

# Interaction between *Aspergillus niger* van Tiegh. and *Glomus mosseae*. (Nicol. & Gerd.) Gerd. & Trappe

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(Received 29 March 1994; accepted 14 October 1994)

## SUMMARY

Percent germination and length of hyphae of germinated *Glomus mosseae* spores, cultivated on water agar, decreased significantly in the presence of *Aspergillus niger*; this decrease was independent of any change in pH of the medium. Soluble and volatile compounds produced by *A. niger* significantly decreased percentage spore germination and the hyphal length of *G. mosseae* on water agar. The decrease caused by volatile compounds was significantly greater when *A. niger* was grown on malt extract agar. Shoot dry weights of maize and lettuce plants cultivated in soil in pots, and percentage arbuscular mycorrhizal (AM) root colonization of plants grown either in sand: vermiculite tubes inoculated with *G. mosseae* spores or in soil in pots with soil inoculum, were unaffected by *A. niger* when this saprobe was inoculated 2 wk after *G. mosseae*. Shoot dry weights and percentage AM colonization of plants decreased when the saprobic fungus was inoculated at the same time or 2 wk before *G. mosseae*. However, the metabolic activity resulting from AM colonization, measured as the percentage of mycelium showing succinate dehydrogenase activity, decreased in all treatments. The population of *A. niger* decreased when inoculated to the rhizosphere of plants at the same time as, or 2 wk after, *G. mosseae*, but not when it was inoculated 2 wk before *G. mosseae*. Our results show that *G. mosseae* decreases the saprobic fungal population through its effect on the plant, whereas *A. niger*, by the production of soluble or volatile substances, inhibits *G. mosseae* in its extramatrical stage.

Key words: Arbuscular mycorrhiza, *Aspergillus niger*, *Glomus mosseae*, saprobic fungus.

## INTRODUCTION

Arbuscular mycorrhizal (AM) symbioses are widespread throughout the vegetable kingdom. They may benefit the host plant primarily by increasing the capability of the root system to absorb and translocate phosphorus through an extensive network of external hyphae (Hayman, 1983). As the arbuscular fungi are partly inside and partly outside the host, external factors such as the presence of soil microorganisms affect the development of the symbiosis. Fungi are frequent and customary inhabitants of the soil rhizosphere. Most studies to date have dealt with interactions with pathogenic fungi (Bagyaraj, 1984; Ocampo, 1993), whereas few studies have investigated interactions with non-pathogenic organisms such as saprobic fungi (Paget, 1975; Calvet, Barea & Pera 1992, 1993). These fungi are important because of the large amount of microbial biomass they supply to soil, and because of their role in litter

decomposition. The saprobe *Aspergillus niger* van Tiegh., a widespread fungus in the rhizosphere of vegetable crops, is known to produce lytic enzymes (Hattori, 1973) and antibiotics (Krasilnikov & Korenyako, 1945; Miller, 1962).

The purpose of this study was to determine the relationships between *G. mosseae* and *A. niger* and to examine some of the possible mechanisms involved in such interactions.

## MATERIALS AND METHODS

### Expt 1. Isolation of saprobic fungi

The active fungi present in the rhizosphere soil and roots were isolated by the particle washing method (Widden & Bisset, 1972) using a multichamber washing apparatus. Thirty washings were necessary to remove fungal sclerotia, spores, etc., from the soil particles and roots of maize. Twenty soil particles (2 mm diam.) were dried on sterilized filter paper

and planted in 2% malt extract agar (MEA) with antibiotics ( $5 \mu\text{g l}^{-1}$  streptomycin and  $10 \mu\text{g l}^{-1}$  tetracycline). From the resulting colonies, *A. niger* (Raper & Fennell, 1965) was selected and transferred to tubes of potato dextrose agar (PDA) and 2% MEA at 4 °C as stock cultures.

