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

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Abstract:	<p>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 <i>Rhizophagus irregularis</i> and the biological control agent <i>Trichoderma asperellum</i> strain T34 on the incidence of fusarium wilt and the growth of tomato plants. Both <i>R. irregularis</i> and T34 lowered disease incidence at similar rates, compared to control plants. <i>R. irregularis</i> added below the seedlings reduced disease incidence more than when it was mixed with the substrate. T34 and <i>R. irregularis</i> increased plant height to the same extent, compared to both control and diseased plants. <i>R. irregularis</i> 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 <i>R. irregularis</i>. T34 and <i>R. irregularis</i> had similar effects on Ca, Mg, S, Mn, B and Si uptake in tomato plants, but <i>R. irregularis</i> 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 <i>R. irregularis</i> or T34 compared to control plants, while the substrate for T34-treated plants had the lowest levels of Fe, Mn, Zn and Cu.</p> <p>Keywords: Biological control, <i>Fusarium oxysporum</i>, <i>Lycopersicon esculentum</i> Mill., mycorrhizae, plant nutrition.</p>	
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1 **Beneficial effects of *Rhizophagus irregularis* and *Trichoderma asperellum* strain**
2 **T34 on growth and fusarium wilt in tomato plants**

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11 Running Title: Tomato plant health is improved by microorganisms

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18 **SUMMARY**

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

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

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42 INTRODUCTION

43 Agricultural production based on high yields, elevated fertiliser concentrations,
44 intensive pest control and high water demand has resulted in soil and ground water
45 contamination, nutrient depletion and reduced numbers of soil microorganisms.
46 Chemical pesticides can successfully control plant diseases; however, their repeated use
47 is not recommended because of the development of pathogen resistance, as well as for
48 its adverse effects on animal and human health and on the environment. In Europe,
49 under Directive 91/414, the use of chemical pesticides has been re-evaluated and 74%
50 of the active ingredients in pesticides have been removed from the market (see
51 <http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/>. Thus, 21st-
52 century agriculture faces the challenge of maintaining food production and quality
53 through safer and more sustainable management that will lead to lower environmental
54 and economic costs. Bacteria and fungi are natural components of soil fertility, involved
55 in fixation of atmospheric N₂ and solubilising phosphates, iron and other nutrients
56 (Altomare and Tringovska, 2013; Azcón-Aguilar and Barea, 2015). A well-documented
57 interaction between mycorrhizal fungi and plants is the exchange of plant carbohydrates
58 for nitrogen, potassium, calcium, iron, copper, etc. (Smith, 1988; Franken *et al.*, 2007).
59 The non-pathogenic free-living fungi of the genus *Trichoderma*, which have previously
60 been associated with mycoparasitism and antibiosis that can control soil-borne plant
61 pathogens, have recently been linked to the promotion of plant growth. The
62 convergence of certain effects of mycorrhizal and *Trichoderma* fungi is now well
63 documented, such as the control of fungal disease by mycorrhizae and root colonisation
64 by different *Trichoderma* spp. that improves nutrient absorption and plant growth
65 (Bigirimana *et al.*, 1997; Harman *et al.*, 2004b; Howell *et al.*, 2000; Segarra *et al.*,
2009; Shores *et al.*, 2005; Yedidia *et al.*, 2003; De Meyer *et al.*, 1998; Djonovic *et*

67 *al.*, 2006, 2007; Harman *et al.* 2004a; Korolev *et al.*, 2008; Segarra *et al.*, 2007; Shoresh
68 *et al.*, 2005). Tomato is one of the most important horticultural crops worldwide, in
69 which several races of *Fusarium oxysporum* f. sp. *lycopersici* cause severe wilt and
70 death, reducing production in warm areas. In Spain, an estimated 1,084,600 tons of
71 tomatoes were produced in 2016 (monthly statistical bulletin from the Ministerio de
72 Agricultura, Alimentación y Medio Ambiente, Spain, June 2017).
73 The objectives of this study were: (i) to evaluate the potential of the arbuscular
74 mycorrhizal fungus *Rhizophagus irregularis* as a biological control agent against
75 fusarium wilt, in relation to the well-studied microbial control agent *Trichoderma*
76 *asperellum* strain T34 (hereinafter T34) against this disease; and (ii) to compare the
77 effects of T34 with *R. irregularis* on nutrient uptake and plant growth in tomato plants.

