



INTERACTIONS BETWEEN *TRICHODERMA KONINGII*, *FUSARIUM SOLANI* AND *GLOMUS MOSSEAE*: EFFECTS ON PLANT GROWTH, ARBUSCULAR MYCORRHIZAS AND THE SAPROPHYTE INOCULANTS

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(Accepted 16 March 1994)

Summary—The effect of inoculation with the saprophytic fungi *Trichoderma koningii* and *Fusarium solani* 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 wt of non-AM inoculated maize and lettuce were unaffected by the presence of *T. koningii* and *F. solani*. In contrast, *T. koningii* decreased plant dry wt and AM colonization when inoculated into the rhizosphere before or at the same time as *G. mosseae*. In addition, the *T. koningii* population was considerably reduced when *G. mosseae* was inoculated 2 weeks before the saprophytic fungus. At this time *T. koningii* did not affect the proportion of the root length colonized by *G. mosseae*, but did adversely affect its metabolic activity assessed as succinate dehydrogenase activity. Although *F. solani* did not affect AM colonization of maize roots, its effect on AM colonization of lettuce roots was similar to that of *T. koningii*. The population of *F. solani* decreased significantly in the rhizosphere of both plants. The possible mechanisms of interaction between the saprophytic fungi and *G. mosseae* are discussed.

INTRODUCTION

There is increasing interest in microbial interactions in the rhizosphere of arbuscular mycorrhizas (AM). Most of the reported interactions have focused on dual inoculations of selected bacteria and mycorrhizal fungi in relation to plant growth enhancement. But even when pathogenic and non-pathogenic bacteria were considered, only interactions with pathogenic fungi were studied in detail (Bagyaraj, 1984; García-Garrido and Ocampo, 1987). Although saprophytic fungi are important and common components of the soil rhizosphere, they have been little studied (Paget, 1975; Calvet *et al.*, 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. Moreover, some saprophytic fungi, such as *Trichoderma* species, are involved in complex interactions such as antibiosis (Cook and Baker, 1983), fungistasis (Pavlica *et al.*, 1978) and mycoparasitism (Elad, 1986). For these reasons these fungi must be considered when AM fungi are inoculated onto host plants. The results of research on the interactions between soil saprophytic fungi and AM differ widely, even when the same genus is involved. For example, *Trichoderma* spp

have been found to have both antagonistic (Chu and Wu, 1981; Cook and Baker, 1983; Camporota, 1985; Wyss *et al.*, 1992) and stimulating (Calvet *et al.*, 1992) effects on AM.

Our study was designed to obtain more detailed knowledge of the interactions between saprophytic fungi and AM.

MATERIALS AND METHODS

The active fungi present in the rhizosphere soil and roots were isolated by the particle washing method (Widden and Bisset, 1972) with a washing multichamber apparatus; 30 washings were necessary to remove fungi, sclerotia, spores, etc., from the soil particles and roots of maize. Twenty soil particles were dried on sterile filter paper and placed on 2% malt extract agar with antibiotics (5 µg streptomycin l⁻¹ and 10 µg tetracycline l⁻¹). From the resulting colonies, *T. koningii* and *F. solani* were selected as the most common fungi. The strains were preserved in tubes of potato dextrose agar (PDA) and 2% malt extract at 4°C as stock cultures.

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 (García-Romera and Ocampo, 1988). It was steam-sterilized and mixed with an equal volume of steril-

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ized quartz sand. 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 conditions. Natural light was supplemented by Sylvania incandescent and cool-white lamps, 400 nmol m⁻² s⁻¹, 400–700 nm; with a 16–8 h light–dark cycle at 25–19°C and 50% r.h. Plants were watered from below using a capillary system, and fed with a nutrient solution (Hewitt, 1952) lacking phosphate for AM-inoculated plants.

The AM inoculum consisted of 5 g of rhizosphere soil from an alfalfa plant pot culture of an isolate of *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe, which contained spores (15 sporocarps g⁻¹ with one–five spores per sporocarp), mycelium 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 AM uninoculated treatment. The filtrate contained common soil microorganisms, but no propagules of *G. mosseae*.