*Expt 2. Effect of G. mosseae on germination of A. niger*

The effect of *G. mosseae* on germination of *A. niger* *in vitro* was tested on 1% sterile water agar, with pH adjusted to 7 with 10% KOH. Sporocarps of *G. mosseae* were isolated by wet sieving the soil (Gerdemann, 1955) from alfalfa plant pot cultures of an isolate of *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe. Spores were obtained by dissecting the sporocarps, stored in water at 4 °C, and used within 1 month. Spores of *G. mosseae* were surface-sterilized as described by Mosse (1962). Twenty-five spores per plate were placed around the perimeter of the Petri dish and a thin streak with spores and mycelium of the saprobic fungi to be assayed was inoculated in the centre. Ten replicates were used and plates with the saprobic fungus alone were used as controls. The plates were sealed to reduce dehydration and contamination, and were kept in the dark at 25 °C. The diameter of the colony of the saprobe was examined at intervals over 15 d.

*Expt 3. Effect of A. niger on development of G. mosseae*

The effect of *A. niger* on spore germination and hyphal length of *G. mosseae* was tested in four different experiments conducted in 9 cm diameter plastic Petri dishes. In the first experiment (Expt 3a) the effect of *A. niger* on spore germination and mycelial length *in vitro* was tested on 1% water agar, with pH adjusted to 7. Five surface-sterilized spores per plate were placed 1 cm from the edge of a Petri dish, and a thin streak of *A. niger* was inoculated opposite and at least 7 cm away from them.

The second experiment (Expt 3b) was designed to test whether the effect of the saprobic fungus on spore germination of *G. mosseae* was an indirect effect due to pH modification caused by the saprobic fungi. Water agar, 1%, was made with 10 mM 2-(N-morpholin) ethane sulfonic acid (MES). This acted to maintain the pH of the medium at 7 through the duration of the experiment. This buffer was previously shown not to affect germination of AM fungal spores *in vitro* (Carr & Hinkley, 1985; Calvet *et al.*, 1992). *G. mosseae* spores and *A. niger* inoculum were placed on water agar with MES, as described before.

The third experiment (Expt 3c) tested the effect of exudates from *A. niger* on germination and hyphal length of the AM fungus *in vitro*. Exudates were

obtained by growing the fungus in 250 ml flasks containing 125 ml of sterile PDA liquid medium in a shaker at 28 °C. After 72 h the culture medium was filtered through a disk of filter paper and sterilized twice by filtration through a  $0.45 \mu\text{m}$  Millipore membrane. These exudates were added, 2 ml to 10 ml of 1% water agar (pH 7), to a Petri dish. Five spores of *G. mosseae* were placed at the vertices of an imaginary pentagon inside the dish. In the control treatment the same volume of sterile distilled water was substituted for the exudates.

In the fourth experiment (Expt 3d) the effect of volatile compounds released by *A. niger* on germination and hyphal length of *G. mosseae* spores was tested in divided plastic Petri dishes. In an initial assay the dishes contained 1% water agar (pH 7) on both sides. On one side, five AM fungus spores were placed near the edge of the plate, and *A. niger* was inoculated on the other side. In a second assay the plates contained 1% water agar (pH 7) on one side and MEA medium on the other. Five AM spores were placed on the water agar, and the saprobic fungus was inoculated on the nutrient agar.

In each of the four experiments, ten replicates were used. The plates were incubated at 25 °C in the dark, and were sealed to reduce dehydration and contamination. Spore germination rate was periodically examined under a light microscope for 16 d, at the end of which the experiment was concluded and hyphal length of the germinated *G. mosseae* spores was assessed using the gridline intersect method (Marsh, 1971).

*Expt 4. Effect of mycorrhizal and non-mycorrhizal root extracts on development of A. niger*

Root extracts of mycorrhizal and non-mycorrhizal plants of maize and lettuce were obtained by two different procedures: (1) roots (2 g) were ground with a mortar in 3 ml Tris-HCl buffer (0.1 M, pH 7), filtered and centrifuged at 20000 g for 10 min. The supernatant was used as root extracts, (2) roots (2 g) were ground in 25 ml 70% methanol using a mortar, and centrifuged at 10000 g for 10 min. The supernatant was dried in vacuum in a rotary evaporator at 40 °C and redissolved in 2 ml 100% methanol. After evaporation to dryness, the extract was again redissolved in 2 ml 100% methanol.

