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Interaction between arbuscular mycorrhizal fungi and *Trichoderma harzianum* under conventional and low input fertilization field condition in melon crops: Growth response and Fusarium wilt biocontrol

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ABSTRACT

The objective of this work was to evaluate the interactions between four arbuscular mycorrhizal fungi (AMF) (*Glomus intraradices*, *Glomus mosseae*, *Glomus claroideum*, and *Glomus constrictum*) and the beneficial fungus *Trichoderma harzianum*, inoculated in a greenhouse nursery, with regard to their effects on melon crop growth under conventional and integrated-system field conditions, and the biocontrol effect against Fusarium wilt. A synergistic effect on AM root colonization due to the interaction between *T. harzianum* and *G. constrictum* or *G.intraradices*, was observed under a reduced fertilizer dosage, while no significant effect was observed for *G. claroideum* or *G. mosseae*. With the reduced fertilizer input, AMF-inoculated plants and *T. harzianum*-inoculated plants had improved shoot weight and nutritional status, but the combined inoculation of AMF and *T. harzianum* did not result in an additive effect. Under the conventional fertilizer dosage, plant growth was not influenced by AM formation; however, it was increased significantly in *T. harzianum*-inoculated plants. The AMF-inoculated plants were effective in controlling Fusarium wilt, *G. mosseae*-inoculated plants showing the greatest capacity for reduction of disease incidence. The *T. harzianum*-inoculated plants were more effective than AMF-inoculated plants with regard to suppressing disease incidence. Co-inoculation of plants with the AMF and *T. harzianum* produced a more effective control of Fusarium wilt than each AMF inoculated alone, but with an effectiveness similar to that of *T. harzianum*-inoculated plants.

1. Introduction

In recent years, low-input agricultural systems have gained increasing importance in many industrialized countries, for reduction of environmental degradation (Mäder et al., 2002). Integrated farming systems with reduced inputs of fertilizers and pesticides have been developed. It is under these conditions that plants are expected to be particularly dependent on beneficial rhizosphere microorganisms (Smith et al., 1997).

Arbuscular mycorrhizal fungi (AMF) are key components of soil microbiota and form symbiotic relationships with the roots of most terrestrial plants, improving the nutritional status of their host and protecting it against several soil-borne plant pathogens (Smith et al., 1997; Harrison, 1999; Bi et al., 2007). The incidence and the effect of root colonization vary depending on the plant species and the AMF (Jeffries and Barea, 2001); they are influenced by soil microorganisms and environmental factors (Azcón-Aguilar and Barea, 1992; Bowen and Rovira, 1999). *Trichoderma* sp. is a common component of rhizosphere soil and has been reported to suppress a great number of plant diseases (Chet, 1987; Harman and Lumsden, 1990; De Meyer et al., 1998; Elad, 2000; Howell, 2003). Some strains, also, have been reported to colonize the root surface, enhancing root growth and development, crop productivity, resistance to abiotic stresses, and the uptake and use of nutrients (Ousley et al., 1994; Björkman et al., 1998; Harman and Björkman, 1998; Rabeendran et al., 2000; Harman et al., 2004).

Several reports have demonstrated that the interaction of these two groups of microorganisms may be beneficial for both plant growth and plant disease control (Linderman, 1992; Barea et al., 1997; Saldajeno et al., 2008; Martínez-Medina et al., 2009a). A synergistic effect of some saprophytic fungi on AMF spore germination and colonization has been confirmed (Calvet et al., 1993; McAllister et al., 1996; Fracchia et al., 1998). For example, it has been reported that some *Trichoderma* strains may influence AMF activity (Calvet et al., 1992, 1993; Brimner and Boland, 2003; Martinez et al., 2004; Martínez-Medina et al., 2009a). Volatile and soluble exudates produced by saprophytic fungi are involved in these effects (McAllister et al., 1994, 1995; Fracchia et al., 1998). Nevertheless, the results

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of research on the interactions between soil saprophytic and AM fungi differ widely, even when the same species of saprophytic fungi are involved. For example, *Trichoderma harzianum* has been found to have antagonistic, neutral, and stimulating effects on AMF (Rousseau et al., 1996; Siddiqui and Mahmood, 1996; Fracchia et al., 1998; Godeas et al., 1999; Green et al., 1999; Martínez-Medina et al., 2009a). Little is known about the interactions between AMF and beneficial saprophytic fungi, and the few studies published on this topic do not provide any conclusive findings (Green et al., 1999; Vázquez et al., 2000). Even more, the beneficial effect attributable to these interactions under controlled experimental conditions may not be reflected in field experiments (Calvet et al., 1992; McAllister et al., 1997; Fracchia et al., 1998; Vázquez et al., 2000; Martinez et al., 2004).

