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Growth response of marigold (*Tagetes erecta* L.) to inoculation with *Glomus mosseae*, *Trichoderma aureoviride* and *Pythium ultimum* in a peat-perlite mixture

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Abstract

The interactions between the mycorrhizal fungus *Glomus mosseae*, the plant pathogen *Pythium ultimum*, and a pathogen-antagonist *Trichoderma aureoviride* in the rhizosphere of *Tagetes erecta* (marigold) were studied for their effects on plant growth in a peat-perlite substrate. Mycorrhizal fungus inoculation protected the plant against *P. ultimum*, since both phytomass production and foliar development were higher in mycorrhizal plants. *T. aureoviride* had no effect on nonmycorrhizal plants in the presence or absence of *P. ultimum*. However, more biomass was produced by mycorrhizal plants when *T. aureoviride* was present, whether or not soil was infested with *P. ultimum*.

Introduction

Some specific interactions have been reported between vesicular-arbuscular mycorrhizal (VAM) fungi (VAMF) and other microbial groups commonly present in the plant rhizosphere: symbiotic and free living N_2 fixing bacteria (Barea et al., 1983), phosphate-solubilizing bacteria (Azcón et al., 1976), plant growth promoting rhizobacteria (Meyer and Linderman, 1986), actinomycetes (Krishna et al., 1982), and soil-borne plant pathogens, including root-pathogenic fungi and plant parasitic nematodes (Perrin, 1991). Since these microbial interactions involving VAMF can affect plant nutrition and health, they must be considered concerning the development and function of the VAM symbiosis (Linderman, 1988).

Horticultural, ornamental, and fruit-tree crops are usually grown in containers on soilless media prepared with organic substrates and inorganic conditioners which lack VAMF propagules. Most of these crops are dependent on VAM symbiosis when they are transplanted to the field from the nursery. Inoculation with mycorrhizae under these controlled conditions is feasible and requires little inoculum.

Biocontrol agents have been successfully used for horticultural crops (Davet et al., 1981; Henis et al., 1979) therefore the interactions between VAMF and any introduced pathogen-suppressor in the rhizosphere should be considered in microbial management of a soil or a planting medium.

Trichoderma spp. are efficient antagonists against a wide range of plant pathogens, including wilt fungi (Trillas-Gay et al., 1986) and fungi causing damping-off (Kwok et al., 1987). Species in this genus are used in biological control against fungal root diseases. The mechanisms of

action can be based on antibiosis (Dennis and Webster, 1971) and mycoparasitism (De Oliveira et al., 1984).

Davey (1971) reviewed the interactions between microorganisms associated with ectomycorrhizae, and reported an increased development of ectomycorrhizae formation when some bacteria and fungi including *Trichoderma* spp. were present at inoculation time, an effect which was probably exerted through the production of plant growth promoting substances that stimulated rooting and mycorrhizal fungi. Their effect on VAM symbiosis has received little attention. For example, Sylvia and Schenck (1983) reported a general inhibitory effect of some contaminating fungi, including *Trichoderma* spp., on spore germination of three species of *Glomus*. Four strains of *Trichoderma* spp. isolated from two organic composts suppressive to *Fusarium oxysporum* f. sp. *dianghi* (Pera and Calvet, 1989) stimulated mycelial growth from *Glomus mosseae* resting spores in monoxenic cultures on water agar (Calvet et al., 1992). Since the same strains had shown antagonism against two wilt fungi in vitro (Calvet et al., 1990), the possibility exists of using fungal populations antagonistic to plant pathogens to stimulate the VAMF in a growing medium.

In this paper, the interactions between one of these strains, a *Trichoderma aureoviride* Rifai isolate, the VAMF *Glomus mosseae* (Nic. and Gerd.) Gerd. and Trappe, and a pathogenic isolate of *Pythium ultimum*, were studied in vivo using marigold (*Tagetes erecta* L.) as test plant. The objective was to test the effect of microbial management in the rhizosphere on the development of a container-grown ornamental through the dual inoculation of a VAMF and a saprophytic fungus in substrate infested or not by a fungal root pathogen.