*A. niger* was grown in a Petri dish containing 20 ml of ten-fold diluted PDA. When the colony was about 3 cm diam., five pits, each of 5 mm diam., were carved in the PDA medium around the colony about 5 mm from its edge. 40  $\mu\text{l}$  of each root extract was poured into the pits, and the fifth pit was filled with Tris-HCl buffer, or methanol as a control.

Plates were incubated 48 h at 28 °C, and the appearance of halos of inhibition of the saprobic colony around the pits was observed. There were five replicates for each extractant.

*Expt 5. Interaction between A. niger and G. mosseae, inoculated as spores, in the rhizosphere of maize under sterile conditions*

This experiment was performed in 20 × 200 mm glass tubes filled with 25 g of a sand/vermiculite mixture (1:1, V/V) and 12 ml diluted (1/2) nutrient solution (Hewitt, 1952) plus 50 µg ml<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub> (pH 7). The tubes were closed with cotton wool and autoclaved at 120 °C for 20 min. In each tube, 30 surface-sterilized spores of *G. mosseae* were placed under the seedlings. Seeds of maize (*Zea mais* cv. Calderon) were surface-sterilized with HgCl<sub>2</sub> for 10 min and thoroughly rinsed with sterilized water. After germination, seedlings were selected for uniformity before planting. Plants were grown in a chamber with supplementary light provided by Sylvania incandescent and cool-white lamps, 400 µE m<sup>-2</sup> s<sup>-1</sup>, 400–700 nm, with a 16/8 h day/night cycle at 25/19 °C and 50% r.h.

*A. niger* was obtained from the rhizosphere and rhizoplane of maize plants as described before. An aqueous suspension in sterile distilled water, containing approx. 2 × 10<sup>8</sup> spores ml<sup>-1</sup>, was prepared from cultures grown in PDA for 1 wk at 27 °C.

Four treatments were used in all experiments: (1) uninoculated controls, (2) inoculated with *A. niger*, (3) inoculated with *G. mosseae*, and (4) inoculated with both *G. mosseae* and *A. niger*. Plants were inoculated at the time of transplanting or after 2 wk of growth. The saprobic fungus was inoculated at the same time and 2 wk after *G. mosseae*.

To evaluate the population of inoculated *A. niger* during the experiments, 1.5 g of sand:vermiculite from five replicate tubes was collected 5 d and 10 wk after the saprobic fungus was inoculated, and the number of saprobic colony forming units (CFU) was counted as described by (Garcia-Garrido & Ocampo, 1988). Ten-fold serial dilutions were prepared for each sample. The CFU in suitable dilutions of such samples were counted on PDA medium. Sand:vermiculite samples were quantified as follows: sand:vermiculite from 10<sup>-1</sup> and 10<sup>-2</sup> dilutions were recovered, dried at 105 °C and weighed. The number of CFU was expressed per g of dry sand:vermiculite. Plants were harvested after 10 wk and shoot dry matter was weighed. After harvesting, the root system in each of the five replicates per treatment was cleared and stained (Phillips & Hayman, 1970) and percentage root colonization was determined as described by Ocampo, Martin & Hayman (1980).

*Expt 6. Interaction between G. mosseae and A. niger in the rhizosphere of maize and lettuce grown in soil pots*

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 & Ocampo, 1988). It was steam-sterilized and mixed 1:1 (V/V) with sterilized quartz sand. Maize and lettuce (*Lactuca sativa* cv. Romana) were used as test plants. Seeds were sown in moistened sand and after 2 wk seedlings were transplanted to the pots and grown under greenhouse conditions with temperature and humidity as described above. Natural light was supplemented by Sylvania incandescent and cool-white lamps as above. Plants were watered from below with a capillary system, and fed with a nutrient solution (Hewitt, 1952) lacking phosphate for AM-inoculated plants.