The aim of this work was to evaluate the effect of *T. harzianum* and four AMF, previously inoculated in a greenhouse nursery, with regard to melon plant growth and their potential biocontrol of Fusarium wilt, under different soil fertilization conditions. To achieve this aim, dual inoculation in a greenhouse nursery with four mycorrhizal fungi from the genus *Glomus* (*G. constrictum*, *G. mosseae*, *G. claroideum* and *Glomus intraradices*) and the fungus *T. harzianum* was evaluated in two field experiments (under conventional conditions and with reduced fertilizer dosage, and under *F. oxysporum* pressure) for its effect on melon crops, with regard to (1) plant growth and (2) biocontrol of Fusarium wilt.

2. Material and methods

2.1. Host plant and fungal inocula

Melon plants (*Cucumis melo* L., cv. "Giotto") were used as the host plants. Plants were inoculated with *T. harzianum* and four different AMF from the genus *Glomus* (*G. constrictum*, *G. mosseae*, *G. claroideum*, and *G. intraradices*) in a greenhouse nursery (Martínez-Medina et al., 2009a). Here, the AM inocula were mixed at a rate of 20 g kg^{-1} of peat, while *T. harzianum* was added to reach a population density of 1×10^6 conidia g^{-1} of peat, according to Martínez-Medina et al. (2009a). The AM fungal inoculum density was found to be 35 infective propagules per gram of inoculum. The isolate of *T. harzianum*, deposited in the Spanish Type Culture Collection (isolate CECT 20714) by Centro de Edafología y Biologia Aplicada del Segura-CSIC (Spain), was chosen for this study owed to its high biocontrol capacity against *F. oxysporum* (Martínez-Medina et al., 2009a). *T. harzianum* inoculum was produced using a specific solid medium, prepared by mixing commercial oats, bentonite and vermiculite (1:2.5:5, w:v:v) according to Martínez-Medina et al. (2009b).

The plants were grown in a peat-vermiculite mixture under natural conditions, for five weeks. They were irrigated manually, as necessary, during this period. Five weeks after planting, the melon plants were transplanted to the field, at the Estación Experimental Cuatro Caminos (Spain) (38°11′N; 1°03′W), where they were arranged in a randomized design.

Monoconidial *Fusarium oxysporum* f.sp. *melonis* was isolated from infected melon plants from a greenhouse nursery. For the production of inocula, the pathogen was cultivated for 5 days on potato dextrose broth (Scharlau Chemie, Barcelona, Spain), at 28 °C in darkness, on a shaker at 120 rpm. After the incubation period, the fungal culture was centrifuged at 193 × *g*, 10 min, re-suspended in sterilized water, and re-centrifuged. The fungal suspension contained 1×10^8 conidia mL^{-1}.

2.2. Experimental design and growth conditions

Two experiments, using a completely randomized design, were conducted separately. The first experiment had three factors, the first factor with five levels: non-inoculation and inoculation with four AMF (*G. constrictum*, *G. mosseae*, *G. claroideum*, or *G. intraradices*) in the greenhouse nursery. The second factor had two levels: non-inoculation and inoculation with *T. harzianum*, in the greenhouse nursery. The third factor had two levels: conventional and reduced fertilization dosage.

To assess the effect of the fertilization on the interactions between the AMF and *T. harzianum*, half of the experiment was fertirrigated with a conventional fertilization dose for melon plants in the Mediterranean area: 0.51 g L^{-1} NH_4NO_3 and 0.51 g L^{-1} $NH_4H_2PO_4$. The other half of the experiment was fertirrigated with 1/3 of this dose. Eight replicates were established for each of the 20 treatments.

The second experiment had three factors, the first factor with five levels: non-inoculation and inoculation in the greenhouse nursery with four AMF (*G. constrictum*, *G. mosseae*, *G. claroideum*, or *G. intraradices*). The second factor had two levels: non-inoculation and inoculation in the greenhouse nursery with *T. harzianum*. The third factor had two levels: non-inoculation and inoculation with *F. oxysporum*. To assess the effect of the interactions between the AMF and *T. harzianum* on the potential biocontrol of Fusarium wilt, four weeks after planting, half of the melon plants were infected by *F. oxysporum* to reach a final concentration of 1×10^4 conidia g^{-1} in the rhizosphere, while the other half was maintained as a control. Eight replicates were established for each of the 20 treatments.

For both experiments, plants were planted 1 m apart, at a depth of 10 cm, in rows. The soil had a pH of 8.04 (1:1 soil:water ratio), the $NaHCO_3$-extractable *P* was 26 μg g^{-1}, total *N* was 1 mg g^{-1}, and extractable *K* was 289 μg g^{-1}. The soil texture was 39 g kg^{-1} coarse sand, 502 g kg^{-1} fine sand, 301 g kg^{-1} silt, and 158 g kg^{-1} clay. Plants were grown for eleven weeks in the field under natural conditions (the climate is semi-arid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2 °C; the potential evapo-transpiration reaches 1000 mm/year). Plants were fertirrigated automatically for 10 min every 12 h with 2.5 L h^{-1} water drippers. The fertilizer was added in fertigation at the following doses: 0.13 g L^{-1} NH_4NO_3 (total nitrogen: 35.5%, nitric nitrogen: 16.9%, ammoniacal nitrogen: 17.6%) and 0.13 g L^{-1} $NH_4H_2PO_4$ (ammoniacal nitrogen: 12%, soluble P_2O_5 in neutral ammonium citrate: 60%).

