Plant and Soil **223:** 175–184, 2000.
© 2000 *Kluwer Academic Publishers. Printed in the Netherlands.*

Effect of the saprophytic fungus *Fusarium oxysporum* on arbuscular mycorrhizal colonization and growth of plants in greenhouse and field trials

S. Fracchia[2], I. Garcia-Romera[1,*], A. Godeas[2] and J.A. Ocampo[1]
[1]*Dept. Microbiología, Estación Experimental del Zaidín, C.S.I.C., Prof. Albareda 1, E-18008 Granada, Spain and*
[2]*Dept. Ciencias Biológicas, 4° II Pabellón, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina*

Received 9 November 1999. Accepted in revised form 16 April 2000

Key words: Arbuscular mycorrhizal fungi, field experiments, *Fusarium oxysporum*, plant growth, saprobe fungi

Abstract

Effects of the saprophytic fungus *Fusarium oxysporum* on arbuscular mycorrhizal (AM) colonization and plant dry matter were studied in greenhouse and field experiments. Host plants: maize (*Zea mays* L.), sorghum (*Sorghum vulgare* L.), lettuce (*Lactuca sativa* L.), tomato (*Lycopersicum esculentum L.)*, wheat (*Triticum vulgare* L), lentil (*Ervum lens* L.) and pea (*Pisum sativum* L.), the AM fungi: *Glomus mosseae*, *G. fasciculatum*, *G. intraradices, G. clarum*, and *G. deserticola* and the carriers for *F. oxysporum* inoculum: aqueous solution, thin agar slices, and pellets of agar and alginate were tested under greenhouse conditions. Greatest plant growth and AM colonization responses in sterilized and unsterilized soils were observed with pea, *Glomus deserticola* and sodium alginate pellets as the carrier for *F. oxysporum* inoculum. Under field conditions, adding *F. oxysporum* increased the survival of transplanted pea, possibly through a beneficial effect on AM fungi. Application of *F. oxysporum* increased shoot dry matter, N and P concentrations of pea and sorghum plants, and the level of AM colonization attained by indigenous or introduced AM fungi. These parameters were similar in plants inoculated with either *G. deserticola* or with the indigenous AM fungi. Application of the saprophytic fungus increased the number of propagules of AM fungi in field plots in which pea was grown, but this increase was not sufficient to increase AM colonization of sorghum after the pea crop.

Introduction

Arbuscular mycorrhizal (AM) fungi can improve plant growth by taking up relatively immobile nutrients such as phosphate (Barea and Jeffries, 1995). AM associations are especially important in degraded soils, because they typically have low fertility. Yet, these soils often have a low density of propagules of AM fungi. Restoration of these soils can be carried out by increasing the population of indigenous AM endophytes, by increasing their effectiveness to sustain plant growth, by reintroducing pre-existing species, or by introduction of effective exotic species (Jeffries and Dodd, 1996; Miller et al., 1994). Inoculation of AM fungi can increase plant and seedling survival in soils of low fertility or with a low density of propagules of AM fungi (Harinikumar and Bagyaraj, 1996; Roldan-Fajardo, 1994). However, the development of AM fungi in plant roots and their effect on plant growth are influenced by the number and type of AM fungi present in soils which may compete with introduced AM fungi (Cardona and Ocampo, 1985).

The importance of the AM association in agriculture has been widely recognised (Bethlenfalvay and Linderman, 1992), but since the AM fungi have not yet been cultured axenically in the absence of plant host roots (Azcon-Aguilar et al., 1999), their field application is limited. Soil micro-organisms affect the development and function of AM symbiosis (Linderman, 1992). Saprophytic fungi are important and common components of rhizosphere soil (Dix and Wester, 1995). Saprophytic and AM fungi are

* FAX No: 958129600. E-mail: IGARCIA@EEZ.CSIC.ES

important because they represent a substantial proportion of microbial biomass in soil. Some experimental results have confirmed the existence of synergistic effects of saprophytic fungi on spore germination of AM fungi and plant root colonization (Calvet et al., 1993; Fracchia et al., 1998; McAllister et al., 1996). For example, *F. oxysporum*-126 has been shown to increase AM colonization and plant growth of plants (García-Romera et al., 1998). However, the beneficial effects attributable to saprophytic fungi under controlled experimental conditions may not reflect their performance in field experiments.

The aim of this work is to evaluate the effect of *F. oxysporum*-126 on AM colonization and plant growth under field conditions. Several experiments were done in the greenhouse to select the *F. oxysporum* inoculum carrier, and the plant and AM endophyte combination to best demonstrate the effects of *F. oxysporum* on AM colonization and host growth.