The arbuscular inoculum consisted of 5 g of rhizosphere soil from alfalfa plant pot cultures of an isolate of *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe which contained spores (10 sporocarps g<sup>-1</sup>, with 1–5 spores per sporocarp), mycelia and colonized root fragments. Uninoculated plants were given filtered leachings from the inoculum soil. Soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of mycorrhizal plants was added to the AM uninoculated treatment. The filtrate contained common soil microorganisms, including bacteria and fungi, but no propagules of AM fungi.

*A. niger* was obtained from the rhizosphere and rhizoplane of maize plants as described before. An aqueous suspension in sterile distilled water containing approximately 2 × 10<sup>8</sup> spores ml<sup>-1</sup>, was prepared from cultures grown in PDA for 1 wk at 27 °C.

Four treatments were used in all experiments: (1) uninoculated controls, (2) inoculated with *A. niger*, (3) inoculated with *G. mosseae*, and (4) inoculated with both *G. mosseae* and *A. niger*. Plants were inoculated at the time of transplanting or after 2 wk growth. The saprobic fungus was inoculated 2 wk before, at the same time as, or 2 wk after *G. mosseae*.

To evaluate the population of inoculated *A. niger* during the experiments, rhizosphere soils were sampled after 0, 3, 7 and 12 wk, and the number of CFU were counted as described before. The number of CFU was expressed per g of dry rhizosphere soil.

Plants were harvested after 10 wk and the dry matter was weighed. After the harvest, the root system in each of the five replicates per treatment was divided into two portions. In the first, mycorrhizal root length was measured; part of the root system was cleared and stained (Phillips & Hayman 1970) and the percentage of root colonization was determined as described by Ocampo *et al.* (1980). In the second, succinate dehydrogenase (EC 1.3.99.1) (SDH) activity was measured in fungal mycelia by the reduction of tetrazolium salts at the expense of added succinate (MacDonald & Lewis, 1978); the percentage of AM mycelia with SDH activity was determined under a compound microscope (Ocampo & Barea, 1985).

### Statistics

Data were analyzed by a one-way analysis of variance. The Duncan multiple range test ( $P \leq 0.05$ ) and the standard errors of means are given.

### RESULTS

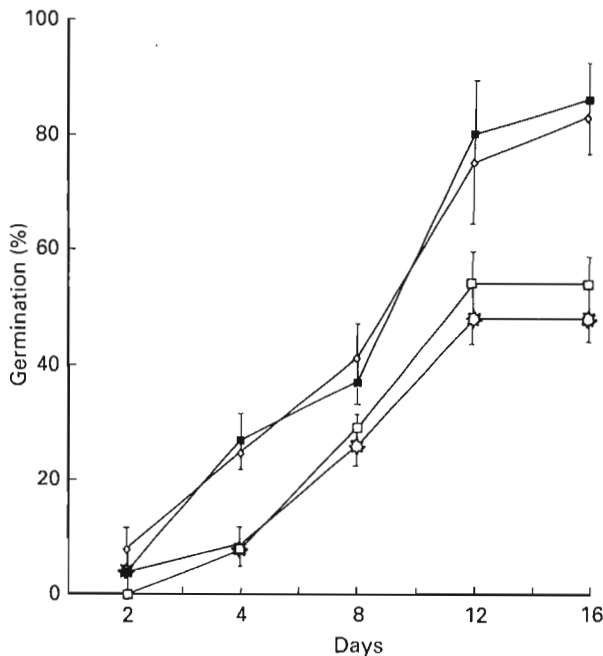
Of the 109 fungal strains isolated from the rhizosphere and rhizoplane of maize, 33% were identified as *A. niger*. This saprobic fungus was not pathogenic to maize or lettuce plants, even when both plants were inoculated with high doses of fungal conidia (data not shown).

Germinated spores of *G. mosseae* did not affect the growth of *A. niger* cultivated on water-agar in Petri dishes (data not shown).