78 MATERIALS AND METHODS

79 **Fungal inoculum preparation.** The pathogen *F. oxysporum* f. sp. *lycopersici* race
80 2, isolate RAF 70, was obtained from the University of Seville and grown in a liquid
81 medium containing 10 g^l-1 of malt. The fungus was grown in a horizontal shaker
82 operating at 150 rpm for seven days at room temperature. The culture was filtered
83 through a 50µm nylon mesh to remove mycelium and centrifuged at 10,000 g (4°C) in a
84 BeckmanJ-21C centrifuge. The pellet was washed twice in sterile distilled water to
85 obtain medium-free conidia. A conidial suspension was prepared in sterile distilled
86 water; the amount of conidia was determined with a haemocytometer and adjusted so as
87 to inoculate at a concentration of 5x10⁵ conidia per ml of substrate.

88 A conidial suspension of the formulated product *Trichoderma asperellum* (Samuels
89 *et al.*, 1999) strain T34 was adjusted so as to inoculate at a concentration of 10⁴ conidia
90 per ml of substrate. The substrate was incubated with T34 at room temperature for
91 seven days, and populations at the beginning and end of the experiment were evaluated

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92 by the dilution plate technique in semi-selective media (Chung, 1990), with 1-2
93 $\times 10^4$ CFU of T34 present per ml of substrate at the beginning of the different
94 experiments.

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95 *R. irregularis* was obtained from Mycosym (Seville, Spain) and used at doses of 2-
96 4% v/v (20-40 cm³ or 6-12 g l⁻¹) in the substrate, corresponding to 8ml per 400ml pot.
97 The substrate used in all the experiments was a peat: vermiculite mixture (1:1, v/v) and
98 the initial pH was adjusted to 6.0–6.5.

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

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111 The substrates were inoculated with the pathogen, mixed vigorously and poured into the
112 pots during transplanting of tomato seedlings. This was considered the beginning of the
113 bioassay. Fifteen pots received substrate inoculated with the pathogen, while the other
114 15 pots were not inoculated and served as controls. *R. irregularis* was either mixed with
115 the substrate or added below the tomato seedlings (two different sets of studies), with
116 half of these pots being infected with the pathogen. T34 was incubated in the substrate

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

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

128 For substrate analysis, 2-3 samples from each treatment were analysed and 1.5 g of
129 dried substrate was ground at room temperature, using a ball mill, to a particle size of
130 less than 150 μm and digested with 10.5 ml HCl and 3.5 ml HNO₃. The solutions were
131 kept for 16 h at room temperature, then heated (to 130°C) at reflux for 2 h and further
132 filtered. For the analysis of nutrients in the leaves, five well-developed leaves per
133 treatment were analysed. For B, Mn, Zn, Cu, Mo and Ni analysis, samples were
134 measured by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin-
135 Elmer ELAN 6000. For Ca, Fe, K, Mg, P, S and Si measurements, leaf samples were
136 assessed by inductively coupled plasma optical emission spectrometry (ICP-OES),
137 using a Perkin-Elmer Optima3200RL. A 45 mg leaf sample, dried at 60°C, was prepared
138 with an agate mortar and pestle and used for all analyses.

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

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

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145 **RESULTS**

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

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

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

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

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

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

200 **DISCUSSION**

201 Strains of *Trichoderma* spp. are registered and used in agriculture as biopesticides
202 under EU Regulation 1107/2009, while mycorrhizal isolates will be used as microbial
203 fertilisers in European agriculture, according to the European Commission Brussels,
204 17.3.2016 COM(2016) 157 final draft 2016/0084 (COD). However, there is a consistent
205 body of evidence demonstrating that some mycorrhizal isolates can protect plants
206 against soil-borne plant pathogens (Martínez-Medina *et al.*, 2011 a, b) and that some
207 *Trichoderma* spp. isolates enhance plant growth and development (López-Bucio *et al.*,
208 2015).