Materials and methods

Greenhouse experiments

Plant seeds were pregerminated, selected for uniformity prior to planting and transplanted into 0.3 L pots. Pots were 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 in a 1:1 soil:water ratio. The P, N and K were determined by methods of the Estacion Experimental del Zaidin using a Technicom autoanalyser. $NaHCO_3$-extractable P was 6.2 μg g^{-1}. N was 0.3 μg g^{-1} and K was 132 μg g^{-1}. The soil texture was 358 g kg^{-1}sand, 436 g kg^{-1}and 205 g kg^{-1}clay. Soil organic matter concentration was 18 g kg^{-1}. The soil was steam-sterilized and mixed with sterilized quartz sand 1:1 by volume.

AM fungal inoculum was a root-and-soil mixture consisting of rhizosphere soil containing spores and colonized root fragments of *Medicago sativa* L. in amounts of 5 g, which was predetermined to produce high levels of root colonization. Plants noninoculated with AM fungi were given a filtrate (passed through Whatman no. 1 paper) of the inoculum containing the common soil microflora, but free of AM fungal propagules.

Plants were grown in a greenhouse with natural light supplemented by Sylvania incandescent and cool-white lamps giving 400 nmol m^{-2} s^{-1} at 400–700 nm; there was a 16–8 h light-dark cycle at 25–19 °C and 500 mL L^{-1} relative humidity. Plants were watered from below, and fed with a nutrient solution lacking phosphate at 10 mL per week (Hewitt, 1952).

The saprophytic fungus, *Fusarium oxysporum* Schlecht. BAFC Cult. No. 126 (Booth, 1977), is present in the rhizosphere soil and roots of maize cultivated in the province of Buenos Aires, Argentina. This fungus was isolated by the particle washing method using a multichamber washing apparatus (Widden and Bisset, 1972). This strain of *F. oxysporum* is kept in the culture collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires in Buenos Aires, Argentina.

To evaluate the population of *F. oxysporum* inoculated into soil, about 1.5 g of rhizosphere soil was taken from each of the experimental pots and 10-fold aqueous dilution series (from 10^{-1} to 10^{-4}) were prepared for each sample. One ml of each solution was plated on potato dextrose agar (PDA). Number of colony forming units (CFUs) in suitable dilutions of such samples, taken from the five replicate pots of each treatment, were counted. Hyaline fungal isolates were identified to genus (Domsch et al., 1980). All the *Fusarium* spp. were identified by using microscopic structures (conidiophores, conidia, chlamydospores) and colony characteristics such as growth rate, aerial mycelium, pigmentation and sclerotial bodies (Booth, 1977, Gerlach and Nirenberg, 1982). Soil was dried at 105 °C and weighed. The number of CFUs was expressed per g of dry soil.

Plants were harvested after 8 weeks and dry mass was determined. Samples of 1 g fresh weight were taken from the entire root system at random and were cleared and stained (Phillips and Hayman, 1970), and the percentage of root colonization was measured by the line-intersect method (Giovannetti and Mosse, 1980).

Experiment 1. Selection of plants and AM endophytes
To select the most appropriate plant (Experiment 1a), maize (*Zea mays* L.), sorghum (*Sorghum vulgare* L.), lettuce (*Lactuca sativa* L.), tomato (*Lycopersicum esculentum* L.), wheat (*Triticum vulgare* L.), lentil (*Ervum lens* L.) and pea (*Pisum sativum* L.) were grown in unsterilized soil and inoculated or not with *F. oxysporum*. Plants were inoculated at the time of transplanting with a thin slice of PDA containing spores and mycelium of *F. oxysporum.*

To select the most appropiate AM endophyte (Experiment 1b), we investigated *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (BEG no. 12) from Rothamsted Experimental Station, *G. fasciculatum*

(Thax. sensu Gerd.) Gerd. and Trappe (BEG 58) from Dijon (INRA), *G. intraradices* (Schenck and Smith) and *G. clarum* (Nicol. and Schenck) from the Instituto Venezolano de Investigaciones Científicas (IVIC) and *G. deserticola* (Trappe, Bloss and Menge) from the Instituto de Investigaciones Agrobiológicas de Galicia (CSIC). Inoculation with *F. oxysporum*-126 was as described before. Pea was selected as the test plant and was grown in sterilized soil. There were: (1) uninoculated controls, (2) soil inoculated with *F. oxysporum* as in the Experiment 1a, (3) soil inoculated with each of the AM endophytes separately and (4) soil inoculated with both *F. oxysporum* and each of the AM endophytes. Seedlings of each plant species were inoculated at the time of transplanting.