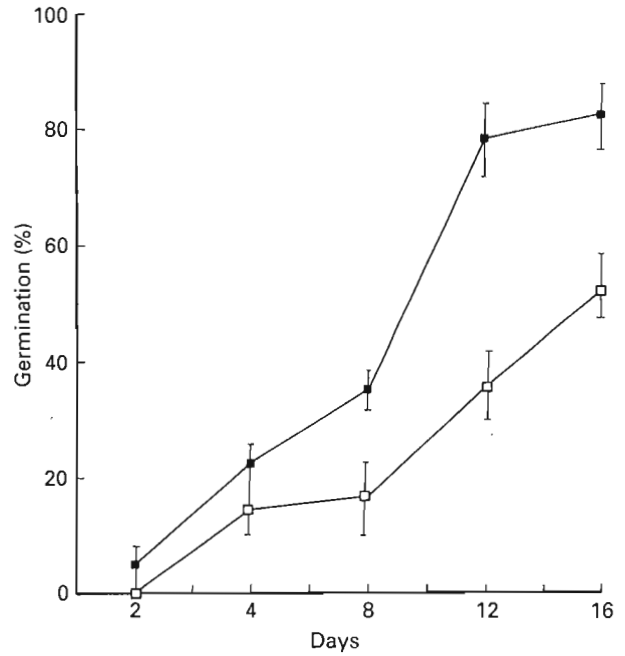
Percent germination of *G. mosseae* spores on water-agar decreased significantly in the presence of *A. niger* (Fig. 1). Similar results were observed for hyphal length (control =  $6.3 \pm 1$  mm; control plus *A. niger* =  $3.1 \pm 0.2$  mm). The saprobic fungus had a similar effect on percentage spore germination (Fig. 1) and hyphal length (control =  $6.1 \pm 0.6$  mm; control plus *A. niger* =  $3.0 \pm 0.2$  mm) of *G. mosseae* when both were grown on water agar buffered with MES.

The exudates of *A. niger* in water agar significantly decreased percentage spore germination (Fig. 2) and hyphal length of *G. mosseae* (control =  $6.6 \pm 1$  mm; plus exudates =  $4.5 \pm 0.5$  mm).

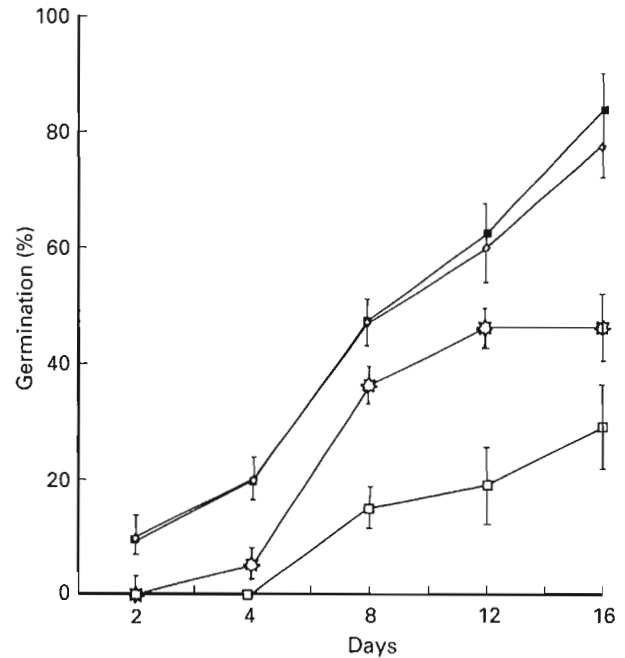
Volatile compounds produced by *A. niger* on water agar significantly decreased percentage spore



**Figure 1.** Effect of *Aspergillus niger* on the germination of *Glomus mosseae* spores on water agar (Expt 3a) and on water agar buffered with MES (Expt 3b). ■, Control; ×, plus *Aspergillus niger* on water agar (Expt 3a); ◇, control; □, plus *Aspergillus niger* on water agar buffered with MES (Expt 3b). Bars show standard errors of means.