Eleven weeks after planting, plants were harvested and rhizosphere samples were taken and stored at 4 °C for biological and biochemical analyses. In the first experiment, the shoot fresh weight and the nitrogen, phosphorus, and potassium contents were recorded as well as the fruit production. In the second experiment, further *F. oxysporum*-infected plants were determined.

2.3. Plant analyses

Plant samples for nutrient content analysis were digested by a microwave technique, using a Milestone Ethos I microwave digestion instrument. A standard aliquot (0.1 g) of dry, finely ground plant material was digested with concentrated nitric acid (HNO_3) (8 mL) and hydrogen peroxide (H_2O_2) (2 mL). Subsequently, the phosphorus and potassium contents were analyzed using ICP (Iris intrepid II XD2 Thermo). Plant nitrogen content was determined using a Flash 1112 series EA carbon/nitrogen analyzer.

Roots were softened with 10% KOH in water bath and stained with 0.05% trypan blue (Phillips and Hayman, 1970). The percentage root length colonized by AMF was calculated by the line intersect method (Giovannetti and Mosse, 1980). Positive counts for AM colonization included the presence of vesicles, arbuscules, or typical mycelium within the roots.

To determine the *F. oxysporum* colonization of inoculated plants, stem segments (~1.5 cm) from inoculated plants were cut imme-

diately above crowns, surface-sterilized by soaking in 1% sodium hypochlorite for 5 min, and rinsed with sterilized water. The segments were incubated on PDA at 28 °C for 6 days, and the appearance of *F. oxysporum* colonies was considered to be indicative of infected plants. The percentage of infected plants was used to determine the disease incidence.

2.4. Soil biological analyses

Serial dilutions of 1 g of rhizosphere soil from the top 0.3 m, in sterile, quarter-strength Ringer solution, were used for quantifying the *T. harzianum* colony forming units (CFU) by a plate count technique using PDA (Scharlau Chemie, Barcelona, Spain) amended with 50 mg L^{-1} rose bengale and 100 mg L^{-1} streptomycin sulfate. The plates were incubated at 28 °C for 5 days. After the incubation period, CFUs were counted. Komada medium (Komada, 1975) was used for quantification of *F. oxysporum*.

2.5. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS software (SPSS system for Windows, version 15.0, SPSS Inc, Chicago, II). The statistical significance of the results was determined by performing Duncan's multiple-range test ($P < 0.05$).

3. Results

3.1. Experiment I

3.1.1. Plant shoot fresh weight

In treatments involving reduced fertilization, plants inoculated with *T. harzianum* alone had significantly increased shoot fresh weight relative to the non-inoculated plants (Table 1). The AMF-inoculated plants also showed increases in fresh weight, with no differences among the AMF. Plants co-inoculated with *T. harzianum* and AMF showed fresh weights similar to those of AMF-inoculated plants. Conventionally fertilized plants had a higher fresh weight than plants receiving a reduced dosage of fertilizer, the factor fertilization being highly significant ($P < 0.001$) (Tables 1 and 4). *T. harzianum*-inoculated plants receiving the conventional fertilizer dosage had an increased fresh weight compared with the non-inoculated plants, while the fresh weight of AMF-inoculated plants did not differ from that of the non-inoculated plants. Co-inoculated (*T. harzianum*-AMF) plants which were fertilized conventionally exhibited fresh weights similar to those of AMF-inoculated plants (Table 1).

Table 1
Fresh shoot weight (g) of plants inoculated or not with *Trichoderma harzianum* and/or *Glomus constrictum*, *Glomus mosseae*, *Glomus claroideum*, or *Glomus intraradices*, under conventional and reduced fertilization dosages.

Treatment	Reduced fertilizer dose	Conventional fertilizer dose
Non-inoculated	772 ± 28 c	1156 ± 27 c
G. constrictum	890 ± 64 b	1160 ± 22 c
G. mosseae	854 ± 69 b	1291 ± 56 abc
G. claroideum	881 ± 22 b	1154 ± 45 c
G. intraradices	884 ± 47 b	1257 ± 19 abc
T. harzianum	1057 ± 35 a	1378 ± 80 ab
G. constrictum × *T. harzianum*	885 ± 9 b	1166 ± 77 c
G. mosseae × *T. harzianum*	978 ± 42 ab	1307 ± 75 abc
G. claroideum × *T. harzianum*	818 ± 19 b	1233 ± 121 bc
G. intraradices × *T. harzianum*	911 ± 14 ab	1288 ± 55 abc

Data are means ± standard error of eight replicates. Values in the same column with the same letters, represent no significant difference between treatments according to Duncan's multiple range test ($P \leq 0.05$), $n = 8$.