Experiment 2. Selection of F. oxysporum *inoculum carriers*

Pea plants were grown in unsterilized soil and inoculated with *F. oxysporum*-126 as: (1) an aqueous solution in sterile distilled water containing approximately 2×10^6 spores mL^{-1} that has been prepared from cultures grown on PDA for 2 weeks at 27 °C, (2) a thin agar slice as described before, (3) 50 mm diam. pellet of agar (Vassileva et al., 1998) containing approximately 5×10^6propagules per pellet, and (4) 0.5 mm diam. pellet of sodium alginate (Lewis and Papavizas, 1987) containing approximately 5×10^6 propagules per pellet. The agar and alginate pellets were prepared by mixing the fungal propagules with a 30 g L^{-1} solution of agar or 30 g L^{-1} sodium alginate at 45 °C . The mixture was homogenized for 180–240 s at low speed of the homogenizer and then dropped into sterile olive oil to form agar pellets or to 0.025 *M* $CaCl_2$ to form alginate pellets. Pellets were separated from the liquid and washed with sterile distilled water before use.

Experiment 3. Interaction between indigenous and introduced AM endophytes

In order to see if there were synergistic, neutral or antagonistic interactions between AM indigenous endophytes and either *G. mosseae* or *G. deserticola*, these AM fungi were inoculated into sterilized and unsterilized soils in presence or in the absence of *F. oxysporum.* Pea plants were grown either in unsterilized or sterilized soil with the following treatments: (1) uninoculated controls, (2) soil inoculated with *F. oxysporum*, (3) soil inoculated with either *G. mosseae* or *G. deserticola*, and (4) soil inoculated with both *G. mosseae* or *G. deserticola* and *F. oxysporum*.

G. mosseae or *G. deserticola* inoculum consisted either of soil inoculum as described before or of a root inoculum of AM colonized root fragments. Root fragments were obtained from lettuce plants inoculated with the same root-and-soil inoculum described before. Six-week-old lettuce roots with 280 and 520 mm m^{-1} root length colonized by *G. mosseae* and by *G. deserticola*, respectively, were washed with sterile water, rinsed with 10 g L^{-1} sodium hypochlorite for 300 s, and rinsed again with sterile water. To each pot, 250 mg of 10- mm root fragments was added. Non-mycorrhizal lettuce roots were used as controls.

Field experiments

Experiment 4. Pea crop

Pea plants were pregerminated, selected for uniformity prior to planting and transplanted into 40-mL pots of a sterilized mix of peat and vermiculite in equal volumes. Plants were grown in a greenhouse under the same conditions described before. AM inoculum consisted of 150 mg of lettuce roots colonized with *G. deserticola* or with the indigenous endophytes. AM root inoculum with indigenous endophytes from the Estación Experimental del Zaidín field were obtained by growing lettuce plants in unsterilized soil for 6 weeks which yielded 400 mm m^{-1} root length colonized. After 2 weeks, the plants and the peat-vermiculite mix in the pots were transferred to 1-m^2 plots (there were 30 plots and 20 plants per plot) at the Estación Experimental del Zaidín field. Plots were separated from others plots by 1.5-m borders. Alginate pellets containing approximately 5×10^6 propagules per pellet of *F. oxysporum*-126, prepared as described before, were inoculated beneath the roots at the time of transplanting. Treatments were: (1) noninoculated controls, (2) plants inoculated with *G. deserticola* or with the indigenous endophytes, (3) plants inoculated with *F. oxysporum*, and (4) plants inoculated with *G. deserticola* or with the indigenous endophytes and *F. oxysporum*. There were five random replicate plots for each treatment and five plants per harvest. Percentage survival after transplant was recorded. Plants were harvested after 1, 2 and 3 months, and roots from the top 0.2 m soil depth were sampled using a 0.15-m diameter soil coring tube placed directly over a plant. Roots were separated from the soil cores by careful washing. Shoot dry matter, AM colonization, the number of rhizobial root nodules and the number of CFUs of *F. oxysporum* were determined. Shoots were analyzed for P and N (Lachica et al., 1973).

Number of effective AM fungal propagules of the plots inoculated with the indigenous AM fungi and with *G. deserticola* was estimated at the beginning and end of the experiment by the most probable number (MPN) method (Porter, 1979) using *M. sativa* as the test plant. To determine the MPN, five samples of 100 cm^3 from each of the plots were used. From each sample, five dilutions (10^{-1}–10^{-5}) were prepared. Each dilution was tested in five 100 cm^3 pots. The *M. sativa* seedlings were harvested after 6 weeks to record the presence or absence of mycorrhizal colonization.

Experiment 5. Sorghum crop

Plots where pea had been grown were cleaned and weeds were controlled by hand weeding. Sorghum seedlings, obtained as described for pea, were transplanted into the same plots where pea had been grown. At the same time, sorghum seedlings were also transplanted into five new replicate plots. Plants were either noninoculated or inoculated with alginate pellets of *F. oxysporum*-126. After 2 months, plants were harvested and the shoot dry matter, shoot P and shoot N concentration and AM colonization were determined.