The saprophytic fungi *T. koningii* and *F. solani* were obtained from the rhizosphere and rhizoplane of maize plants as described above. An aqueous suspension in sterile distilled water containing ca 2 × 10³ spores ml⁻¹ was prepared from cultures grown in PDA for 1 week at 27°C.

Four treatments were used in all experiments: (1) uninoculated controls; (2) inoculated with *T. koningii* or with *F. solani*; (3) inoculated with *G. mosseae*; and (4) inoculated with *G. mosseae* and *T. koningii* or *F. solani*. Plants were inoculated at the time of transplanting or after 2 weeks of growth. The saprophytic fungi were inoculated 2 weeks before, at the same time as and 2 weeks after *G. mosseae*.

To evaluate the population of inoculated *T. koningii* or *F. solani* during the experiments, rhizosphere soils were sampled after 0, 3, 7 and 12 weeks, as described by García-Garrido and Ocampo (1988). About 1.5 g of rhizosphere soil was taken from each of the experimental pots. Then 10-fold dilution series 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 plus streptomycin and tetracycline. 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 g⁻¹ of dry rhizosphere soil.

Plants were harvested after 10 weeks and the dry matter wt was determined. After the plants were harvested, the root system in each of the five replicates per treatment was divided into two portions to record the following: (i) 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); (ii) mycorrhizal fungus with

succinate dehydrogenase (SDH) activity: (EC 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). Roots were cut into ca 5 mm segments and kept at 25°C for 16 h in 50 mM of Tris–HCl buffer at pH 7.4 containing 0.25 M sodium succinate and 10 mg ml⁻¹ of nitroblue tetrazolium. Positive results for enzyme activity produced a violet-coloured deposit of reduced tetrazolium salts in the fungal mycelium. The percentage of AM mycelium with SDH activity was measured under a compound microscope (Ocampo and Barea, 1985).

The results were evaluated statistically with Duncan's multiple range test.

RESULTS

From 109 fungal strains isolated from the rhizosphere and rhizoplane of maize, 23% of the rhizosphere fungi were *T. koningii* and 35% of the rhizoplane fungi were *F. solani*. The *T. koningii* and the *F. solani* isolates were not pathogenic to maize or lettuce plants, even when both plants were inoculated with high concentration of fungal conidia (data not shown).

Table 1 shows that plant dry wt of maize and lettuce were unaffected by the presence of *T. koningii* and *F. solani*; however, *G. mosseae* increased shoot dry wt of both plants. When *T. koningii* was inoculated 2 weeks after *G. mosseae*, shoot dry wt of maize and lettuce plants were similar to those in plants inoculated with *G. mosseae* alone. However, when *T. koningii* was inoculated at the same time as or 2 weeks before *G. mosseae*, shoot dry wt of maize and lettuce plants were similar to those in non-AM inoculated

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 uninoculated with *T. koningii* and *F. solani*

Inoculation time	Treatments	Dry weight (mg)			
		Maize		Lettuce	
		Shoot	Root	Shoot	Root
Saprophytic fungi inoculated at the same time as <i>G. mosseae</i>	C	690 ^a	570 ^a	0.38 ^a	0.31 ^a
	Tk.	670 ^a	587 ^a	0.48 ^a	0.42 ^a
	Fs.	780 ^a	637 ^a	0.52 ^a	0.42 ^a
	M	1095 ^b	690 ^a	0.78 ^b	0.49 ^a
Saprophytic fungi inoculated 2 weeks after <i>G. mosseae</i>	M + Tk.	750 ^a	580 ^a	0.32 ^a	0.30 ^a
	M + Fs.	970 ^b	720 ^a	0.51 ^a	0.46 ^a
	C	658 ^a	592 ^a	0.46 ^a	0.41 ^a
	Tk.	644 ^a	578 ^a	0.42 ^a	0.37 ^a
Saprophytic fungi inoculated 2 weeks before <i>G. mosseae</i>	Fs.	816 ^a	640 ^a	0.49 ^a	0.38 ^a
	M	1116 ^b	705 ^a	0.86 ^c	0.48 ^a
	M + Tk.	980 ^b	620 ^a	0.84 ^b	0.50 ^a
	M + Fs.	1050 ^b	640 ^a	0.94 ^b	0.55 ^a
Saprophytic fungi inoculated 2 weeks before <i>G. mosseae</i>	C	690 ^a	570 ^a	0.38 ^a	0.31 ^a
	Tk.	570 ^a	746 ^a	0.34 ^a	0.31 ^a
	Fs.	690 ^a	720 ^a	0.52 ^a	0.50 ^a
	M	1095 ^b	690 ^a	0.78 ^b	0.49 ^a
	M + Tk.	818 ^a	635 ^a	0.40 ^a	0.31 ^a
	M + Fs.	985 ^b	790 ^a	0.49 ^a	0.39 ^a