3.1.2. Nutrient content

With the low fertilizer dosage, total plant nitrogen was increased ($P < 0.001$) in AMF-inoculated plants (Tables 2 and 4). Inoculation of plants with *T. harzianum* increased the nitrogen content significantly. No additive effect for nitrogen content was observed in plants co-inoculated with *T. harzianum* and AMF; even negative effect could be observed in the case of plants co-inoculated with *G. constrictum* and *T. harzianum*. The plants co-inoculated with *T. harzianum* and *G. mosseae* showed higher nitrogen contents than plants co-inoculated with any other AMF. The conventional fertilization dose increased the plant nitrogen concentration in all treatments ($P < 0.001$) (Tables 3 and 4). The nitrogen content was increased in *T. harzianum*-inoculated plants, while no differences in nitrogen content were found in AMF-inoculated plants, compared to the non-inoculated plants. Co-inoculation with *T. harzianum* and AMF gave nitrogen values similar to those of plants inoculated with the AMF alone.

Under reduced fertilizer dosage, the phosphorus concentration was increased ($P < 0.001$) in plants which had been inoculated in the greenhouse nursery with *G. mosseae*, *G. claroideum*, or *G. intraradices* alone, but it was unaffected in *G. constrictum*-inoculated plants (Tables 2 and 4). The phosphorus content of *T. harzianum*-inoculated plants at the reduced fertilizer dosage was increased with respect to the non-inoculated plants. The plants co-inoculated with *T. harzianum* and AMF showed phosphorus levels similar to those of plants inoculated with the AMF alone. The conventional fertilizer application increased ($P < 0.001$) the shoot phosphorus level in all the treatments compared with the lower dose (Tables 3 and 4). The phosphorus content of *T. harzianum*-inoculated plants was not altered with respect to non-inoculated plants. A decreased phosphorus level was observed in AMF-inoculated plants, and in plants co-inoculated with AMF and *T. harzianum*, relative to non-inoculated plants. Co-inoculated plants showed lower phosphorus contents than *T. harzianum*-inoculated plants.

With the low fertilizer dosage, the plant potassium content was increased in plants which had been inoculated in the greenhouse nursery with the AMF (Table 2). The potassium content of *T. harzianum*-inoculated plants was increased with respect to the non-inoculated plants, at the reduced fertilizer dosage. The potassium contents of plants co-inoculated with *T. harzianum* and AMF were similar to those of plants inoculated with each AMF alone, with no differences among them or with respect to *T. harzianum*-inoculated plants. The factor fertilization was not significant for the plant potassium content (Table 4). At the conventional fertilizer dosage, no differences in plant potassium content were found among the treatments (Table 3).

3.1.3. AM root colonization

Inoculation in the greenhouse nursery with the different AMF produced a significant increase ($P < 0.001$) in the AM root colonization under field conditions (Fig. 1). Under low fertilizer dosage, *G. constrictum*- and *G. intraradices*-inoculated plants showed higher percentages of AM root colonization than any other AMF tested. The lowest percentage of AM colonization was observed in *G. claroideum*-inoculated plants. Under the reduced fertilizer dosage, AM root colonization by *G. constrictum* or *G. intraradices* was increased ($P < 0.001$) in plants which were also co-inoculated with *T. harzianum*, with respect to plants inoculated with AMF alone, but it was unaffected in plants co-inoculated with *G. mosseae* or *G. claroideum* and *T. harzianum*. The conventional fertilizer dosage produced, in general, a decreased percentage of AM root colonization ($P < 0.001$) compared with the reduced fertilizer dose.

Table 2
Shoot nitrogen, phosphorus, and potassium contents (g per plant) of melon plants inoculated or not with *Trichoderma harzianum* and/or *Glomus constrictum*, *Glomus mosseae*, *Glomus claroideum*, or *Glomus intraradices*, under reduced fertilization dosage.

Treatment	Nitrogen	Phosphorous	Potassium
Non-inoculated	1.90 ± 0.01 c	0.37 ± 0.02 c	1.18 ± 0.09 c
G. constrictum	2.11 ± 0.01 b	0.36 ± 0.02 c	1.56 ± 0.16 ab
G. mosseae	2.49 ± 0.01 ab	0.62 ± 0.17 ab	1.73 ± 0.40 a
G. claroideum	2.08 ± 0.16 b	0.47 ± 0.05 b	1.51 ± 0.16 ab
G. intraradices	2.05 ± 0.05 b	0.45 ± 0.02 b	1.34 ± 0.21 b
T. harzianum	2.42 ± 0.06 ab	0.88 ± 0.09 a	1.51 ± 0.13 ab
G. constrictum × *T. harzianum*	1.82 ± 0.29 c	0.39 ± 0.09 bc	1.52 ± 0.05 ab
G. mosseae × *T. harzianum*	2.63 ± 0.07 a	0.48 ± 0.05 b	1.66 ± 0.44 ab
G. claroideum × *T. harzianum*	1.94 ± 0.11 bc	0.49 ± 0.19 b	1.29 ± 0.59 b
G. intraradices × *T. harzianum*	2.07 ± 0.23 b	0.41 ± 0.01 bc	1.33 ± 0.46 b

Data are means ± standard error of eight replicates. Values in the same column with the same letters, represent no significant difference between treatments according to Duncan's multiple range test ($P \leq 0.05$), $n = 8$.