209 In the present study, inoculation with either T34 or *R. irregularis* had a markedly
210 positive influence on the health of tomato plants by reducing the DI caused by *F.*
211 *oxysporum* f. sp. *lycopersici*. Furthermore, *R. irregularis* added directly below the plant
212 performed better in reducing DI (by 77%) than when it was mixed with the substrate
213 (55% reduction in DI). However, T34, which was always mixed with the substrate,
214 produced reductions in DI of 57% and 100%. Similar results have previously been
215 reported for T34 on the same disease and crop (Cotxarrera *et al.*, 2002; Nogues *et al.*,
216 2002; Borrero *et al.*, 2012) and also on carnation wilt (Sant *et al.*, 2010), as well as

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217 against other soil plant pathogens (Trillas *et al.*, 2006; Segarra *et al.*, 2013). Several
218 studies demonstrate similar effects of other strains of different *Trichoderma* spp. on
219 fusarium wilt and other soil diseases (Harman *et al.*, 2004a; Howell, 2003; Vinale,
220 2008; Dubey, 2007; Verma, 2007; Singh *et al.*, 2014).

221 The performance of different arbuscular mycorrhizal strains against fusarium wilt in
222 melon plants varies widely: a DI of around 40% for *Glomus mosseae*, and DI of around
223 50% for *Glomus intraradices* (now known as *R. irregularis*), compared to a DI of over
224 80% for the control (Martínez-Medina, 2011a). Inoculation with each of these
225 mycorrhizae together with *T. harzianum* (CECT 20714) further reduced DI to around
226 20% (Martínez-Medina, 2011a). In another study by the same author (Martínez-
227 Medina, 2011b), the combination of *T. harzianum* and *G. intraradices* reduced DI to
228 13%, while *G. Mosseae* or *T. harzianum* or their combination did not significantly
229 improve DI. In a study of *Phytophthora parasitica* infection in papaya, *G. mosseae* or
230 *T. harzianum* (strain IIHR-Th49) reduced DI by 75.5%, while their combination
231 reduced DI by up to 90% (Sukhada *et al.*, 2011).

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

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

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

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

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266 deficient media, but has no significant effect in Fe-enriched soil (de Santiago *et al.*,
267 2011).

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

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

284

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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	<i>T. asperellum</i> (T34) mixed in substrate	<i>R. irregularis</i> mixed in substrate	<i>R. irregularis</i> 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 ($\mu\text{g g}^{-1}$) 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	<i>T. asperellum</i> (T34) mixed in substrate	<i>R. irregularis</i> mixed in substrate	<i>R. irregularis</i> 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 ($\mu\text{g g}^{-1}$)	0.27 ± 0.00c	0.21 ± 0.00a	0.26 ± 0.00c	0.23 ± 0.00b
B ($\mu\text{g g}^{-1}$)	5.01 ± 1.08a	3.68 ± 1.10a	11.55 ± 0.12b	12.64 ± 0.05b
Zn ($\mu\text{g g}^{-1}$)	35.79 ± 0.84b	26.68 ± 0.07a	60.87 ± 0.14d	57.33 ± 1.20c
Cu ($\mu\text{g g}^{-1}$)	6.85 ± 0.14b	5.22 ± 0.03a	10.40 ± 0.05d	9.42 ± 0.18c
Mo ($\mu\text{g g}^{-1}$)	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.