Statistical treatments

Percentage AM colonization values were arcsine transformed. Data obtained for dry weight, percentage of AM colonization, CFU of saprophytic fungi, shoot N and P concentrations and the most probable number of propagules of AM fungi were subjected to ANOVA. Mean values of five replicate pots or plots were compared using Duncan's multiple range test ($P = 0.05$).

Results

Greenhouse experiments

Experiment 1. Selection of plants and AM endophytes

Fusarium oxysporum-126 was not pathogenic to the hosts tested even when plants were inoculated with a high concentration of fungal conidia (data not shown).

Inoculation of unsterilized soil with *F. oxysporum*-126 increased the shoot dry weight and the percentage of AM root length colonized by the indigenous endophytes of sorghum, tomato and pea plants (Table 1). Root dry weight did not vary among treatments. From these results, we selected pea for the following experiments. *G. mosseae* and *G. deserticola* in the presence of *F. oxysporum* increased shoot dry weight and AM colonization of pea grown in sterilized soil (Table 2). Root dry weight of pea inoculated with *G. mosseae* and *G. fasciculatum* was higher than that of plants inoculated with *G. deserticola* and *G. clarum*. *F. oxysporum* increased root dry weight of pea (Table 2). Number of CFUs of *F. oxysporum* was similar among *Fusarium*-inoculated treatments (Table 2).

Table 1. Shoot and root dry weights and mycorrhizal colonization of different plants grown in nonsterilized soils inoculated or not with *Fusarium oxysporum*-126

Plants	Dry weight (mg)		mm m^{-1} root length colonized
	Shoot	Root	
Maize	430a	440a	670a
Maize + *F. oxysporum*	503a	462a	690a
Sorghum	420a	450a	570a
Sorghum + *F.oxysporum*	530b	470a	700b
Wheat	152a	173a	410a
Wheat + *F. oxysporum*	156a	170a	660b
Lettuce	232a	133a	670a
Lettuce + *F. oxysporum*	246a	143a	670a
Tomato	163a	103a	280a
Tomato + *F. oxysporum*	372b	80a	420b
Pea	200a	193a	680a
Pea + *F. oxysporum*	383b	203a	860b
Lentil	260a	150a	750a
Lentil + *F. oxysporum*	286a	143a	740a

Within each plant species, column values followed by the same letter are not significantly different as determined by Duncan's multiple range test ($P = 0.05$).

Experiment 2. Selection of F. oxysporum *inoculum carriers*

Higher shoot dry weight and AM colonization of pea were obtained when *F. oxysporum* was inoculated as alginate pellets, compared to when it was inoculated as agar pellets, aqueous solution or agar slices (Table 3). Root dry weight and the number of CFUs of *F. oxysporum* did not vary among inoculum treatments. These results show that *G. mosseae* and *G. deserticola* were the most effective endophytes (Table 2) and that alginate pellets were the best carrier for *F. oxysporum* (Table 3).

Experiment 3. Interaction between indigenous and introduced AM endophytes

In sterilized soil, *G. mosseae* and *G. deserticola* in the presence or absence of *F. oxysporum* increased AM colonization and shoot dry weight of pea (Table 4).

Table 2. Number of CFUs of *Fusarium oxysporum*-126, shoot and root dry weight and mycorrhizal colonization of pea (*Pisum sativum*) plants grown in sterilized soil inoculated with different *Glomus* endophytes and in the presence or absence of *F. oxysporum*

Plants	Dry weight (mg)		mm m^{-1} root length colonized	CFU × 10^3 g-^{1} soil
	Shoot	Root		
Non-inoculated	333a	243ab	0	0
Non-inoculated + *F. oxysporum*	335a	486c	0	2.4a
G. mosseae	400b	313b	480c	0
G. mosseae + *F. oxysporum*	510c	261ab	740d	1.9a
G. fasciculatum	300a	314b	210b	0
G. fasciculatum + *F. oxysporum*	325a	242ab	170ab	2.8a
G. intraradices	403b	293ab	760d	0
G. intraradices + *F. oxysporum*	410b	230a	840d	2.2a
G. deserticola	423b	226a	830d	0
G. deserticola + *F. oxysporum*	642d	303ab	970e	1.7a
G. clarum	373ab	231a	60a	0
G. clarum + *F. oxysporum*	346ab	220a	160ab	3.4a

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P = 0. 05).

Table 3. Effect of different *Fusarium oxysporum*-126 carriers on the number of CFUs, shoot and root dry weights and mycorrhizal colonization of *Pisum sativum* grown in nonsterilized soil

Treatments	Dry weight (mg)		mm m^{-1} root length colonized	CFU × 10^3 g^{-1} soil	
	Shoot	Root		Day 1	Day 42
Control	336a	203a	450a	0	0
Aqueous solution	441b	173a	630b	5.2a	1.3a
Agar slice	542c	163a	630b	2.7a	0.8a
Agar pellet	586cd	166a	610b	3.5a	0.6a
Alginate pellet	650d	173a	770c	4.3a	0.7a

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P = 0. 05).