**Figure 2.** Effect of exudates of *Aspergillus niger* on the germination of *Glomus mosseae* spores on water agar (Expt 3c). ■, Control; □, plus exudates. Bars show standard errors of means.



**Figure 3.** Effect of volatile compounds produced by *Aspergillus niger* on the germination of *Glomus mosseae* spores on water agar and on MEA (Expt 3d). ■, Control; ×, plus volatiles on water agar. ◇, Control; □, plus volatiles on MEA. Bars show standard errors of means.

germination (Fig. 3) and hyphal length (control =  $6.2 \pm 0.7$  mm; plus volatiles =  $2.5 \pm 0.2$  mm) of *G. mosseae*. The decreases in spore germination (Fig. 3) and hyphal length (control =  $6.4 \pm 1$  mm; plus volatiles =  $1.0 \pm 0.2$  mm) were significantly greater when *A. niger* was grown on MEA medium.

**Table 1.** Percentage of root length colonized by *Glomus mosseae* and colony-forming units (CFU) of *Aspergillus niger* from the rhizosphere ( $\text{g}^{-1}$  d. wt of sand:vermiculite) of maize (*Zea mays*) plants, grown under sterile conditions in tubes, inoculated with the two fungi at different times (Expt 5)

Inoculation time for <i>A. niger</i>	Inoculation treatment	Root length colonized (%)	CFU $\times 10^6 \text{ g}^{-1}$ sand:vermiculite after (wk)	
			0	10
Inoculated at the same time as <i>G. mosseae</i>	An		135 a	37.9 a
	M	64.3 a		
	M + An	14.6 b	135 a	30.2 a
Inoculated 2 wk after <i>G. mosseae</i>	An		108 a	36.4 a
	M	56.4 a		
	M + An	46.2 a	108 a	18.6 b

An, inoculated with *A. niger*; M, inoculated with *G. mosseae*. Each figure is the mean of five pots. Column values followed by the same letter are not significantly different according to Duncan's multiple range test ( $P = 0.05$ ).

**Table 2.** Plant dry weight of maize (*Zea mays*) and lettuce (*Lactuca sativa*) in presence or in absence of *Glomus mosseae* and inoculated or uninoculated with *Aspergillus niger* (Expt 6)

Inoculation time for <i>A. niger</i>	Inoculation treatments	Dry weight (mg)			
		Maize		Lettuce	
		Shoot	Root	Shoot	Root
Inoculated at the same time as <i>G. mosseae</i>	C	690 a	570 a	0.38 a	0.31 a
	An	747 a	587 a	0.48 a	0.42 a
	M	1095 c	590 a	0.78 b	0.49 a
	M + An	902 b	580 a	0.34 a	0.30 a
Inoculated 2 wk after <i>G. mosseae</i>	C	658 a	592 a	0.46 a	0.41 a
	An	755 a	578 a	0.46 a	0.37 a
	M	1116 c	605 a	0.86 b	0.48 a
	M + An	1017 c	540 a	0.82 b	0.55 a
Inoculated 2 wk before <i>G. mosseae</i>	C	690 a	570 a	0.38 a	0.31 a
	As	744 a	546 a	0.48 a	0.31 a
	M	1095 c	590 a	0.78 b	0.49 a
	M + An	810 a	535 a	0.46 a	0.31 a

C, Control; An, inoculated with *A. niger*; M, inoculated with *G. mosseae*. Each value is the mean of five pots. Column values followed by the same letter are not significantly different according to Duncan's multiple range test ( $P = 0.05$ ).

Under our experimental conditions extracts of mycorrhizal roots had no obvious inhibitory effect on development of *A. niger* in Petri dishes.

The dry weight of maize plants grown in tubes under axenic conditions were similar in all treatments (data not shown). In maize, the inoculation of *A. niger* at the same time as *G. mosseae* significantly decreased the percentage of root length colonized by the AM fungus, but *A. niger* had no effect when inoculated two weeks after *G. mosseae* (Table 1). The population of *A. niger* decreased from 0 to 10 wk when the saprobe was inoculated two weeks after *G. mosseae*, but was not changed when it was inoculated at the same time as *G. mosseae* (Table 1).

