Journal of Plant Pathology

BENEFICIAL EFFECTS OF RHIZOPHAGUS IRREGULARIS AND TRICHODERMA ASPERELLUM STRAIN T34 ON GROWTH AND FUSARIUM WILT IN TOMATO PLANTS

--Manuscript Draft--

Manuscript Number:	JPPY-D-18-00296R1
Full Title:	BENEFICIAL EFFECTS OF RHIZOPHAGUS IRREGULARIS AND TRICHODERMA ASPERELLUM STRAIN T34 ON GROWTH AND FUSARIUM WILT IN TOMATO PLANTS
Article Type:	Review
Funding Information:	Agència de Gestió d'Ajuts Universitaris i de Recerca (2014SGR863) — Doctoral Student Boubker Bidellaoui-Idrissi Filali; Dr Maria Isabel Trillas; Dr Guillem Segarra; Dr Abdelkader Hakkou
Abstract:	Fusarium tomato wilt is one of the most prevalent and damaging diseases wherever tomatoes are grown intensively. Progress in agriculture in the 21st century is set to be based on lowering agrochemical inputs (implementation of Directive 2009/128/EC on sustainable use of pesticides), which can be achieved to some extent through the use of beneficial microorganisms. This study aimed at comparing the effects of the mycorrhizal fungus Rhizophagus irregularis and the biological control agent Trichoderma asperellum strain T34 on the incidence of fusarium wilt and the growth of tomato plants. Both R. irregularis and T34 lowered disease incidence at similar rates, compared to control plants. R. irregularis added below the seedlings reduced disease incidence more than when it was mixed with the substrate. T34 and R. irregularis increased plant height to the same extent, compared to both control and diseased plants. R. irregularis gave the highest levels of chlorophyll, followed by T34 and control plants; however, the measures for infected plants were slightly better for T34 than for R. irregularis. T34 and R. irregularis had similar effects on Ca, Mg, S, Mn, B and Si uptake in tomato plants, but R. irregularis induced a greater P, K, Zn, Cu and Mo accumulation than T34. Interestingly, at the end of the experiment, the depletion of the substrate was lower on Ca, Mg and S for plants inoculated with either R. irregularis or T34 compared to control plants, while the substrate for T34-treated plants had the lowest levels of Fe, Mn, Zn and Cu. Keywords: Biological control, Fusarium oxysporum, Lycopersicon esculentum Mill., mycorrhizae, plant nutrition.
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Response to Reviewers:	Hello, Your Honor; Mr. Prof. Giovanni P. Martelli Editor in Chief Journal of Plant Pathology I have reviewed the text, I have made the necessary changes that you have advised, to correspond to the references, I have put the missing ones and those that do not match and are deleted, as well as the formats that have to be changed. I hope this document is in good condition. Greetings. Regards.

Beneficial effects of *Rhizophagus irregularis* and *Trichoderma asperellum* strain T34 on growth and fusarium wilt in tomato plants

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SUMMARY

Fusarium tomato wilt is one of the most prevalent and damaging diseases wherever tomatoes are grown intensively. Progress in agriculture in the 21st century is set to be based on lowering agrochemical inputs (implementation of Directive 2009/128/EC on sustainable use of pesticides), which can be achieved to some extent through the use of beneficial microorganisms. This study aimed at comparing the effects of the mycorrhizal fungus *Rhizophagus irregularis* and the biological control agent *Trichoderma asperellum* strain T34 on the incidence of fusarium wilt and the growth of tomato plants. Both *R. irregularis* and T34 lowered disease incidence at similar rates, compared to control plants. *R. irregularis* added below the seedlings reduced disease incidence more than when it was mixed with the substrate. T34 and *R. irregularis* increased plant height to the same extent, compared to both control and diseased plants. *R. irregularis* gave the highest levels of chlorophyll, followed by T34 and control plants; however, the measures for infected plants were slightly better for T34 than for *R. irregularis*. T34 and *R. irregularis* had similar effects on Ca, Mg, S, Mn, B and Si uptake in tomato plants, but *R. irregularis* induced a greater P, K, Zn, Cu and Mo accumulation than T34. Interestingly, at the end of the experiment, the depletion of the substrate was lower on Ca, Mg and S for plants inoculated with either *R. irregularis* or T34 compared to control plants, while the substrate for T34-treated plants had the lowest levels of Fe, Mn, Zn and Cu.

Keywords: Biological control, *Fusarium oxysporum*, *Lycopersicon esculentum* Mill., mycorrhizae, plant nutrition.