When *F. oxysporum*, *G. mosseae* and *G. deserticola* were inoculated into unsterilized soil, increases in shoot dry weight and AM colonization of pea were observed. Both *G. mosseae* or *G. deserticola* reached the same root colonization level in unsterilized soil as in sterilized soil. The highest shoot dry weight and AM colonization response of pea was when *F. oxysporum* was co-inoculated with *G. deserticola* (Table 4). Percentage of root colonized and plant growth were not increased when *G. mosseae* and *G. deserticola* were inoculated together as soil-and-root inoculum (data not presented) or as root inoculum.

Field experiments

From the results obtained in the greenhouse experiments, for field experiments we selected the combination of pea, *G. deserticola*, and *F. oxysporum* inoculated in alginate pellets.

Experiment 4. Pea crop

Percentage of survival of uninoculated pea transplanted to plots was significantly lower than when plants were inoculated with *F. oxysporum*, with indigenous endophytes in the absence or in the presence of *F. oxysporum*, or with *G. deserticola* alone or together with *F. oxysporum* (Table 5).

Table 4. Number of CFUs of *Fusarium oxysporum*-126, shoot and root dry weights, mycorrhizal colonization of pea (*Pisum sativum)* grown in the greenhouse in nonsterilized and sterilized soil, inoculated or not with *Glomus mosseae* or *Glomus deserticola* and in presence or absence of *F. oxysporum* inoculated with alginate carrier

Treatments	Dry weight (mg)		mm m^{-1} root length colonized	CFU $\times$ 10^3 g^{-1} soil
	Shoot	Root		
Sterilized soil				
Noninoculated	213ab	281abc	0	0
Noninoculated + *F. oxysporum*	200a	373c	0	3.5a
G. mosseae	372cd	186ab	450b	0
G. mosseae + *F. oxysporum*	412ef	193ab	580cd	2.5a
G. deserticola	413ef	292abc	650de	0
G. deserticola + *F. oxysporum*	443fg	156a	710ef	1.9a
Unsterilized soil				
Noninoculated	250b	140a	250a	0
Noninoculated + *F. oxysporum*	352c	143a	380b	2.7a
G. mosseae	360cd	176ab	490bc	0
G. mosseae + *F. oxysporum*	396de	186ab	500bc	2.9a
G. deserticola	410ef	200ab	610cde	0
G. deserticola + *F. oxysporum*	472g	160a	780f	2.4a

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

Table 5. Percentage of survival of *Pisum sativum* transplanted to soil plots inoculated with indigenous arbuscular mycorrhizal fungi AMF or with *Glomus deserticola* in presence or absence of *Fusarium oxysporum*-126

Treatments	Without *F. oxysporum*	With *F. oxysporum*
Control	76.1a	96.1b
Indigenous AMF	94.6b	97.3b
G. deserticola	97.3b	95.7b

Values followed by the same letter are not significant as determined by Duncan's multiple range test ($P = 0.05$).

Inoculation of *F. oxysporum* increased shoot dry weight of pea and increased AM colonization of 1- and 2-month-old pea plants. AM root colonization after 2 months was highest when *F. oxysporum* was inoculated together with *G. deserticola*. However, 3-month-old pea had similar AM colonization in all treatments. Number of rhizobial root nodules (data not shown) and CFUs of *F. oxysporum* were similar among treatments (Table 6).

Combination *G. deserticola* and *F. oxysporum* increased the concentration of shoot N and P in 1- and 2-month-old pea plants. *G. deserticola* significantly increased P concentration compared to the control at one and two months. The other treatments were similar (Table 7).

Number of AM fungal propagules in soil plots at the beginning of field experiments was 50.7±15. After harvest of 3-month-old pea, the number of propagules had increased in those plots that had been inoculated with AM plus *F. oxysporum* (Table 8).

Experiment 5. Sorghum crop

Sorghum plants cultivated after pea in the same plots had similar shoot dry weights, AM colonization, and N and P concentrations in the different treatments (Table 9a). However, the inoculation of sorghum grown with *F. oxysporum* in new plots in the same experimental field increased the shoot dry weight and the percentage of AM colonization of plants (Table, 9b).