Materials and methods

Container medium

A steamed-sterilized sphagnum peat-perlite mixture (1:1 v/v) was used as growing medium for plants. $CaCO_3$ was added to reach a neutral pH.

Host plant and growth conditions

Seeds of *Tagetes erecta* L. 'Hawai orange' (Royal Fleur) were surface sterilized with 0.5% NaClO before sowing in autoclaved quartz sand. Two week old seedlings were then transplanted to 650 mL volume containers filled with the soilless mixture and different inocula. Two weeks after transplanting, plants received 20 mL of Hoagland's nutrient solution without phosphorus weekly.

Saprophytic fungi

A *T. aureoviride* strain isolated from composted olive pumice (Pera and Calvet, 1989), had shown a strong stimulatory effect on spore germination rate and mycelial growth of the mycorrhizal fungus *G. mosseae* under axenic conditions (Calvet et al., 1992). A preliminary experiment was conducted to determine conditions for growth of *T. aureoviride* in peat perlite (1:1 v/v). One hundred mL test tubes containing 25 mL substrate were inoculated with conidial suspensions in sterile distilled water from potato dextrose agar (PDA, Difco) tube cultures of *T. aureoviride*, at the same density detected in composted olive pumice: 9×10^7 cfu mL^{-1}. Growth data for *T. aureoviride*, obtained from periodical plate dilution of the substrate in 4 PDA tubes, indicated that *T. aureoviride* could develop and maintain a constant population level (10^7 cfu mL^{-1} after 20 days of incubation) in the steamed-sterilized peat-perlite.

Pathogenicity test of Pythium *spp. on* Tagetes erecta

The pathogenic potential of three *Pythium* spp. isolates was tested on marigold (Hwang, 1988) but using millet (*Sorghum bicolor* L) instead of cornmeal to produce inocula. These were *P. ultimum* var. *ultimum*, *P. ultimum* var. *sporangiferum* (strains PUU4 and PUS4 from INRA Dijon, France) and *Pythium* spp (isolate from Centro de Investigación y Tecnologia Agraria, Tenerife, Spain). The inoculum of each *Pythium* sp. isolate was mixed with the peat-perlite mixture at 1/8 and 1/4 dilutions by volume. Ten marigold seeds were sown in each container. The

seeded containers were maintained under greenhouse conditions (25°C, 16 h light/day) for 21 days, at which time percentage survival and the plant height were measured for each treatment. *P. ultimum* var. *ultimum* was the most effective strain for decreasing height of marigold plantlets, with no differences between both inoculum dilutions.

Interaction experiments

The sterilized peat-perlite mixture (1:1 v/v) was inoculated with spore suspensions of *T. aureoviride* obtained from PDA tube cultures. Inoculated and noninoculated batches of the growing medium were incubated for two weeks at 24°C in a dark chamber. They were shaken periodically to obtain a homogeneous distribution of the saprophyte.

Soil samples from a stock pot culture with *Allium cepa L.* of the VAMF *G. mosseae*, containing 4.2 sporocarps g^{-1} soil plus mycorrhizal root pieces, were used as mycorrhizal inoculum. This inoculum was mixed with peat-perlite at a 15 g L^{-1}. To establish the accompanying non-mycorrhizal microorganisms, 10 mL of a filtrate (Whatman No 1), obtained by mixing 100 g of inoculum with 1 L water, were added to each container in the non-mycorrhizal treatments.

P. ultimum var. *ultimum* inoculum, prepared in the same way as for the pathogenicity test, was added homogenously at a 1/8 dilution to the peat-perlite mixture.