INTRODUCTION

Agricultural production based on high yields, elevated fertiliser concentrations, intensive pest control and high water demand has resulted in soil and ground water contamination, nutrient depletion and reduced numbers of soil microorganisms. Chemical pesticides can successfully control plant diseases; however, their repeated use is not recommended because of the development of pathogen resistance, as well as for its adverse effects on animal and human health and on the environment. In Europe, under Directive 91/414, the use of chemical pesticides has been re-evaluated and 74% of the active ingredients in pesticides have been removed from the market (see http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/. Thus, 21st-century agriculture faces the challenge of maintaining food production and quality through safer and more sustainable management that will lead to lower environmental and economic costs. Bacteria and fungi are natural components of soil fertility, involved in fixation of atmospheric N_2 and solubilising phosphates, iron and other nutrients (Altomare and Tringovska, 2013; Azcón-Aguilar and Barea, 2015). A well-documented interaction between mycorrhizal fungi and plants is the exchange of plant carbohydrates for nitrogen, potassium, calcium, iron, copper, etc. (Smith, 1988; Franken *et al*., 2007). The non-pathogenic free-living fungi of the genus *Trichoderma*, which have previously been associated with mycoparasitism and antibiosis that can control soil-borne plant pathogens, have recently been linked to the promotion of plant growth. The convergence of certain effects of mycorrhizal and *Trichoderma* fungi is now well documented, such as the control of fungal disease by mycorrhizae and root colonisation by different *Trichoderma* spp. that improves nutrient absorption and plant growth (Bigirimana *et al*., 1997; Harman *et al*., 2004b; Howell *et al*., 2000; Segarra *et al*., 2009; Shoresh *et al*., 2005; Yedidia *et al*., 2003; De Meyer *et al*., 1998; Djonovic *et*

al., 2006, 2007; Harman *et al*. 2004a; Korolev *et al*., 2008; Segarra *et al*., 2007; Shoresh *et al*., 2005). Tomato is one of the most important horticultural crops worldwide, in which several races of *Fusarium oxysporum*f. sp. *lycopersici* cause severe wilt and death, reducing production in warm areas. In Spain, an estimated 1,084,600 tons of tomatoes were produced in 2016 (monthly statistical bulletin from the Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain, June 2017).

The objectives of this study were: (i) to evaluate the potential of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* as a biological control agent against fusarium wilt, in relation to the well-studied microbial control agent *Trichoderma asperellum* strain T34 (hereinafter T34) against this disease; and (ii) to compare the effects of T34 with *R. irregularis* on nutrient uptake and plant growth in tomato plants.

MATERIALS AND METHODS

Fungal inoculum preparation. The pathogen *F. oxysporum* f. sp. *lycopersici* race 2, isolate RAF 70, was obtained from the University of Seville and grown in a liquid medium containing 10 $gl^{-1}$of malt. The fungus was grown in a horizontal shaker operating at 150 rpm for seven days at room temperature. The culture was filtered through a 50µm nylon mesh to remove mycelium and centrifuged at 10,000 *g* (4°C) in a BeckmanJ-21C centrifuge. The pellet was washed twice in sterile distilled water to obtain medium-free conidia. A conidial suspension was prepared in sterile distilled water; the amount of conidia was determined with a haemocytometer and adjusted so as to inoculate at a concentration of $5x10^5$ conidia per ml of substrate.

A conidial suspension of the formulated product *Trichoderma asperellum* (Samuels *et al.*, 1999) strain T34 was adjusted so as to inoculate at a concentration of 10^4 conidia per ml of substrate. The substrate was incubated with T34 at room temperature for seven days, and populations at the beginning and end of the experiment were evaluated

by the dilution plate technique in semi-selective media (Chung, 1990), with 1-2 x10^4CFU of T34 present per ml of substrate at the beginning of the different experiments.

R. irregularis was obtained from Mycosym (Seville, Spain) and used at doses of 2-4% v/v (20-40 cm^3or 6-12 g l^{-1}) in the substrate, corresponding to 8ml per 400ml pot. The substrate used in all the experiments was a peat: vermiculite mixture (1:1, v/v) and the initial pH was adjusted to 6.0–6.5.

Plant material and bioassays. Tomato plants (*Lycopersicon esculentum* Mill. cv. 'Roma') from Semillas Fitó (Barcelona, Spain) were first germinated in substrate for 10 days. The plants were treated as described in Segarra *et al.* (2010), with some modifications. After the appearance of the second or third true leaf, four tomato seedlings were transplanted into 400ml pots. Five pots were used for each treatment, representing 20 plants per treatment, and each disease study was repeated three times. The pots were placed in a walk-in growth chamber at 25°C ± 2°C, under 16 h of light at an intensity of 150-210 µE m^{-2} s^{-1} PAR (Photosynthetically Active Radiation). The transplanted pots were irrigated daily with 100 or 200 ml of a nutrient solution, depending on the rate of plant development. The nutrient solution applied was Hoagland for all treatments, except for plants inoculated with mycorrhizal fungi, which were fertilised with Hoagland containing 68 ppm of phosphorous.