Discussion

Saprophytic fungi can influence AM colonization and host plant response (Calvet et al., 1993; Fracchia et al., 1998; García-Romera et al., 1998). Ability of

Table 6. Number of CFUs of *Fusarium oxysporum*, shoot dry weight and mycorrhizal colonization of pea (*Pisum sativum*) grown in field plots indigenous arbuscular mycorrhizal inoculated with fungi AMF or with *Glomus deserticola* in the presence or absence of *Fusarium oxysporum*-126

Treatments	1 month			2 months			3 months		
	Shoot Dry weight (mg)	mm m^{-1} root length colonization	CFU g^{-1} soil	Shoot dry weight (mg)	mm m^{-1} root length colonization	CFU g^{-1} soil	Shoot dry weight (mg)	mm m^{-1} root length colonization	CFU g^{-1} soil
Control	110a	68a		2335a	270a		7390a	730a	
F. oxysporum	179bc	171b	27.5a	4841b	520b	12.7a	10272b	750a	12.6a
Indigenous AMF	170ab	172b		2952a	280a		8301a	690a	
Indigenous AMF + *F. oxysporum*	220c	273c	25.2a	6182c	470b	9.9a	12814c	730a	10.4a
G. deserticola	191bc	178b		5932c	530bc		12249c	770a	
G. deserticola + *F. oxysporum*	230c	271c	27.6a	6345c	640c	15.1a	13306c	730a	17.1a

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

Table 7. Shoot N and P concentration of pea (*Pisum sativum*) grown in field plots inoculated with indigenous arbuscular mycorrhizal fungi AMF or with *Glomus deserticola* in the presence or absence of *Fusarium oxysporum*-126

Treatments	1 month		2 months		3 months	
	mg g^{-1}P	mg g^{-1}N	mg g^{-1}P	mg g^{-1}N	mg g^{-1}P	mg g^{-1}N
Control	1.2a	60.7a	1.4a	64.3a	1.2a	23.7a
F. oxysporum	1.3a	60.3a	1.4a	66.3a	1.3a	26.0a
Indigenous AMF	1.5ab	59.7a	1.8ab	66.3a	1.3a	21.7a
Indigenous AMF + *F.oxysporum*	1.7 ab	59.0a	1.6ab	62.7a	1.9a	25.0a
G. deserticola	2.0b	67.0a	2.1b	71.0a	1.5a	25.3a
G. deserticola + *F. oxysporum*	3.5c	83.7b	3.7c	89.7b	1.2a	25.0a

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

Fusarium to increase plant dry matter and AM root colonization varied markedly depending on the plant and AM endophyte (McAllister et al., 1994). In this study *F. oxysporum*-126 increased AM colonization and shoot dry weight of sorghum, tomato and pea, but not maize, wheat, lettuce and lentil when grown in unsterilized soil. In the same way, *G. mosseae* and *G. deserticola* increased shoot dry weight and AM root colonization of pea in the presence of *F. oxysporum* more than the other AM fungi tested. In spite of the stimulatory effect of *F. oxysporum* on the colonization of pea root by AM fungi, no influence of AM fungi on the CFUs of *F. oxysporum* was detected. This lack of effect has been observed for several *Fusarium* strains co-inoculated wih *G. mosseae* (Garcia-Romera et al., 1998).

Beneficial effects of *F. oxysporum* on shoot dry matter and AM root colonization of pea varied with the carrier of the *Fusarium* inoculum. Sodium alginate pellets were a better carrier than agar pellets,

Table 8. The most probable number of propagules of arbuscular mycorrhizal fungi AMF in soil plots inoculated with indigenous AMF or with *Glomus deserticola* in presence or absence of *Fusarium oxysporum*-126 after the *Pisum sativum* harvest

Treatments	Number of propagules × 10 g^{-1} soil	
	Without *F. oxysporum*	With *F. oxysporum*
Control	5a	17b
Indigenous AMF	8a	14b
G. deserticola	5a	35c

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

Table 9. Shoot dry weight, colonization by arbuscular mycorrhizal fungi (AMF) and shoot N and P concentrations of *Sorghum vulgare* grown in plots either (a) in the same plots in which pea had been grown or (b) in new plots inoculated or not with *Fusarium oxysporum*

Treatments	Shoot dry weight (mg)	mm m^{-1} root length colonized	N (mg g^{-1})	P (mg g^{-1})
a				
Control	12153a	375a	68a	1.2a
F. oxysporum	1245a	382a	72a	1.5a
Indigenous AMF	11280a	278a	66a	1.9a
Indigenous AMF + *F. oxysporum*	11523a	394a	65a	1.5a
G. deserticola	12066a	306a	62a	1.7a
G. deserticola + *F. oxysporum*	12219a	368a	68a	1.6a
b				
Control	14451a	325a	61a	1.2a
F. oxysporum	46820b	612b	68a	1.3a

Within sorghum plants grown in old plots and within sorghum plants grown in new plots, column values followed by the same letter are not significant as determined by Duncan's multiple range test ($P = 0.05$).

aqueous solution or agar slices. Sodium alginate has been shown to overcome some of the problems associated with survival, stability, efficacy and ease of application of nitrogen-fixing, rock phosphate solubilizing and biocontrol microorganisms (Van Elsas and Heijnen, 1990; Vassileva et al., 1998).