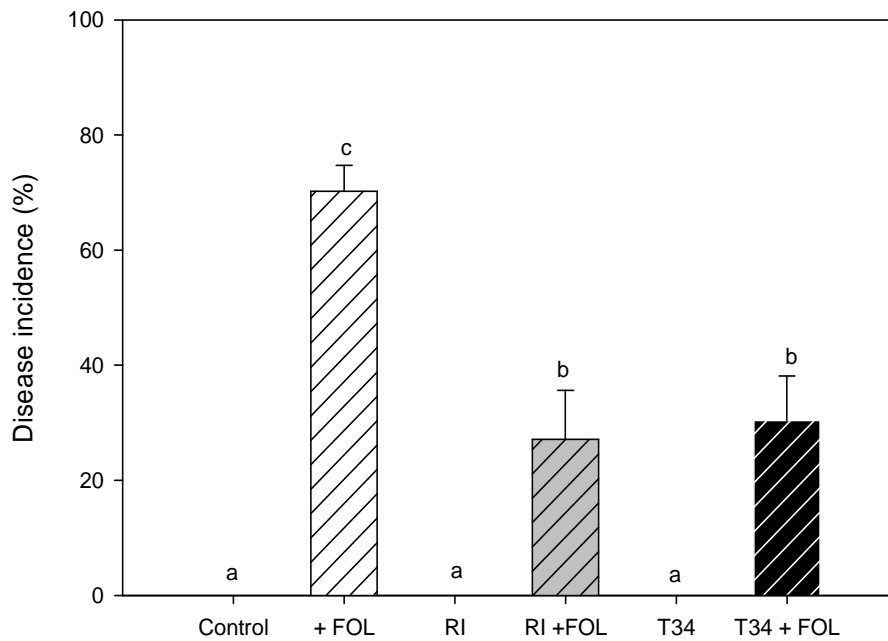


Fig. 1. Disease incidence (%) in tomato plants *Lycopersicon esculentum* Mill. cv. Roma. Control, non-infected plants; FOL, plants infected with *F. oxysporum* f. sp. *lycopersici* at a concentration of 5×10^5 conidia per ml of substrate; RI, plants inoculated with *Rhizophagus irregularis*; RI + FOL, 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 cm³ or 6-12 g l⁻¹), while *T. asperellum* strain T34 was incubated in the substrate for 7 days at a concentration of 10^4 cfu per ml of substrate.

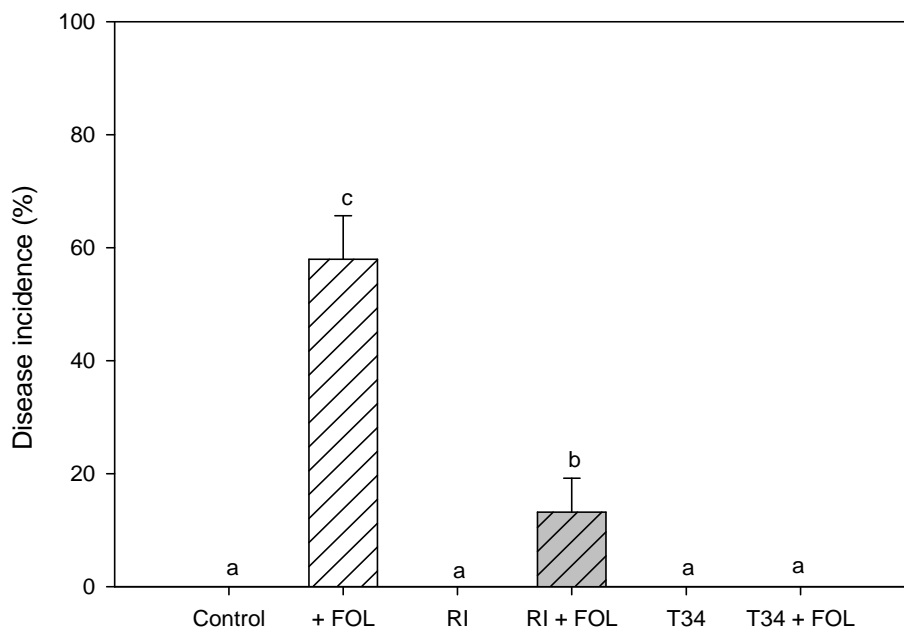


Fig. 2. Disease incidence (%) in tomato plants *Lycopersicon esculentum* Mill. cv. Roma. Control, non-infected plants; FOL, plants infected with *F. oxysporum* f. sp. *lycopersici* at a concentration of 5×10^5 conidia per ml of substrate; RI, plants inoculated with *Rhizoglyphus irregularis*; RI + FOL, 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 cm³ or 6-12 g l⁻¹), while *T. asperellum* strain T34 was incubated in the substrate for 7 days at a concentration of 10^4 cfu per ml of substrate.