Table 2 shows that plant dry weights of maize and lettuce grown in soil in pots were unaffected by *A. niger*. In contrast, *G. mosseae* increased shoot dry weight in both plants. When *A. niger* was inoculated 2 wk after *G. mosseae*, shoot dry weights of maize and lettuce plants were similar to those of plants inoculated with *G. mosseae* alone. Shoot dry weights of maize and lettuce plants decreased when the saprobic fungus was inoculated at the same time or 2 wk before *G. mosseae*.

Percentage root length colonization by *G. mosseae* in maize and lettuce plants was decreased significantly when the saprobic fungus was inoculated at the same time or 2 wk before *G. mosseae*, but was

**Table 3.** Percentage of root length colonized by *Glomus mosseae* and percentage of AM fungus-mycelium with SDH in maize (*Zea mays*) and lettuce (*Lactuca sativa*) plants inoculated or not with *Aspergillus niger* at different times (Expt 6)

Inoculation time for <i>A. niger</i>	Inoculation treatments	Root length colonized (%)		% AM mycelium with SDH activity	
		Maize	Lettuce	Maize	Lettuce
Inoculated at the same time as <i>G. mosseae</i>	M	40.4 a	84.4 a	77.8 a	86.0 a
	M + An	26.5 b	31.6 b	31.3 b	33.3 c
Inoculated 2 wk after <i>G. mosseae</i>	M	38.7 a	76.2 a	72.2 a	80.3 a
	M + An	43.8 a	81.6 a	27.4 b	40.0 b
Inoculated 2 wk before <i>G. mosseae</i>	M	40.4 a	84.4 a	77.8 a	86.0 a
	M + An	15.1 c	16.8 c	19.2 c	24.7 c

Abbreviations as in Table 1.

**Table 4.** Colony-forming units (CFU  $g^{-1}$  d. wt of soil) of *Aspergillus niger* from the rhizosphere of maize (*Zea mays*) and lettuce (*Lactuca sativa*) plants inoculated or not with *Glomus mosseae* at different times (Expt 6)

Inoculation time for <i>A. niger</i>	Inoculation treatments	CFU $\times 10^6 g^{-1}$ soil after (wk)			
		0	3	7	12
(a) Maize					
Inoculated at the same time as <i>G. mosseae</i>	An	134 a	83 b	76.7 b	31 c
	M + An	155 a	34 c	25.6 c	10.6 d
Inoculated 2 wk after <i>G. mosseae</i>	An	132 a	73 b	70.4 b	36.5 d
	M + An	143 a	17.2 c	11.7 c	2.3 e
Inoculated 2 wk before <i>G. mosseae</i>	M	139 a	88 b	65.7 c	12.5 d
	M + An	149 a	63.8 c	59.3 c	8.2 d
(b) Lettuce					
Inoculated at the same time as <i>G. mosseae</i>	An	90 a	72.4 b	62.7 c	42.6 d
	M + An	105 a	69.4 b	40.6 d	22.6 e
Inoculated 2 wk after <i>G. mosseae</i>	An	89 a	67 b	48.2 c	42.6 c
	M + An	94 a	69 b	27.3 d	18.9 e
Inoculated 2 wk before <i>G. mosseae</i>	M	95 a	63.2 b	39.9 c	29.3 d
	M + An	102 a	58.8 b	34.9 c	22.4 d

Abbreviations as in Table 1.

unaffected when the saprobic fungus was inoculated after *G. mosseae*. However, metabolic activity after AM colonization, measured as the percentage of mycelium of SDH activity, decreased in all treatments (Table 3).

The number of CFU of *A. niger*  $g^{-1}$  rhizosphere soil decreased through the 12 wk of the experiments. Table 4 *a b* shows that when *A. niger* was inoculated to the rhizosphere of maize and lettuce at the same time or 2 wk after *G. mosseae*, the number of CFU decreased significantly after 3 wk of plant growth. However, when *A. niger* was inoculated 2 wk before *G. mosseae*, the final population of the saprobic fungus was similar to that in control plants.