The substrates were inoculated with the pathogen, mixed vigorously and poured into the pots during transplanting of tomato seedlings. This was considered the beginning of the bioassay. Fifteen pots received substrate inoculated with the pathogen, while the other 15 pots were not inoculated and served as controls. *R. irregularis* was either mixed with the substrate or added below the tomato seedlings (two different sets of studies), with half of these pots being infected with the pathogen. T34 was incubated in the substrate

and added to a different set of pots, half of which were inoculated with the pathogen. Thus, there were six treatments: control; pathogen (*F. oxysporum* f. sp. *lycopersici*); *R. irregularis*; *R. irregularis* + pathogen; T34; andT34 + pathogen. Disease incidence (DI) was measured as the percentage of diseased plants out of the total number of plants evaluated five weeks after the beginning of the bioassay: scored as 0 for non-diseased plants and 1 for plants showing wilts symptom.

Plant growth measurements and substrate analysis. At the end of the experiment, the height of the plants, and their chlorophyll content together with the macro- and micronutrient levels in tomato leaves and substrate were recorded. For chlorophyll measurements, four expanded leaves from the same stage and treatment were analysed with a Minolta SPAD-502 chlorophyll meter (Plainfield, USA).

For substrate analysis, 2-3 samples from each treatment were analysed and 1.5 g of dried substrate was ground at room temperature, using a ball mill, to a particle size of less than 150 µm and digested with 10.5 ml HCl and 3.5 ml HNO_3. The solutions were kept for 16 h at room temperature, then heated (to 130ºC) at reflux for 2 h and further filtered. For the analysis of nutrients in the leaves, five well-developed leaves per treatment were analysed. For B, Mn, Zn, Cu, Mo and Ni analysis, samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin-Elmer ELAN 6000. For Ca, Fe, K, Mg, P, S and Si measurements, leaf samples were assessed by inductively coupled plasma optical emission spectrometry (ICP-OES), using a Perkin-Elmer Optima3200RL. A 45 mg leaf sample, dried at 60ºC, was prepared with an agate mortar and pestle and used for all analyses.

Statistical analysis. Analysis of variance (ANOVA) was performed using data on plant height, chlorophyll content and percentage of diseased plants, as well as on macro- and micronutrient levels in tomato plant leaves and substrate. When significant

differences were observed ($P<0.05$), Duncan's multiple range test was applied. Data were analysed with SPSS statistical software package version 18.

RESULTS

At the doses of phosphorus used, 68 ppm, tomato plants treated with *R. irregularis* achieved adequate growth over the duration of the study; below 50 ppm these plants showed lower growth rates. Of the plants inoculated with *R. irregularis* or T34 in the substrate, 30% and 32%, respectively, showed signs of disease, compared with 70% of the plants not inoculated with a beneficial fungus, which displayed fusarium wilt (Fig.1). The reductions in DI were thus 57% and 54% for plants inoculated with *R. irregularis* and T34, respectively. Similar results were attained in another set of studies where T34 was also mixed with the substrate, but *R. irregularis* added below the seedlings at transplantation. In these plants, the overall DI was lower. Plants not inoculated with either T34 or *R. irregularis* showed a DI of 58%, while those treated with T34 showed no DI and those exposed to *R. irregularis* presented a DI of 13% (Fig.2). The reductions in DI were thus 78% for *R. irregularis* and 100% for T34-treated plants. No plants grown in substrates without FOL inoculation developed any symptoms of fusarium wilt.

All plants inoculated with *R. irregularis* or T34 and not infected with the pathogenic *F. oxysporum* f. sp. *lycopersici* were taller than the control plants not inoculated with any of the fungi (Fig.3). The plants treated with either one of the beneficial fungi as well as FOL were also taller than FOL-infected plants not inoculated with *R. irregularis* or T34 (Fig.3). The plants treated with *R. irregularis* or T34 and not infected with FOL also exhibited significantly higher shoot dry weight (53% and 52% for *R. irregularis* added below the seedlings and T34, respectively; and 26% for *R.*

irregularis mixed into the substrate) than the non inoculated control plants (data not shown). *R. irregularis*-treated plants had the highest chlorophyll content, followed by those inoculated with T34 and by control plants (Fig. 4). *R. irregularis* or T34-treated plants infected with the pathogen showed the same pattern for chlorophyll content; with *F. oxysporum* f. sp. *lycopersici*-infected plants presenting the lowest chlorophyll content (Fig. 4).