C = Control; Tk = PLANTS inoculated with *T. koningii*; Fs = PLANTS inoculated with *F. solani*. 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 Duncan's multiple range test ($P = 0.05$).

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

Inoculation time	Treatments	Root length colonized by AM (%)		% AMF mycelium with SDH activity	
		Maize	Lettuce	Maize	Lettuce
Saprophytic fungi inoculated at the same time as <i>G. mosseae</i>	M	40.4 ^a	84.4 ^a	77.8 ^a	86.0 ^a
	M + Tk.	3.9 ^b	37.3 ^b	31.3 ^b	40.1 ^b
	M + FS.	32.8 ^a	51.6 ^b	63.2 ^a	42.7 ^b
Saprophytic fungi inoculated 2 weeks after <i>G. mosseae</i>	M	38.7 ^a	76.2 ^a	72.2 ^a	80.3 ^a
	M + Tk.	31.0 ^a	72.3 ^a	27.4 ^b	38.6 ^b
	M + FS.	42.6 ^a	81.2 ^a	66.1 ^a	75.0 ^a
Saprophytic fungi inoculated 2 weeks before <i>G. mosseae</i>	M	40.4 ^a	84.4 ^a	77.8 ^a	86.0 ^a
	M + Tk.	3.7 ^b	26.3 ^b	19.2 ^b	18.3 ^c
	M + FS.	31.4 ^a	28.5 ^c	62.7 ^a	21.7 ^c

C = Control; Tk = PLANTS inoculated with *T. koningii*; Fs = PLANTS inoculated with *F. solani*. 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 Duncan's multiple range test ($P = 0.05$).

controls. *F. solani* alone did not affect shoot dry wt in maize, but when inoculated with *G. mosseae* the results were similar to those in plants inoculated with the endophyte only at all three times of inoculation. However, when *F. solani* was inoculated before or at the same time as *G. mosseae*, shoot dry wt of lettuce plants were significantly lower than in plants inoculated with *G. mosseae* alone. When lettuce plants were inoculated with *F. solani* 2 weeks after *G. mosseae*, shoot dry wt were similar to those in plants inoculated with *G. mosseae* alone.

As Table 2 shows, *T. koningii* 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*. *T. koningii* decreased the percentage of AM fungal mycelium with SDH activity in all the treatments tested.

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. solani* in the rhizosphere. However, *F. solani* decreased both the percentage of AM root colonization in lettuce plants and the percentage of AM fungal mycelium with SDH activity when this saprophytic fungus was inoculated at the same time as or before *G. mosseae*.

The number of CFUs of saprophytic fungi g^{-1} rhizosphere soil decreased throughout the experiments. When *T. koningii* was inoculated into the rhizosphere of maize and lettuce at the same time as or before *G. mosseae*, the number of CFUs was similar to that in the rhizosphere of plants inoculated with *T. koningii* alone [Figs 1(a) and 1(b)]. When *T. koningii* was inoculated 2 weeks after *G. mosseae*, the saprophytic fungus population decreased significantly after 3 weeks of plant growth. The population

of *F. solani* [Fig. 2(a)] in the rhizosphere of maize was similar when inoculated alone and when it was inoculated before or at the same time as *G. mosseae*. When *F. solani* was inoculated after *G. mosseae*, the saprophytic fungus population decreased significantly after 3 weeks. In the lettuce rhizosphere [Fig. 2(b)] the population of *F. solani* was affected only when *G. mosseae* was inoculated before the saprophytic fungus, which decreased significantly.