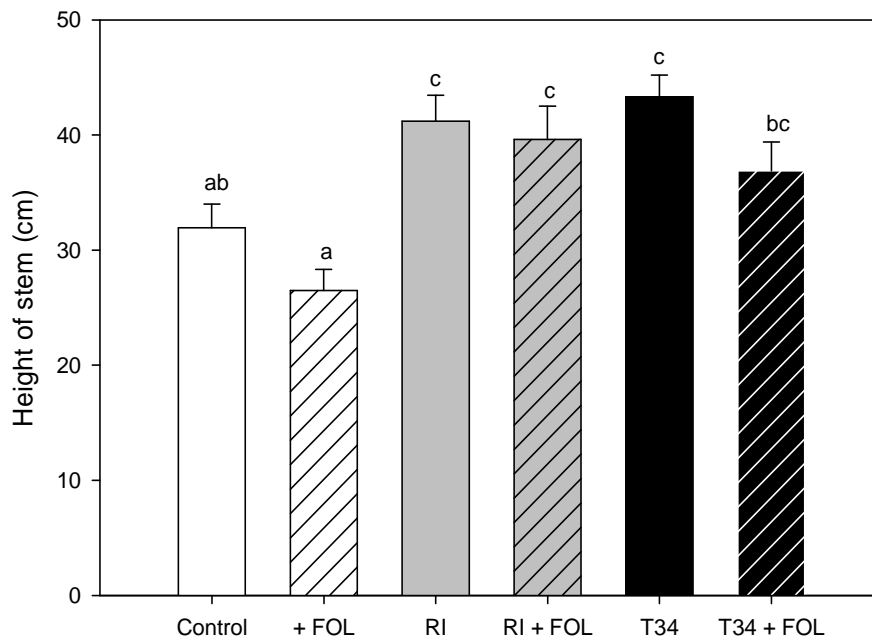


Fig. 3. Height of tomato plants *Lycopersicon esculentum* Mill. cv. Roma. Control, non-infected plants; FOL, plants infected with *F. oxysporum* f. sp. *lycopersici* at a concentration of 5×10^5 conidia per ml of substrate; RI, plants inoculated with *Rhizoglyphus irregularis*; RI + FOL, plants inoculated 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 cm³ or 6-12 g l⁻¹), while *T. asperellum* strain T34 was incubated in the substrate for 7 days at a concentration of 10^4 cfu per ml of substrate.

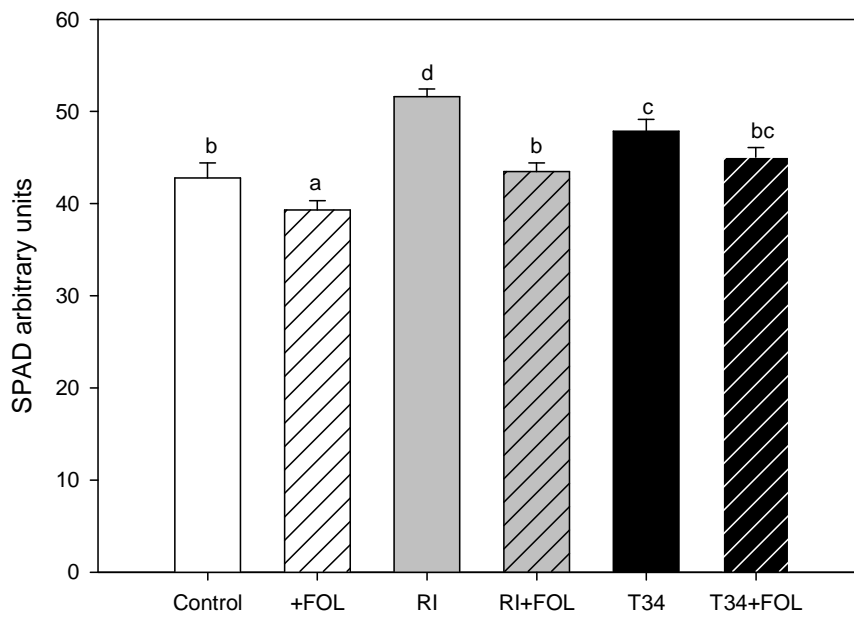


Fig. 4. Leaf chlorophyll content of tomato plants *Lycopersicon esculentum* Mill. cv. Roma, as measured by a SPAD chlorophyll meter. Control, non-infected plants; FOL, plants infected with *F. oxysporum* f. sp. *lycopersici* at a concentration of 5×10^5 conidia per ml of substrate; RI, plants inoculated 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 ($20-40 \text{ cm}^3$ or $6-12 \text{ g l}^{-1}$), while *T. asperellum* strain T34 was incubated in the substrate for 7 days at a concentration of 10^4 cfu per ml of substrate.