R. irregularis-treated tomato plants not infected with *F. oxysporum* f. sp. *lycopersici* and fertilised with Hoagland solution containing low phosphorus content accumulated more P and K at the end of the experiment than T34-inoculated plants and the non-inoculated control plants (Table 1). T34-treated plants accumulated the same levels of Ca and Mg as those inoculated with *R. irregularis* in the substrate, which were higher than the amounts determined in control plants. However, S levels in plants treated with *R. irregularis* in the substrate were higher than, but not significantly different from those in T34-inoculated plants, which exhibited S levels that were similarly higher, but not significantly different from those of controls plants (Table 1). The levels of Fe and Cu were also the same in control and *R. irregularis* and T34-treated plants. Control plants showed lower levels of Mn, B, Zn and Si than inoculated plants, with the highest accumulation of Mn and B observed in plants treated with *R. irregularis* mixed with the substrate. Control and T34-inoculated plants showed the same levels of Mo, with *R. irregularis*-treated plants presenting a higher Mo concentration (Table 1).

At the end of the bioassays, nutrient levels were also different in the substrates inoculated with *R. irregularis*, T34 or not treated with either (the control substrate). The levels of P and K were higher in the *R. irregularis*-treated substrate than in the T34-treated and control substrates (Table 2). However, the control substrate accumulated the

highest concentrations of Ca, Mg and S. Meanwhile, Ca and Mg levels were higher in the substrate with *R. irregularis* mixed into it than for both *R. irregularis* added below the plants and T34; with S levels being the same for all the inoculated substrates (Table 2). Substrate amounts of Fe, Mn and Cu were highest for *R. irregularis* mixed into the substrate, followed by the control, then by *R. irregularis* added below the seedlings, and finally by T34. The levels of B were higher in the *R. irregularis*-inoculated substrate than the T34-treated and control substrates. Mo concentrations were the same in all the substrates.

DISCUSSION

Strains of *Trichoderma* spp. are registered and used in agriculture as biopesticides under EU Regulation 1107/2009, while mycorrhizal isolates will be used as microbial fertilisers in European agriculture, according to the European Commission Brussels, 17.3.2016 COM(2016) 157 final draft 2016/0084 (COD). However, there is a consistent body of evidence demonstrating that some mycorrhizal isolates can protect plants against soil-borne plant pathogens (Martínez-Medina *et al.*, 2011 a, b) and that some *Trichoderma* spp. isolates enhance plant growth and development (López-Bucio *et al.*, 2015).

In the present study, inoculation with either T34 or *R. irregularis* had a markedly positive influence on the health of tomato plants by reducing the DI caused by *F. oxysporum* f. sp. *lycopersici*. Furthermore, *R. irregularis* added directly below the plant performed better in reducing DI (by 77%) than when it was mixed with the substrate (55% reduction in DI). However, T34, which was always mixed with the substrate, produced reductions in DI of 57% and 100%. Similar results have previously been reported for T34 on the same disease and crop (Cotxarrera *et al.*, 2002; Nogues *et al.*, 2002; Borrero *et al.*, 2012) and also on carnation wilt (Sant *et al.*, 2010), as well as

against other soil plant pathogens (Trillas *et al*., 2006; Segarra *et al*., 2013). Several studies demonstrate similar effects of other strains of different *Trichoderma* spp. on fusarium wilt and other soil diseases (Harman *et al.*, 2004a; Howell, 2003; Vinale, 2008; Dubey, 2007; Verma, 2007; Singh *et al*., 2014).

The performance of different arbuscular mycorrhizal strains against fusarium wilt in melon plants varies widely: a DI of around 40% for *Glomus mosseae*, and DI of around 50% for *Glomus intraradices* (now known as *R. irregularis*), compared to a DI of over 80% for the control (Martínez-Medina, 2011a). Inoculation with each of these mycorrhizae together with *T. harzianum* (CECT 20714) further reduced DI to around 20% (Martínez-Medina, 2011a). In another study by the same author (Martínez-Medina, 2011b), the combination of *T. harzianum* and *G. intraradices* reduced DI to 13%, while *G. Mosseae* or *T. harzianum* or their combination did not significantly improve DI. In a study of *Phytophthora parasitica* infection in papaya, *G. mosseae* or *T. harzianum* (strain IIHR-Th49) reduced DI by 75.5%, while their combination reduced DI by up to 90% (Sukhada *et al*., 2011).

T34 and *R. irregularis* promoted plant growth (height and dry weight) to the same extent, compared to control; but their effects on chlorophyll levels were not the same. The chlorophyll content in leaves was highest in *R. irregularis*-inoculated plants, followed by T34-treated plants and control. Diseased control plants displayed the lowest chlorophyll content.