Experiments were conducted to test the effect of *T. aureoviride* on growth of marigold when added either (i) alone, treatments T (*T. aureoviride*) and C (control), (ii) in combination with *G. mosseae*, treatments GM (*G. mosseae*) and T + GM (*T. aureoviride* plus *G. mosseae*), and (iii) in combination with *G. mosseae* in substrate infested by *P. ultimum* var. *ultimum*, treatments T + P (*T. aureoviride* plus *P. ultimum*), GM + P (*G. mosseae* plus *P. ultimum*) and T + GM + P (*T. aureoviride* plus *G. mosseae* plus *P. ultimum*).

In treatments involving more than one microorganism, the inoculation sequence was as follows: after two-week incubation period of *T. aureoviride* in the growing media, the corresponding containers were inoculated with *G. mosseae* alone or together with *P. ultimum* var. *ultimum*. There were 9 pots per treatment, with 5 plantlets per pot, in a randomized design in the greenhouse. Plants grew for two months and once harvested, shoot dry weight and foliar area were measured. Roots from each pot were cleared with 10% KOH and stained with 0.05% trypan blue in lactic acid after Phillips and Hayman (1970), and the percentage of VAM internal colonization was determined by a grid-line intersect method (Giovannetti and Mosse, 1980). Data recorded for dry weight and foliar area were statistically analyzed by a one way ANOVA and Tukey's multiple range test ($p = 0.05$).

Results

Biomass of non-mycorrhizal marigolds was similar whether or not the substrate was inoculated with *T. aureoviride* (data not shown). However, *T. aureoviride* enhanced the dry weight and the foliar area of mycorrhizal plants (Fig. 1).

Inoculation of the substrate with *T. aureoviride* prior to introduction of *G. mosseae* increased the percentage of VAM internal colonization by *G. mosseae*. Average VAM internal colonization percentage was 20.10 ± 4.76 for mycorrhizal plants in GM treatment and 35.43 ± 5.81 for plants in GM + T treatment (see Fig. 1 for symbols).

When the growth medium was infested by *P. ultimum* var. *ultimum*, inoculation with *T. aureoviride* did not increase growth of *T. erecta* (Fig. 2), but introduction of *G. mosseae* alone significantly increased plant growth, while inoculation with *T. aureoviride* did not increase biomass of mycorrhizal plants, foliar area was increased (Fig. 2).

The internal VAM colonization caused by *G. mosseae* in the medium infested with *P. ultimum* var. *ultimum* was lower than that in pathogen-free peat-perlite, $10.05 \pm 2.70\%$ in GM and $13.72 \pm 1.30\%$ in GM + T treatments, respectively.