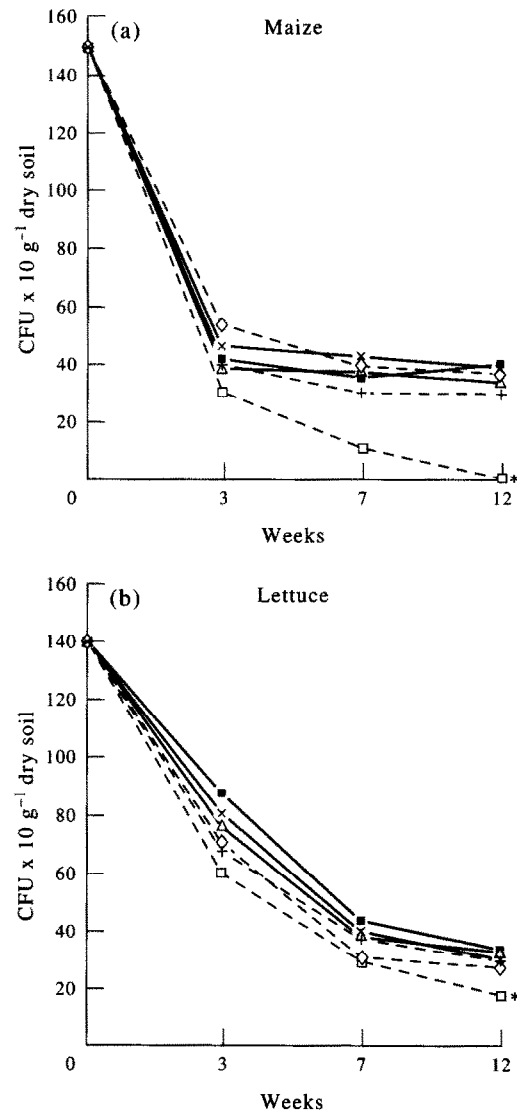


Fig. 1. Colony-forming units (CFUs) of *T. koningii* from the rhizosphere (g^{-1} dry wt of soil) of maize (*Z. mays*) (a) and lettuce (*L. sativa*) (b) plants inoculated or not with *G. mosseae* at different times. (—): *T. koningii* inoculated at the same time as *G. mosseae* (First treatment); (---): *T. koningii* inoculated alone (First treatment); (—): *T. koningii* inoculated 2 weeks after *G. mosseae* (Second treatment); (---): *T. koningii* inoculated alone (Second treatment); (—): *T. koningii* inoculated 2 weeks before *G. mosseae* (Third treatment); (---): *T. koningii* inoculated alone (Third treatment). Asterisks indicated values that are significantly different according to Duncan's multiple range test ($P = 0.05$).