Our results showing that *R. irregularis* promotes plant nutrient uptake are in agreement with those of other studies. It has been well established that *R. irregularis* significantly increases uptake of the macronutrients P, K and S. as well as uptake of the micronutrient B in tomato plants (Cardoso and Kuyper, 2006; Altomare and Tringovska, 2011; Smith and Smith, 2015). Moreover, studies of mycorrhizal fungi

have also reported increased uptake of Ca, Mg, Mn and Si. The reported effects of arbuscular mycorrhizae on Fe uptake vary (Altomare and Tringovska, 2011); and this is consistent with our observations of *R. irregularis* leading to an increase (when mixed with the substrate) or decrease (when added under the seedlings) in Fe uptake, compared to control plants. T34, with respect to the control plants, also significantly improved the uptake of Ca, Mg, Mn, B and Si, while moderately improving S uptake. Similar results have been obtained for other *Trichoderma* strains (*T. asperellum* T203 and *T. harzianu*m T22 (Yedidia *et al*., 2001; Kaya *et al*., 2009). It has been reported that strain T22 produces diffusible metabolites that can reduce Fe(III) and Cu(II) (Altomare and Tringovska, 1999); however, that study was performed in sucrose yeast extract rather than plants.

In the conditions studied (non-restrictive nutrition), T34 did not mobilise, solubilise or improve P or K uptake by tomato plants, as observed with other *Trichoderma* spp. However, in a siliceous growing medium fertilised with low P levels, T34 and *Bacillus subtilis* strain QST713 have been shown to significantly increase total P levels in the shoots (García-López *et al*., 2016). Our observation of depleted macroelements (Ca and Mg) and microelements (Fe, Mn, Zn and Cu) in the substrate of T34-inoculated plants is very important and constitutes new information concerning the mechanisms where by T34 reduces fusarium wilt. Competition for iron leading to reduced incidence of fusarium wilt has previously been established for T34 (Segarra *et al*., 2010). Furthermore, non-pathogenic *Fusarium* species produce more siderophores than the pathogenic species and are able to compete more effectively for iron, thus, suppressing pathogenic species (Lemanceau *et al*., 1985). It has also been reported that in calcareous soil, T34 increases the Fe concentration in wheat plants grown in Fe-

deficient media, but has no significant effect in Fe-enriched soil (de Santiago *et al*., 2011).

The use of *Trichoderma* spp. in commercial greenhouses for intensive crop production is widely accepted; however, the utilisation of mycorrhizae has certain restrictions due to the effect of fertiliser dose on mycorrhizal activity (Martínez-Medina *et al*., 2011b). According to the draft of the directive concerning fertilisers in Europe, mychorrizal fungi *per se* will be employed as microbial fertilisers, while *Trichoderma*, *Bacillus* and *Pseudomonas* species will be used as plant protection agents. It would be interesting to study nutrient uptake in plants (Kragelund and Nybroe, 1996; Raaijmakers *et al*., 1995) and/or DIs (Nahalkova *et al*., 2008; Olivain *et al*., 2006) in agricultural systems with a complex and rich microbial community using lower levels of chemical fertilisers or pesticides.

In summary, plants inoculated with *R. irregularis* under the seedling and fertilised with Hoagland solution containing half the phosphorus content showed improved growth and nutrient uptake. Furthermore, *R. irregularis* protected plants against fusarium wilt at a rate similar to that achieved with the biological control agent T34. T34 also promoted the solubilisation of mineral elements, enhancing plant nutrient absorption and growth.

ACKNOWLEDGEMENTS

This research was partially funded by AGAUR (*Generalitat de Catalunya* regional authority) via the project 2014SGR863: *Fisiologia de les plantes enrelació amb l'ambient.*

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Table 1. Mineral elements in tomato plants *Lycopersicon esculentum* Mill. cv. Roma, at the end of the bioassays. Plants were grown in a substrate that was not inoculated (control) or inoculated with either *Rhizophagus irregularis* or *Trichoderma asperellum* strain T34. Plants inoculated with *R. irregularis* were cultivated with Hoagland solution containing half the phosphorus content.