Table 3
Shoot nitrogen, phosphorus, and potassium contents (g per plant) of melon plants inoculated or not with *Trichoderma harzianum* and/or *Glomus constrictum*, *Glomus mosseae*, *Glomus claroideum*, or *Glomus intraradices*, under conventional fertilization dosage.

Treatment	Nitrogen	Phosphorous	Potassium
Non-inoculated	4.74 ± 0.01 bcd	1.49 ± 0.15 a	1.20 ± 0.14 ab
G. constrictum	4.39 ± 0.05 bcde	1.11 ± 0.40 bc	1.56 ± 0.49 ab
G. mosseae	4.80 ± 0.49 bcd	1.16 ± 0.21 bc	1.55 ± 0.46 ab
G. claroideum	4.19 ± 0.34 cde	0.95 ± 0.06 c	1.19 ± 0.28 ab
G. intraradices	5.46 ± 0.27 ab	1.21 ± 0.09 bc	1.70 ± 0.62a
T. harzianum	5.72 ± 0.81 a	1.59 ± 0.57 a	1.78 ± 0.84a
G. constrictum × *T. harzianum*	3.73 ± 0.32 de	0.98 ± 0.42 c	1.00 ± 0.21ab
G. mosseae × *T. harzianum*	5.02 ± 0.64 abc	1.10 ± 0.36 bc	1.47 ± 0.35ab
G. claroideum × *T. harzianum*	3.56 ± 0.04 e	0.99 ± 0.14 c	0.99 ± 0.03 b
G. intraradices × *T. harzianum*	4.98 ± 0.17 abc	0.93 ± 0.02 c	1.77 ± 0.03 a

Data are means ± standard error of eight replicates. Values in the same column with the same letters, represent no significant difference between treatments according to Duncan's multiple range test ($P \leq 0.05$), $n = 8$.

3.1.4. *T. harzianum* population

T. harzianum was detected in the rhizosphere, reaching values around 1×10^4 CFU g^{-1} and showing similar CFU values in all the treatments which included inoculation with *T. harzianum*; in non-inoculated treatments, its density was below 1×10^2 CFU g^{-1} (data not shown).

3.1.5. Number of fruits

With the reduced fertilizer dosage, AMF-inoculated plants had an increased number of fruits ($P < 0.01$) compared with non-inoculated plants (Fig. 2). *T. harzianum*-inoculated plants did not differ in their fruit number relative to non-inoculated plants. At the reduced fertilizer dose, and compared with the AMF-inoculated plants, the number of fruits was decreased significantly by *T. harzianum*–*G. constrictum* or *T. harzianum*–*G. intraradices* co-inoculation, whereas *T. harzianum*–*G. mosseae* co-inoculation significantly increased the number of fruits.

The conventional fertilization dose reduced significantly the number of fruits ($P < 0.001$), compared with the lower dose (Fig. 2). No. significant differences in fruit number were produced by AMF or *T. harzianum* inoculation, alone or in combination, at the conventional fertilizer dosage, compared to non-inoculated plants.

3.2. Experiment II

3.2.1. *T. harzianum* population

T. harzianum was detected in the rhizosphere, reaching values around 1×10^4 CFU g^{-1} and showing similar CFU values in all the treatments which included inoculation with *T. harzianum*; in non-inoculated treatments, its density was below 1×10^2 CFU g^{-1} (data not shown).

3.2.2. Disease incidence

The disease incidence in AMF-inoculated plants was reduced by up 25–50%, *G. mosseae*-inoculated plants showing the lowest percentage of infection (Fig. 3). The disease incidence in *T. harzianum*-inoculated plants was reduced by 60% with respect to non-inoculated plants. Plants co-inoculated with *T. harzianum* and AMF showed a lower percentage infection than AMF-inoculated plants.

Table 4
The three-factor ANOVA (arbuscular mycorrhizal fungi (AMF) inoculation, *Trichoderma harzianum* inoculation, and fertilization (F) level) for all parameters studied. *P* significant values.

Parameters studied	AMF inoculation AM	*T. harzianum* inoculation Th	Fertilization F	Interaction AM × Th	Interaction AM × F	Interaction Th × F	Interaction AM × Th × F
Shoot fresh weight	0.029	0.008	<0.001	0.020	0.005	NS	NS
Nitrogen content	<0.001	0.05	<0.001	<0.001	<0.001	NS	NS
Phosphorus content	<0.001	0.045	<0.001	NS	NS	NS	NS
Potassium content	0.03	0.05	NS	0.045	NS	NS	NS
AM root colonization	<0.001	NS	<0.001	<0.001	<0.001	<0.001	NS
Fruit number	0.006	0.027	<0.001	0.017	NS	NS	NS
T. harzianum population	NS	<0.001	NS	<0.001	NS	<0.001	NS

NS: non-significant.

