions between AM and saprophytic fungi were observed (McAllister et al. 1994; Tarafdar and Marschner 1995).

The negative effect of *A. alternata* and *F. equiseti* on the development of *G. mosseae* when the saprophytic fungi were inoculated before or at the same time as the arbuscular endophyte suggests that there may have been a direct interaction between the mycorrhizal and the saprophytic fungi in the extramatrical phase of the former. Similar interactions have been proposed for other microorganisms (Caron et al. 1985; Garcia-Garrido and Ocampo 1988, McAllister et al. 1994). However, when *G. mosseae* was inoculated 2 weeks before the saprophytic fungi, i.e., when the AM fungus mycelium was developed in the rhizosphere or when the AM fungus was established in the root, the AM fungus was less affected by the saprophytic fungi. *A. alternata* and *F. equiseti* inhibited the germination of spores of *G. mosseae* but did not reduce the hyphal length of the arbuscular fungus (McAllister et al. 1996). This may be one of the reasons why the saprophytic fungi inhibited mycorrhization of plants when *G. mosseae* was inoculated after or at the same time as the saprophytic fungi, whereas no inhibition was observed when the saprophytic fungi were inoculated after *G. mosseae*. On the other hand, in previously AM colonized plants the endophyte was more resistant to the action of pathogenic microorganisms (Ocampo 1993). However, despite its advantageous location in the root, the endophyte may be influenced by soil microorganisms (Linderman 1988). The saprophytic fungus not only affected percentage root length colonized by *G. mosseae* but also affected metabolic activity (assessed as SDH activity), as was observed in other saprophytic fungi (McAllister et al. 1994). The decrease in metabolic activity was accompanied by the formation of septae in the intraradical hypha (results not shown). This effect has also been observed when mycorrhizal plants were subjected to stress situations (Kinden and Brown 1975).

A. alternata inhibits *G. mosseae* both in plants cultivated in tubes inoculated with surface-sterilized spores (McAllister et al. 1996) and in pots where the inoculum consisted of spores, mycelia and pieces of colonized root. These results suggest that the effect of *A. alternata* on *G. mosseae* was independent of the plant, the growth medium

Table 3 Colony-forming units (CFU) of *A. alternata* or *F. equiseti* from the rhizosphere (g^{-1} dry weight soil) of maize (*Zea mays*) and lettuce (*Lactuca sativa*) plants inoculated or not with *Glomus mosseae* at different times

Inoculation time with saprophytes	Treatments	CFU $\times 10^6$ g $^{-1}$ soil after (weeks)			
		0	3	7	12
Maize					
Two weeks before <i>G. mosseae</i>	Aa	108 a	67 a	30 a	21 a
	Fe	116 a	48 a	35 a	30 a
	M+Aa	118 a	69 a	27 a	19 a
	M+Fe	110 a	39 a	40 a	29 a
At same time as <i>G. mosseae</i>	Aa	110 a	72 a	30 a	17 a
	Fe	112 a	51 a	31 a	21 a
	M+Aa	100 a	63 a	39 a	16 a
	M+Fe	116 a	34 a	26 a	31 a
Two weeks after <i>G. mosseae</i>	Aa	110 a	78 a	34 a	22 a
	Fe	120 a	37 a	35 a	29 a
	M+Aa	126 a	67 a	31 a	20 a
	M+Fe	110 a	41 a	28 a	23 a
Lettuce					
Two weeks before <i>G. mosseae</i>	Aa	98 a	64 a	27 a	18 a
	Fe	114 a	84 a	47 a	20 a
	M+Aa	102 a	66 a	24 a	16 a
	M+Fe	118 a	70 a	36 a	23 a
At same time as <i>G. mosseae</i>	Aa	94 a	70 a	29 a	15 a
	Fe	122 a	82 a	40 a	24 a
	M+Aa	94 a	61 a	37 a	14 a
	M+Fe	109 a	76 a	36 a	21 a
Two weeks after <i>G. mosseae</i>	Aa	96 a	76 a	32 a	20 a
	Fe	112 a	75 a	43 a	27 a
	M+Aa	100 a	65 a	29 a	18 a
	M+Fe	120 a	67 a	33 a	15 a

See note to Table 1 for explanation of abbreviations and significance values

and the kind of *G. mosseae* inoculum used. However, *F. equiseti* seems to affect mycorrhization in a very different way depending on the host plant considered, the substrate where the plant was grown, or the kind of inoculum used; similar findings have been reported for plant pathogens and other biotic factors (El Atrach et al. 1989; Ocampo 1993). In fact, whereas *F. equiseti* had no effect on maize root mycorrhization by *G. mosseae* when both microorganisms were inoculated at the same time, its effect on AM colonization of lettuce roots was similar to that observed with *A. alternata*. *F. equiseti* decreased maize root colonization when the plant was grown in sand-vermiculite (McAllister et al. 1996), but not in soil.

In contrast with other saprophytic fungi (McAllister et al. 1994), neither the population of *A. alternata* nor that of *F. equiseti* was affected by the presence of *G. mosseae*. However, the population of *A. alternata* decreased when the saprophyte was inoculated to the rhizosphere of maize grown in sand: vermiculite medium 2 weeks after *G. mosseae* (McAllister et al. 1996). The plant growth medium may have some influence on this effect, as has been observed with other AM-microorganism interactions (Ocampo 1993).

The variability of effects of *F. equiseti* on mycorrhization will be investigated with different AM inocula, soils and plants.

Acknowledgements The authors thank Karen Shashok for revising the English translation of the text. Financial support for this study was provided by the Comision Interministerial de Ciencia y Tecnologia, Spain, and by the European Union (PVD Program).

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