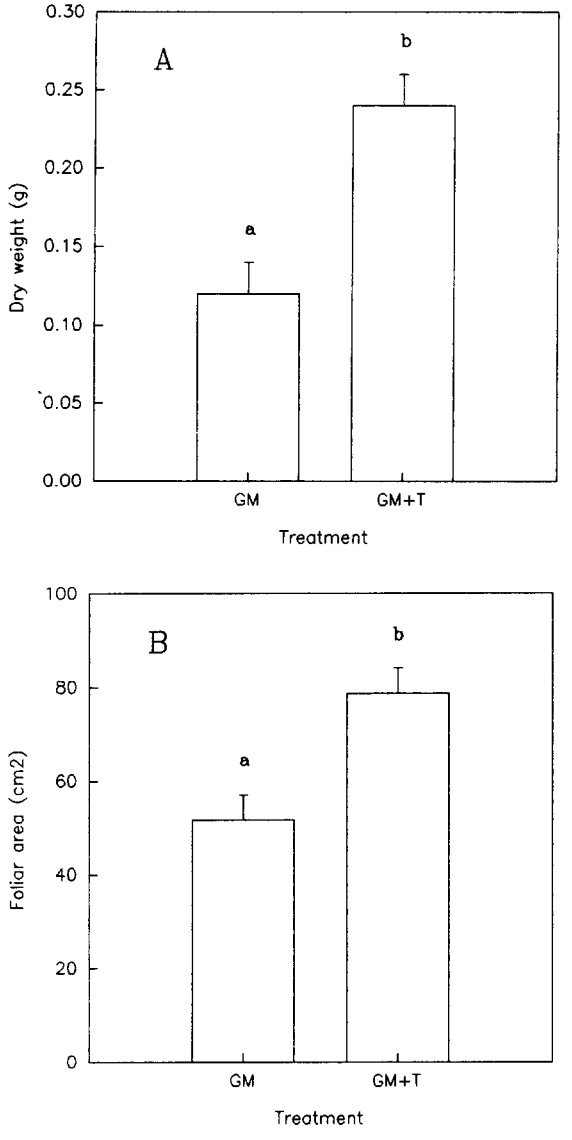

Fig. 1. (**A**, **B**) Interactions between *Glomus mosseae* (GM) and *Trichoderma aureoviride* (T) and their effect on the development of *Tagetes erecta* after two months growth in peat/perlite (1:1 v/v). Data were subjected to a one way ANOVA plus Tukey's multiple range test ($p = 0.05$). Different letters show significant differences between treatments.

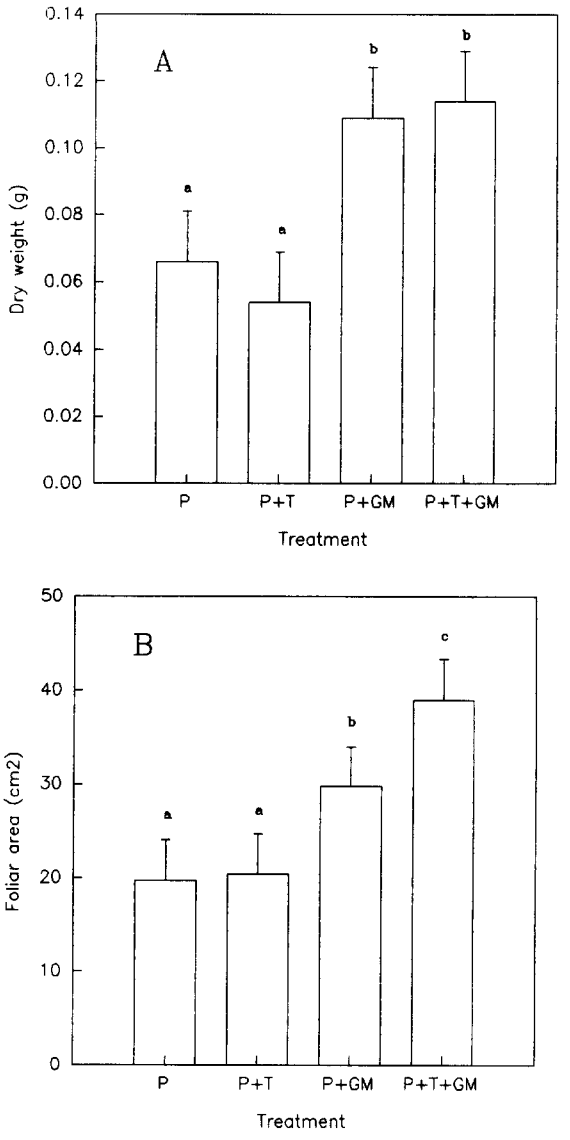

Fig. 2. (**A**, **B**) Effect of *Glomus mosseae* (GM) and *Trichoderma aureoviride* (T) on *Tagetes erecta* after two months growth in peat/perlite (1:1 v/v) infested with *Pythium ultimum* var. *ultimum* (P). Data were subjected to a one way ANOVA plus Tukey's multiple range test ($p = 0.05$). Different letters show significant differences between treatments.