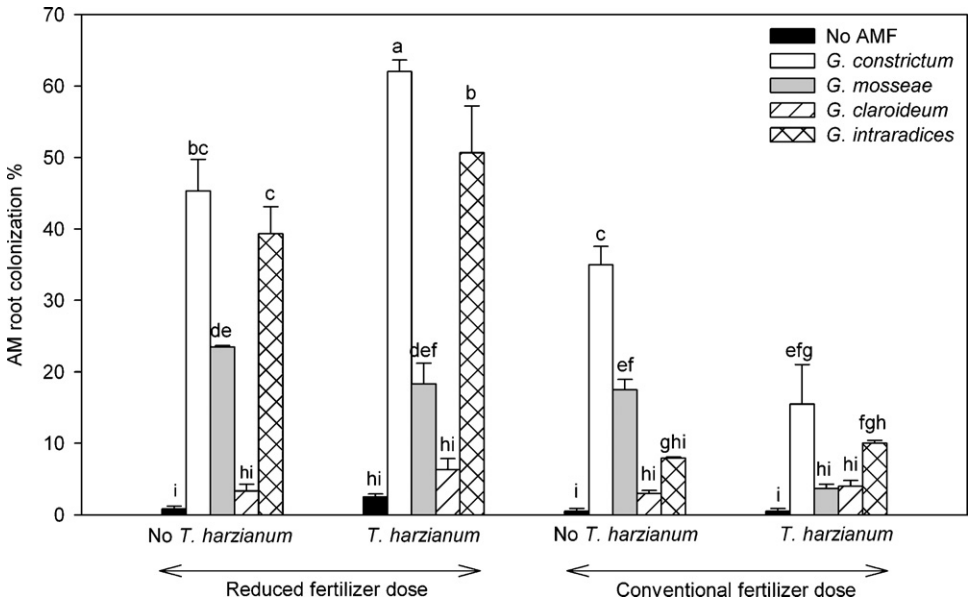

Fig. 1. Percentage of root length colonized by *Glomus constrictum*, *Glomus mosseae*, *Glomus claroideum*, and *Glomus intraradices* in melon plants receiving conventional or reduced fertilization and co-inoculated or not with *Trichoderma harzianum*. Bars indicate standard error of eight replicates. Values with the same letter do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$), $n = 8$.

4. Discussion

The results show a synergistic effect on AM root colonization due to the interaction between *T. harzianum* and *G. constrictum* or *G.intraradices*, while no significant effect was observed for *G. claroideum* and *G. mosseae*. Although saprophytic fungi have been reported to influence AM colonization and host plant response (Fracchia et al., 2000), the effects of the saprophytic fungi on AM formation differ depending on the inherent characteristic of both agents (Martinez et al., 2004; Saldajeno et al., 2008; Martínez-Medina et al., 2009a). A synergistic interaction between *T. aureoviride* and *G. mosseae* has been reported for AM root colonization (Calvet et al., 1993). Fracchia et al. (1998) found that *T. harzianum* did not affect the percentage of soybean root length colonized by *G. mosseae*, whereas *T. pseudokoningii* increased it. Calvet et al. (1992) reported a stimulation of *G. mosseae* spore germination by *T. harzianum* and *T. aureoviride*. The synergistic effect produced by the interaction between *T. harzianum* and *G. constrictum* or *G. intraradices* in our experiment could have been caused by a direct beneficial action of soluble exudates and volatile compounds produced by the saprophytic fungus (Calvet et al., 1992). No negative interaction was observed in our results, in contrast to previous results (McAllister et al., 1996; Green et al., 1999; Martinez et al., 2004).

Our results further demonstrate that, under reduced fertilizer dosage, AMF and *T. harzianum* inoculation resulted in an improvement in shoot weight and nutritional status. Soil microorganisms and their activities play important roles in the transformation