Mineral element in plant (dry weight)	Control	*T. asperellum* (T34) mixed in substrate	*R. irregularis* mixed in substrate	*R. irregularis* below seedlings
P (mg/plant)	6.33 ± 0.27a	8.63 ± 0.61a	18.95 ± 1.40b	17.52 ± 1.39b
K (mg/plant)	26.51 ± 5.47a	24.12 ± 4.43a	48.99 ± 3.90b	65.36 ± 4.26c
Ca (mg/plant)	39.92 ± 0.17a	69.29 ± 9.62b	62.49 ± 1.70b	63.87 ± 2.75b
Mg (mg/plant)	22.52 ± 0.31a	31.61 ± 3.21b	30.54 ± 0.62b	28.93 ± 1.23b
S (mg/plant)	11.87 ± 0.30a	19.91 ± 1.92ab	24.61 ± 2.42b	27.27 ± 2.34b
Fe (mg/plant)	0.14 ± 0.05a	0.16 ± 0.03a	0.24 ± 0.04a	0.23 ± 0.01a
Mn (mg/plant)	0.30 ± 0.01a	0.42 ± 0.00b	0.54 ± 0.02c	0.43 ± 0.02b
B (mg/plant)	0.08 ± 0.00a	0.14 ± 0.02b	0.19 ± 0.00c	0.18 ± 0.00bc
Zn (µg/plant)	19.94 ± 6.07a	16.68 ± 1.08a	45.79 ± 7.77b	47.57 ± 3.39b
Cu (µg/plant)	3.60 ± 0.92a	4.16 ± 1.52a	8.75 ± 1.59b	8.58 ± 1.08b
Mo (µg/plant)	5.77 ± 0.48a	7.78 ± 0.48a	9.17 ± 0.59ab	13.12 ± 1.76b
Si (µg/plant)	1.38 ± 0.10a	3.10 ± 0.63b	3.84 ± 0.15b	3.34 ± 0.25b

Values for macronutrients and micronutrients are given as mean ± standard error of 6 leaves per treatment collected from 3 replicates. Different letters indicate statistically significant differences, $P<0.05$, according to Duncan's multiple range test.

Table 2. Macronutrient (mg g^{-1}) and micronutrient (µg g^{-1}) concentrations per dry weight of substrate at the end of the bioassays. Substrates were not inoculated (control) or inoculated with either *Rhizophagus irregularis* or *Trichoderma asperellum* strain T34. Plants inoculated with *R. irregularis* were cultivated with Hoagland solution containing half the phosphorus content.

Mineral element in substrate	Control	*T. asperellum* (T34) mixed in substrate	*R. irregularis* mixed in substrate	*R. irregularis* below seedlings
P (mg g^{-1})	0.45 ± 0.02a	0.45 ± 0.00a	2.20 ± 0.02b	2.22 ± 0.02b
K (mg g^{-1})	0.45 ± 0.01a	0.46 ± 0.00a	1.50 ± 0.02b	1.61 ± 0.00c
Ca (mg g^{-1})	15.64 ± 0.15c	12.06 ± 0.03a	14.33 ± 0.06b	11.89 ± 0.14a
Mg (mg g^{-1})	72.99 ± 0.58d	55.04 ± 0.58b	57.09 ± 0.32c	46.22 ± 0.52a
S (mg g^{-1})	0.96 ± 0.05b	0.58 ± 0.13a	0.64 ± 0.05a	0.34 ± 0.02a
Fe (mg g^{-1})	17.50 ± 0.15c	13.36 ± 0.15a	19.35 ± 0.13d	16.38 ± 0.15b
Mn(µg g^{-1})	0.27 ± 0.00c	0.21 ± 0.00a	0.26 ± 0.00c	0.23 ± 0.00b
B(µg g^{-1})	5.01 ± 1.08a	3.68 ± 1.10a	11.55 ± 0.12b	12.64 ± 0.05b
Zn(µg g^{-1})	35.79 ± 0.84b	26.68 ± 0.07a	60.87 ± 0.14d	57.33 ± 1.20c
Cu(µg g^{-1})	6.85 ± 0.14b	5.22 ± 0.03a	10.40 ± 0.05d	9.42 ± 0.18c
Mo(µg g^{-1})	1.62 ± 0.41a	0.96 ± 0.00a	1.08 ± 0.00a	0.98 ± 0.03a

Values for macronutrients and micronutrients are given as mean ± standard error of 3 replicates of substrate samples per treatment. Different letters indicate statistically significant differences, $P<0.05$, according to Duncan's multiple range test.