Discussion

Many experimental results confirm the importance of microbial activity in the soil and rhizosphere to mycorrhizal colonization. There are not only antagonistic effects, but also stimulatory effects (De Oliveira and Garbaye, 1989). Garbaye and Bowen (1987) suggested that microbial interactions with ectomycorrhizal fungi in the rhizosphere should not be interpreted only as antagonistic, but that synergistic effects should be considered as well. They described inhibitory and stimulatory effects of a mixture of microorganisms on mycorrhizal infection of *Pinus*. Azcón-Aguilar and Barea (1985), found an increase in mycorrhizal infection of luzerne

(*Medicago sativa* L.) by *G. mosseae* when microorganisms were introduced in axenic growing medium. The inoculation of *Pseudomonas putida* in nonsterile soil increased VAMF colonization in clover plants, and there was a synergistic action of both microorganisms on plant growth (Meyer and Linderman, 1986).

In the present experiments, the combined inoculation of *T. aureoviride* and *G. mosseae* has a synergistic effect on the growth of marigold. The interaction between a saprophytic fungus, known to act in vitro as a potential antagonist of plant pathogens (Calvet et al., 1990) and a VAMF, was beneficial for plant growth. The percentage of VAMF internal colonization in the present study was also increased in the presence of *T. aureoviride*. The dual inoculation evidenced a stimulatory effect, even though the *T. aureoviride* isolate alone did not stimulate plant growth. It is possible that plant growth promoting effect attributed to *Trichoderma* spp. (Baker, 1989) may be linked to the effect of these fungi on mycorrhizal infection. The use of selected *Trichoderma* strains in biological control of plant pathogens (Cook and Baker, 1983) suggested that their influence on VAMF is antagonistic as well, however an antagonistic action of *Trichoderma* against VAMF has not been previously demonstrated. The research of Summerbell (1987) on ectomycorrhizal fungi demonstrated the inhibitory effect of *Trichoderma* spp. on in vitro formation of mycorrhizae by *Laccaria bicolor*. This is not necessarily related to in vivo interactions. Also, *Trichoderma* spp. have been isolated in the rhizosphere of ectomycorrhizal plants in forest nursery soils (Pera, personal communication).

T. aureoviride was not an antagonist of *P. ultimum* var. *ultimum*. Nelson and Hoitink (1983), showed that suppressive properties of a growing medium against *R. solani* requires that such medium favour the activities of the antagonistic agent to be introduced. Kuter and Hoitink (1985), proved that populations of *Trichoderma* spp. are not only responsible for the reduction of diseases in container suppressive substrates, but also that the suppressive effect is not generally associated with a single antagonistic fungus.

When peat-perlite was infested by *P. ultimum* var. *ultimum*, VAM inoculation improved plant growth. Tolerance to diseases in plants inoculated with VAMF can be due to increased nutrient uptake, antibiotic production, altered root exudation, and changes in microbial populations in the rhizosphere. Dual inoculation of *G. mosseae* and a *Pseudomonas syringae* isolate made tomato plants more tolerant to the bacterial wilt (García-Garrido and Ocampo, 1989). Mycorrhizal inoculation of plants in medium infested by *Pythium paroecandrum* increased shoot height, dry weight and fresh weight compared with non VAM plantlets (Hwang, 1988). The interaction between *G. mosseae* and *P. ultimum* var. *ultimum* produced similar results on the growth of *T. erecta* compared to non mycorrhizal plantlets growing on pathogen-infested peat-perlite.

In this paper, a synergistic action between a VAMF and a potential antagonistic agent, *T. aureoviride*, was detected on growth stimulation of marigold, a container grown ornamental. Furthermore, mycorrhizal inoculation had a beneficial effect on the growth of plants in peat-perlite infested by *P. ultimum* var. *ultimum* causing damping-off, an effect that was improved by the presence of *Trichoderma* concerning some parameters of plant growth. Bowen and Theodorou (1979) have proposed practical utilization of accompanying beneficial microbiota as done in biological control of plant pathogens. Our results prove that it is possible to design, through microbial manipulation, a rhizosphere favouring VAM.

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