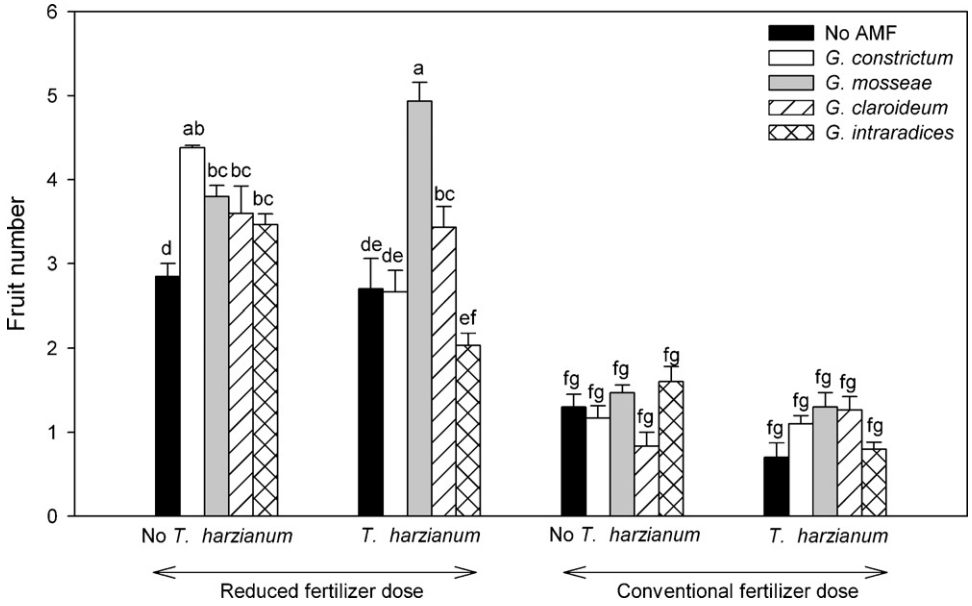

Fig. 2. Number of fruits produced by plants inoculated with *Trichoderma harzianum* and/or *Glomus constrictum*, *Glomus mosseae*, *Glomus claroideum*, or *Glomus intraradices*, under conventional and reduced fertilization doses. Bars indicate standard error of eight replicates. Values with the same letter do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$), $n = 8$.

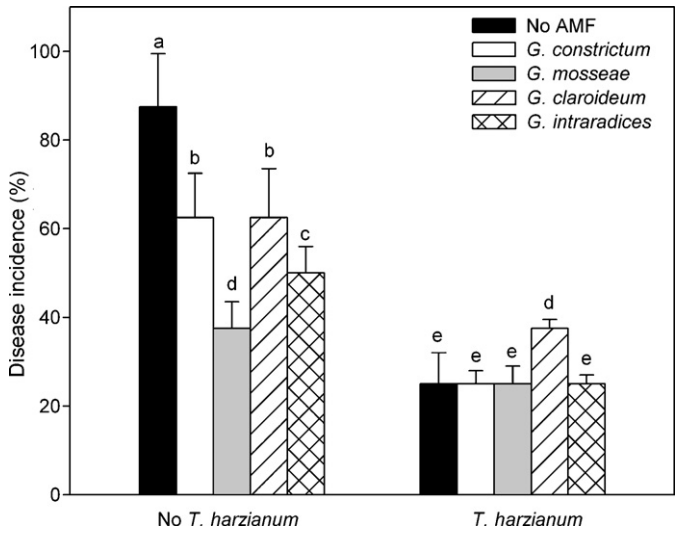

Fig. 3. Disease incidence (%) in plants inoculated with *Trichoderma harzianum* and/or *Glomus constrictum*, *Glomus mosseae*, *Glomus claroideum*, or *Glomus intraradices*, seven weeks after pathogen inoculation. Bars indicate standard error of eight replicates. Values with the same letter do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$), $n = 8$.

of plant nutrients from unavailable to available forms and the improvement of soil fertility (Adesemoye and Kloepper, 2009). The capacity of AMF to promote plant growth and enhance phosphorous availability and uptake has been widely reported over the years (Ames et al., 1983; Smith et al., 1997; Barea et al., 2002; Tawaraya et al., 2006). Several investigations indicated as well, that plant interaction with *Trichoderma* sp. correlates with improved phosphorous availability and plant growth (Harman and Björkman, 1998; Altomare et al., 1999). However, the combined inoculation of AMF and *T. harzianum* did not result in an additive effect. In general, for co-inoculated plants, both growth and nutrient uptake were maintained at values similar to those of plants inoculated with the AMF alone. In contrast to our results, Haggag and Abd-El latif (2001) found that the combined inoculation of *G. mosseae* and *T. harzianum* enhanced growth of geranium plants. Similarly, combined inoculation of *T. aureoviride* and *G. mosseae* had a synergistic effect on the growth of marigold plants (Calvet et al., 1993). However, root and shoot weights of soybean were decreased by co-inoculation with *T. pseudokoningii* and *Gigaspora rosea* (Martinez et al., 2004). The interaction between AMF and *T. harzianum* and its effect on plant growth may vary depending on the inherent characteristics of the AMF and the *T. harzianum* strain (Saldajeno et al., 2008). In our experiment, this interaction was in fact negative in the case of plants co-inoculated with *G. constrictum* and *T. harzianum*, which showed a decrease in nitrogen content relative to plants inoculated with the AMF or *T. harzianum* alone. However, an increase in the plant nitrogen content was observed in plants which had been co-inoculated with *T. harzianum* and *G. mosseae* in the greenhouse nursery, relative to plants inoculated with the saprophyte alone. Co-inoculation with *T. harzianum* and *G. mosseae* was more effective than any other combination tested with regard to increases in the uptake of nitrogen.