In field plots, *F. oxysporum* increased the infectivity and effectiveness of indigenous and introduced AM endophytes and growth and nutrient concentration of pea plants. AM colonization of pea was increased by *F. oxysporum* during the early stage of root colonization. A direct beneficial action of saprophytic fungi on their extramatrical phase of AM fungi has been hypothesized (Fracchia et al., 1998; García-Romera et al., 1998). Uptake of metabolites produced by saprophytic fungi may be enhanced by AM fungi which increases their mycotrophic effect (Tarafdar and Marschner, 1995). On the other hand, *F. oxysporum* also contributed to the survival of plants transplanted to the field, possibly through an effect on AM fungi.

The N content of leguminous and nonleguminous mycorrhizal plants was increased, but the number of rhizobial root nodules of AM pea plants was not increased. These results suggest a general improvement in growth by AM fungi more than an effect on the N_2-fixing system (George et al., 1995).

No negative interaction between *G. mosseae* and *G. deserticola* was found with the indigenous endophytes. These data suggest that indigenous and introduced endophytes do not compete. It is remarkable that plants responded to introduced endophytes even in the presence of infective and active native AM fungi. These results corroborate those obtained under greenhouse conditions here and elsewhere (García-Romera et al., 1998). The effectiveness of the introduced indigenous endophytes was similar to *G. deserticola*, indicating that the low efficiency of indigenous AM fungi was probably caused by the lower population than with inoculation in these soils. Application of *F. oxysporum* increased the number of propagules of indigenous AM fungi, but no increase of AM colonization and plant growth of sorghum cultivated after in these plots were observed. Similar results have been found in other experiments (Abbott et al., 1983; Harinikumar and Bagyaraj, 1988). Competition between some soil micro-organisms and AM fungi may decrease the infectivity and effectivity of AM fungi (Bethlenfalvay et al., 1983; Ruiz-Lozano and Azcon, 1993). Our results show that dual inoculation of *G. mosseae* or *G. deserticola* and *F. oxysporum* led to additive AM colonization of root and enhanced growth of plants. Therefore, *F. oxysporum* might be exploited to improve the colonization of introduced AM fungi, especially in plant nurseries. However, one of the most important limitations in the use of AM fungi for field crops in agriculture is culturing the AM fungus in the absence of plant roots. Beneficial effects of application of *F. oxysporum* on root colonization by

indigenous endophytes and on plant growth indicate the possibility of using this micro-organism to increase the population and effectiveness of AM fungi at least during one crop. These findings may 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 (Jeffries and Dodd, 1996).

In future studies, the beneficial effect of *F. oxysporum* on AM symbiosis will be investigated by determining what points in the root colonization process in spore germination, penetration of plant root, and extramatrical hyphal development are affected by the saprophytic fungus.

Acnowledgements

Financial support for this study was provided by the Comisión Interministerial de Ciencia y Tecnología, Spain and CONICET and UBA, Argentina.

References

Abbott L K, Robson A D and Hall I R 1983 Introduction of vesicular arbuscular mycorrhizal fungi into agricultural soils. Aust. J. Agric. Res. 34, 741–743.

Azcon-Aguilar C, Bago B and Barea J M 1999 Saprophytic growth of arbuscular mycorrhizal fungi. *In* Mycorrhiza. 2nd Edn. Eds. H. Varma and B. Hock. pp 341–408. Springer-Verlag, Berlin.

Barea J M and Jeffries P 1995 Arbuscular mycorrhizas in sustainable soil-plant systems. *In* Mycorrhiza. Eds. H. Varma and B. Hock. pp 521–560. Springer-Verlag, Berlin.

Bethlenfalvay G J, Bayne H G and Pacovsky R S 1983 Parasitic and mutualistic associations between a mycorrhizal fungus and soybean. The effect of phosphorus on host plant-endophyte interactions. Physiol. Plant. 57, 543–548.

Bethlenfalvay G J and Linderman R G 1992 Mycorrhizae and crop productivity. *In* VA Mycorrhizae in Sustainable Agriculture. Eds. G J Bethlenfalvay and R G Linderman. pp 1–27. American Society Agronomy Special Publ. No. 54, Madison.

Booth C 1977 Fusarium. Laboratory guide to the indentification of the major species. C. M. I., Kew, England. 237 p.

Calvet C, Barea J M and Pera J 1993 Growth response of marigold (*Tagetes erecta* L.) to inoculation with *Glomus mosseae*, *Trichoderma aureoviride* and *Pythium ultimum* in a peat-perlite mixture. Plant Soil 148, 1–6.

Cardona L F and Ocampo J A 1985 Estudio de la posible utilización de micorrizas VA como fertilizantes biológicos en dos suelos. An. Edafol. Agrobiol. 44, 457–462.