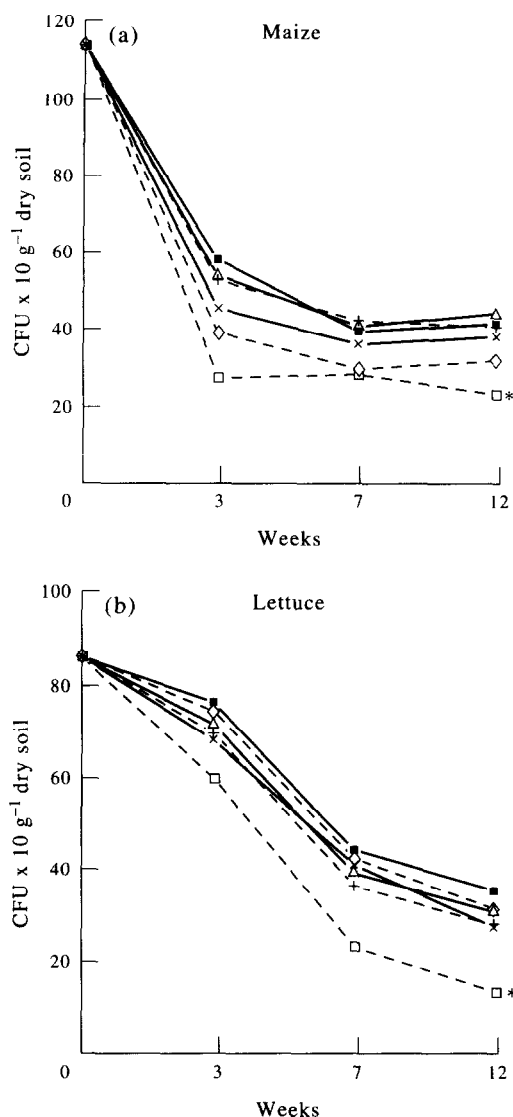


Fig. 2. Colony-forming units (CFUs) of *F. solani* from the rhizosphere (g^{-1} dry wt of soil) of maize (*Z. mays*) (a) and lettuce (*L. sativa*) (b) plants inoculated or not with *G. mosseae* at different times. (—): *F. solani* inoculated at the same time as *G. mosseae* (First treatment); (—): *F. solani* inoculated alone (First treatment); (Δ): *F. solani* inoculated 2 weeks after *G. mosseae* (Second treatment); (\square): *F. solani* inoculated alone (Second treatment); (\blacksquare): *F. solani* inoculated 2 weeks before *G. mosseae* (Third treatment); (\diamond): *F. solani* inoculated alone (Third treatment). Asterisks indicated values that are significantly different according to Duncan's multiple range test ($P = 0.05$).

DISCUSSION

Most recent studies of interaction between non-pathogenic rhizosphere fungi and AM fungi have focused on microorganisms enhancing AM formation (De Oliveira and Garbaye, 1989). Neither fungi tested in our assays behaved in this way, as *T. koningii* and *F. solani* had no positive effect on AM formation.

The decrease in dry wt of plants caused by saprophytic fungi seems to be a direct consequence of the

action of these fungi on root colonization by AM as plant dry wt decreased only when the saprophytic fungi were inoculated before or at the same time as *G. mosseae*. This suggests a negative effect of the saprophytes on the extramatrical phase of the endophyte. As a matter of fact, *T. koningii* reduced mycorrhizal formation, whether it was present in the rhizosphere before or at the same time as *G. mosseae* was established on the roots. This result contrasts markedly with the absence of competition observed in other studies, such as that of Calvet *et al.* (1993) with *T. aureoviride* on *Tagetes erecta*. A noteworthy fact is that the effects of interaction between *G. mosseae* and microorganisms may be very different between different species of the same genus.

The population of *T. koningii* was considerably reduced when *G. mosseae* was established on the roots (i.e., when *G. mosseae* was inoculated 2 weeks before *T. koningii*). Modification of root exudates due to AM fungus may explain this finding (Ratnayake *et al.*, 1978; Schwab *et al.*, 1983). However, the endophyte may benefit from an advantageous situation in the root, and be favorably influenced by soil microorganisms (Linderman, 1988). Under these circumstances saprophytic fungi scarcely affect the proportion of the root length colonized by *G. mosseae*, but do affect metabolic activity assessed as SDH activity. This decrease in metabolic activity was observed along with the formation of septa in the intraradical hypha (results not shown), an effect that has been observed when mycorrhizal plants were subjected to stress (Kinden and Brown, 1975). Ours is thus the first report of decreased SDH as a consequence of AM fungus-microorganism interaction, although this decrease is a well-known response to herbicide application (Ocampo and Barea, 1985). Despite these results, plant growth was not affected. This histochemical technique detects fungal metabolic function; however, the assessment of mycorrhizal root length on the basis of this activity is not indicative of fungal efficiency in terms of host plant growth (Kough *et al.*, 1987; Vierheilg and Ocampo, 1991). The same species of saprophytic fungus seems to affect mycorrhizal formation in a very different way, depending on the host plant considered. Whereas *F. solani* had no effect on maize root mycorrhizal formation by *G. mosseae*, its effect on AM colonization of lettuce roots was similar to that observed with *T. koningii*.

The population of *F. solani* was significantly reduced. This fact may be due not only to the changes produced by the AM fungus on root physiology, but also to the ability to colonize primary roots shown by some species of *Fusarium*, which makes it difficult to establish whether any other fungus has previously colonized the substrate (Cook and Bruhl, 1968).

Future studies will investigate whether AMF are inhibited directly by saprophytic fungi, or whether this effect is mediated through the plant.

Acknowledgements—We thank Karen Shashok for revising the English translation of the text. Financial support for this study was provided by the Comisión Interministerial de Ciencia y Tecnología, Spain, and by the CEE (PVD Program).

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