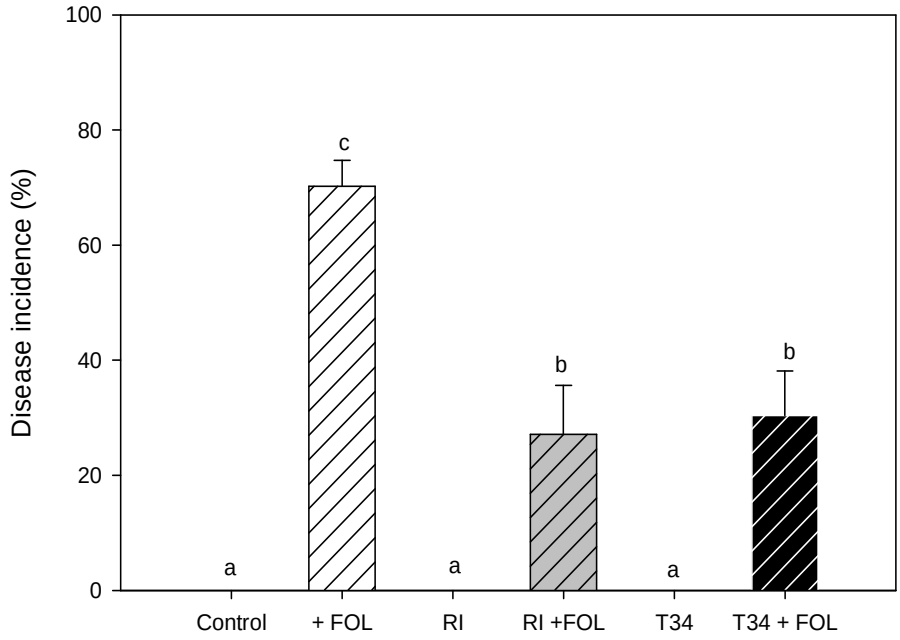
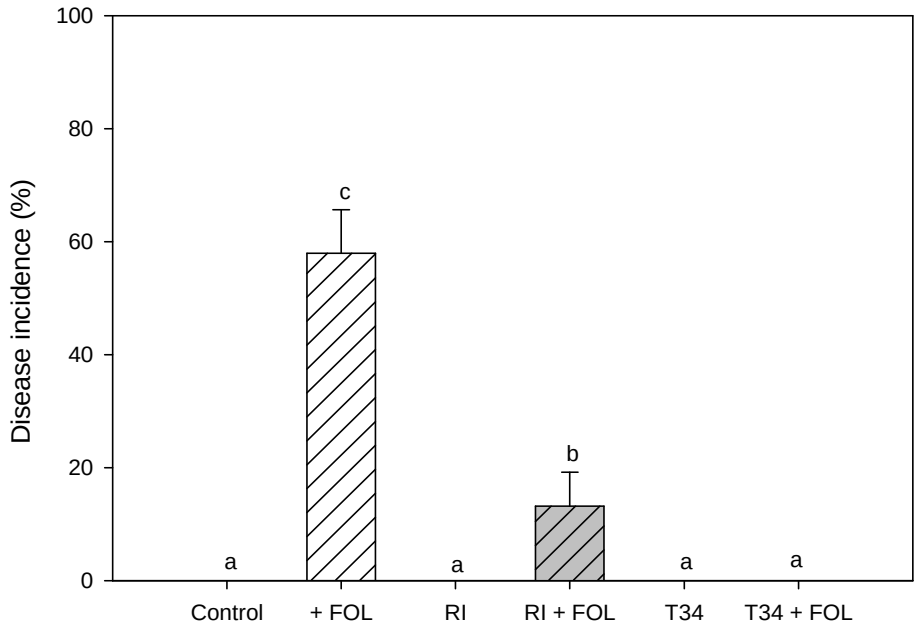


Fig. 1. Disease incidence (%) in tomato plants *Lycopersicon esculentum* Mill. cv. Roma. Control, non-infected plants; FOL, plants infected with *F. oxysporum* f. sp. *lycopersici* at a concentration of $5x10^5$ conidia per ml of substrate; RI, plants inoculated with *Rhizophagus irregularis*; RI + FOL, plants inoculated with *R. irregularis* and infected with FOL; T34, plants inoculated with *Trichoderma asperellum* strain T34; T34 + FOL, plants inoculated with *T. asperellum* strain T34 and infected with FOL. *R. irregularis* was mixed into the substrate at doses of 2%-4% v/v (20-40 cm^3 or 6-12 $g\ l^{-1}$), while *T. asperellum* strain T34 was incubated in the substrate for 7 days at a concentration of 10^4cfu per ml of substrate.