Under the conventional fertilizer dose, plant growth was not influenced by AM formation, but it was significantly increased when *T. harzianum* was inoculated alone. However, the growth promotion mediated by *T. harzianum* was decreased at this fertilizer rate. Rabeendran et al. (2000) hypothesized that when plants are grown under optimal conditions growth promotion by *Trichoderma* is unlikely, whereas under suboptimal conditions enhanced growth can be achieved. Our results show that differences in growth promotion by *T. harzianum* and AMF are related to differences in growing conditions, being more pronounced in soils relatively poor in nutrients. It is noteworthy that plants co-inoculated with the AMF and *T. harzianum* had growth which was similar to that of non-inoculated plants under these conditions.

The negative impact of high *N* and *P* levels on mycorrhizal root colonization has been reported (Rubio et al., 2002; Kohler et al., 2006). In our experiment, under the higher fertilization dose, the beneficial effect of the AMF disappeared and the effect was even negative in the case of phosphorus uptake. The suppression of extraradical mycelium development, which occurs in soil following a high fertilizer application (Azcón et al., 2003), could not explain our findings, since under this condition no effect on plant growth should be expected. This negative effect may be explained by an alteration in the rhizosphere microbial population due to the nutrient supply (Liu et al., 2000; Rengel and Marschner, 2005). Stimulation of the rhizospheric population may increase competition between plant roots and the microbial population, which has particular nutrient requirements (Germida et al., 1998; Griffiths et al., 1999), microorganisms being, in many cases, superior competitors (Kaye and Hart, 1997; Hodge et al., 2000). Similar results have been reported by Azcón et al. (2003), who observed that a higher application of nitrogen and phosphorus to the soil reduced the nutrient uptake in AM- compared with non-AM-lettuce plants. Our findings may indicate not only the lack of mycorrhizal benefit at these high fertilizer doses, but also a negative influence of AMF on mechanisms associated with the mineral nutrition of plants when grown in a highly fertilized soil. These results suggest that the beneficial mycorrhizal effect on plant nutrition is only evident under lower fertility levels and that fertilizer application can reduce or even eliminate it. Interestingly, a decrease in fruit number was observed due to an increase in the fertilizer dose. Imbalanced fertilizer use in soil has been reported to cause yield decline (Manna et al., 2005). In our experiment, *T. harzianum* was able to increase plant nitrogen uptake even at the higher fertilizer level. Our findings indicate an improvement in plant active-uptake mechanisms, and an increase in the effectiveness of nitrogen-containing fertilizer. These results contrast markedly with the absence of effects observed in tomato plants under fertilized conditions: no effects on plant nutritional status occurred following inoculation with *Trichoderma* (Inbar et al., 1994). The AMF-inoculated plants showed a significant decrease in Fusarium wilt incidence, *G. mosseae*-inoculated plants showing the greatest reduction. AM symbiosis has been shown to reduce the damage caused by soil-borne plant pathogens (Azcón-Aguilar and Barea, 1996; Bi et al., 2007). Competition for host photosynthates or sites, microbial changes in the mycorrhizosphere due to AM, and induction of local and systemic defense responses have been proposed (Azcón-Aguilar and Bago, 1994; Caron, 1989; Liu et al., 2007). With regard to suppressing disease incidence, *T. harzianum* was more effective than the AMF. Several studies report the biocontrol capacity of *Trichoderma* sp. (Chet, 1987; Chet et al., 1997; De Meyer et al., 1998; Yedidia et al., 1999; Harman, 2000; Howell et al., 2000; Yedidia et al., 2003; Shoresh et al., 2005; Martínez-Medina et al., 2009b). Various mechanisms of biocontrol have been reported, such as mycoparasitism, antibiotic production, competition, or induction of local and systemic defense responses (Howell, 2003; Yedidia et al., 2003; Harman et al., 2004). Co-inoculated plants showed disease suppression similar to that of *T. harzianum*-inoculated plants. Datnoff et al. (1995) reported a higher suppressive effect against Fusarium crown and root rot of tomato with the combination of *T. harzianum* and *G. intraradices* than with each biological agent applied alone. However, there are several examples of combinations of different biocontrol agents providing no better or, in some cases, worse biocontrol than the isolates used singly (Larkin and Fravel, 1998; de Boer et al., 1999).

In conclusion, the reduced-input system was more dependent on AMF than the conventionally managed, higher-input system. Co-inoculation of plants with AMF and *T. harzianum* did not result in an additive effect on plant growth and nutritional status. With respect to enhancement of nutrient uptake, co-inoculation with *T. harzianum* and *G. mosseae* resulted more effective than any other combination tested. The effect of the AMF was influenced by the fertilizer dose. Combinations of the AMF and *T. harzianum* were able to control Fusarium wilt more effectively than each AMF applied alone, but their effectiveness was similar to that of *T. harzianum* applied alone.

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