Dix N J and Webster J 1995 Fungal Ecology. Chapman and Hall, England. 594 p.

Domsch K H, Gams W and Anderson T H 1980 Compendium of Soil Fungi. Vol. 1. Academic Press, London. 859 p.

Fracchia S, Mujica M T, García-Romera I, García-Garrido J M, Martín J, Ocampo J A and Godeas A 1998 Interactions between *Glomus mosseae* and arbuscular mycorrhizal sporocarp-associated saprophytic fungi. Plant Soil 200, 131–137.

García-Romera I, García-Garrido J M, Martín J, Fracchia S, Mujica M T, Godeas A and Ocampo J A 1998 Interactions between saprotrophic *Fusarium* strains and arbuscular mycorrhizas of soybean plants. Symbiosis 24, 235–246.

George E, Marschner H and Jakobsen I 1995 Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit. Rev. Biotechnol. 15, 257–270.

Gerlach W and Nirenberg H 1982. The genus Fusarium-a pictorial atlas. Mitteilungen aus der Biologischen Budensanstalt für Land- und Forstwirtschaft, Berlin. 405 p.

Giovannetti M and Mosse B 1980 An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.

Harinikumar K M and Bagyaraj D J 1988 Effect of crop rotation on native vesicular arbuscular mycorrhizal propagules in soil. Plant Soil 110, 77–80.

Harinikumar K M and Bagyaraj D J 1996 Persistence of introduced *Glomus intraradices* in the field as influenced by repeated inoculation and cropping system. Biol. Fert. Soils 21, 184–188.

Hewitt E J 1952 Sand water culture methods used in the study of plant nutrition. C.A.B. Tech. Co. No. 22.

Jeffries P and Dodd J C 1996 Functional ecology of mycorrhizal fungi in sustainable soil-plant systems. *In* Mycorrhizas in Integrated Systems from Genes to Plant Development. Eds. C Azcon-Aguilar and J M Barea. pp 497–501. European Commission Report, Brussels.

Lachica M, Aguilar A and Yañez J 1973 Análisis foliar. Métodos utilizados en la Estación Experimental del Zaidín. An. Edafol. Agrobiol. 32, 1033–1047.

Lewis J and Papavizas G 1987 Application of *Trichoderma* and *Gliocadium* in alginate pellets for control of *Rhizoctonia* damping-off. Plant Pathol. 36, 438–446.

Linderman R G 1992 Vesicular-arbuscular mycorrhizae and soil microbial interactions. *In* VA Mycorrhizae in Sustainable Agriculture. Eds. G J Bethlenfalvay and R G Linderman. pp 45–70. American Society Agronomy Special Publ. No. 54, Madison.

McAllister C B, García-Romera I, Godeas A and Ocampo J A 1994 Interaction between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*: Effect on plant growth, arbuscular mycorrhizas and the saprophytic population. Soil Biol. Biochem. 26, 1363–1367.

McAllister C B, García-Garrido J M, García-Romera I, Godeas A and Ocampo J A 1996 Interactions between *Alternaria alternata*, *Fusarium equiseti* and *Glomus mosseae*. I. Endophyte-saprophyte interactions *in vitro.* Symbiosis 20, 163–174.

Miller M, McGonigle T and Addy H 1994 An economic approach to evaluate the role of mycorrhizas in managed ecosystems. Plant Soil 159, 27–35.

Phillips J M and Hayman D S 1970 Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161.

Porter W M 1979 "The most probable number" method of enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. Aust. J. Soil Res. 17, 515–518.

Roldan-Fajardo B E 1994 Effect of indigenous arbuscular mycorrhizal endophytes on the development of six wild plants colonizing a semi-arid area in south-east Spain. New Phytol. 127, 115–121.

Ruiz-Lozano J M and Azcon R 1993 Specific and functional compatibility of VA mycorrhizal endophytes in association with *Bradyrhizobium* strains in *Cicer arietinum*. Symbiosis 15, 217–226.

Tarafdar J C and Marschner H 1995 Dual inoculation with Aspergillus fumigatus and *Glomus mosseae* enhances biomass production and nutrient uptake in wheat (*Triticum aestivum* L.) supplied with organic phosphorus as Naphytate. Plant Soil 173, 92–102.

Van Elsas J D and Heijnen C E 1990 Methods of introduction of bacteria into soil. A review. Biol. Fert. Soils, 10, 127–133.

Vassileva M, Azcon R, Barea J M and Vassilev N 1998 Application of an encapsulated filamentous fungus in solubilization of inorganic phosphate. J. Biotechnol. 63, 67–72.

Widden P and Bisset J 1972 An automatic multichamber soil washing apparatus for removing fungal spores from soil. Can. J. Microbiol. 18, 1399–1404.

Section editor: J H Graham