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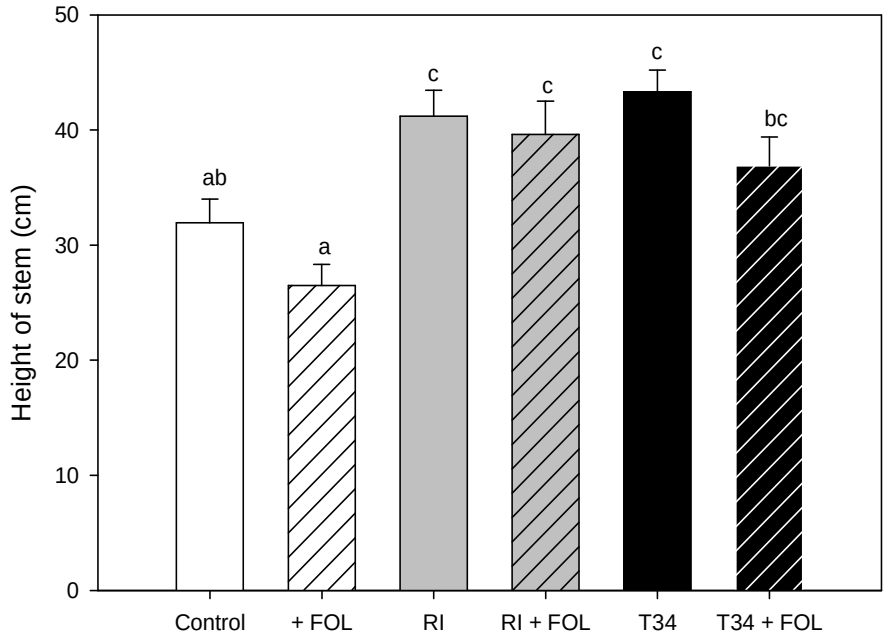

Fig. 3. Height of tomato plants *Lycopersicon esculentum* Mill. cv. Roma. Control, non-infected plants; FOL, plants infected with *F. oxysporum* f. sp. *lycopersici* at a concentration of $5x10^5$ conidia per ml of substrate; RI, plants inoculated with *Rhizophagus irregularis*; RI + FOL, plants inoculated with *R. irregularis* and infected with FOL; T34, plants inoculated with *Trichoderma asperellum* strain T34; T34 + FOL, plants inoculated with *T. asperellum* strain T34 and infected with FOL. *R. irregularis* was added below the seedlings during transplantation at doses of 2%-4% v/v (20-40 cm^3 or 6-12 g l^{-1}), while *T. asperellum* strain T34 was incubated in the substrate for 7 days at a concentration of 10^4cfu per ml of substrate.

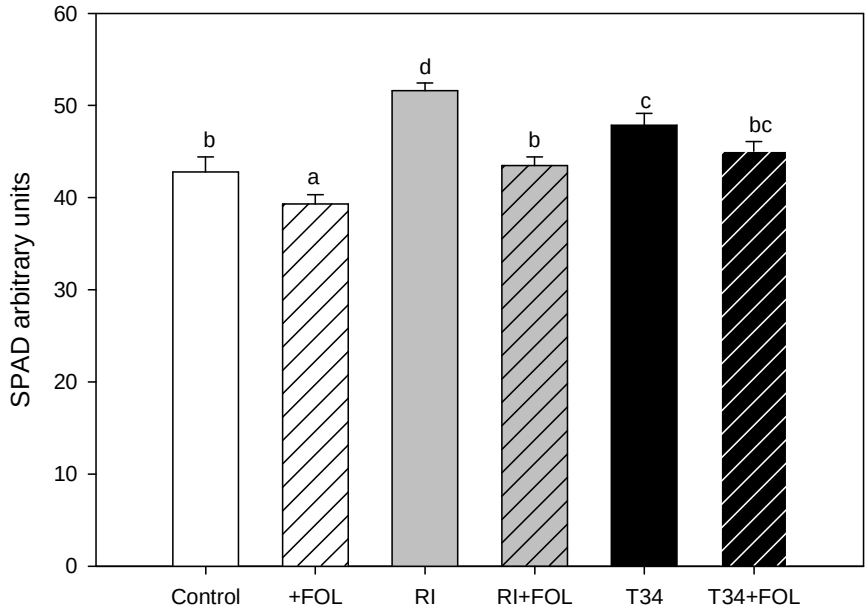

Fig. 4. Leaf chlorophyll content of tomato plants *Lycopersicon esculentum* Mill. cv. Roma, as measured by a SPAD chlorophyll meter. Control, non-infected plants; FOL, plants infected with *F. oxysporum* f. sp. *lycopersici* at a concentration of $5x10^5$ conidia per ml of substrate; RI, plants inoculated with *Rhizophagus irregularis*; RI + FOL, plants inoculated with *R. irregularis* and infected with FOL; T34, plants inoculated with *Trichoderma asperellum* strain T34; T34 + FOL, plants inoculated with *T. asperellum* strain T34 and infected with FOL. *R. irregularis* was added below the seedlings during transplantation at doses of 2%-4% v/v (20-40 cm^3 or 6-12 g l^{-1}), while *T. asperellum* strain T34 was incubated in the substrate for 7 days at a concentration of 10^4cfu per ml of substrate.