## DISCUSSION

*A. niger*, like some other species of this genus (Calvet, 1989), inhibited the germination and pre-symbiotic development of *G. mosseae*. The inhibition by *A. niger* of germination and hyphal growth from *G. mosseae* spores in water agar was independent of the pH of the medium, as shown by the similarity of the results obtained in water-agar with or without MES. Soluble substances present in the exudates of *A. niger* decreased percentage spore germination of *G. mosseae*, and hyphal growth in *G. mosseae*. *Aspergillus* spp. produce antibiotics that can affect the development of other fungal species (Miller,

1962). Moreover, our results indicate that *A. niger* produced volatile substances that inhibited the germination of spores and hyphal growth of *G. mosseae*; moreover, inhibition increased as a result of the increased metabolic activity when the saprobic fungus grew in a richer medium. The nature of these volatile inhibitors is unknown. These *in vitro* observations indicate that *A. niger* can inhibit development of *G. mosseae* in its extramatrical stage.

The decrease in plant dry weight caused by *A. niger* seems to be a direct consequence of the action of this saprobic fungus on root colonization by the AM fungus. On the other hand, AM fungi are able to protect plants against the pathogenic effect of bacteria, fungi and nematodes when both the VA fungus and the plant pathogen are inoculated at the same time, i.e. when the effect of the mycorrhiza is unlikely to be via host nutrition or a general change in host plant physiology induced by the establishment of symbiosis (Ocampo, 1993). However, plant dry weight and percentage AM colonization decreased only when *A. niger* was inoculated before or at the same time as *G. mosseae*, suggesting a negative effect of the saprobe on the extramatrical phase of the endophyte, as was observed with the pathogenic fungus *Aphanomyces euteiches* in pea plants (Rosendhal, 1985). Previously colonized plants are more resistant to the action of plant pathogenic microorganisms (Ocampo, 1993); indeed, *A. niger* did not reduce mycorrhization when *G. mosseae* was established in the root.

Despite its advantageous location in the root, the endophyte may be influenced by soil microorganisms (Linderman, 1988). The saprobic fungus not only affected percentage root length colonized by *G. mosseae* but also affected its metabolic activity, assessed as SDH activity. The decrease in metabolic activity was observed along with the formation of septae in the intraradical hyphae (results not shown). This effect has been observed when mycorrhizal plants are subjected to stress situations (Kinden & Brown, 1975). SDH activity is also known to decrease in the AM mycelium of plants as a consequence of herbicide application (Ocampo & Barea, 1985). Despite these results, the assessment of mycorrhizal root length with SDH activity is not always an accurate indicator of fungal efficiency in terms of promoting host plant growth (Kough, Gianinazzi-Pearson & Gianinazzi, 1987; Vierheilg & Ocampo, 1991).

The population of *A. niger* was considerably reduced when *G. mosseae* was established in the root (i.e. when *G. mosseae* was inoculated 2 wk before *A. niger*). Modification to root exudates due to the AM fungus has been proposed as an explanation for such finding (Ratnayake, Leonard & Menge, 1978; Schwab, Menge & Leonard, 1983).

Our results show that *G. mosseae* decreases the saprobic fungal population through its effect on the

plant, whereas *A. niger* inhibits *G. mosseae* in its extramatrical stage, both in plants cultivated in tubes inoculated with surface-sterilized spores and in pots where the inoculum consists of spores, mycelia and pieces of colonized root. These results suggest that the effect of the interaction between both microorganisms was independent of the kind of *G. mosseae* inoculum used.

The absence of a clear effect of extracts of mycorrhizal plant roots on development of the saprobic fungus may be a result of the extraction procedure used in our experiments. Further studies will investigate these effects with different methods of plant root extraction.

#### ACKNOWLEDGEMENTS

The authors thank Karen Shashok for grammatical correction of the text. Financial support for this study was provided by the Comision Interministerial de Ciencia y Tecnologia, Spain and by the EEC (PVD programme).

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