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RESEARCH ARTICLE

Effects of dual inoculation of mycorrhiza and endophytic, rhizospheric or parasitic bacteria on the root-knot nematode disease of tomato

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The effects of mycorrhisation and inoculation with soil bacteria on the disease caused by *Meloidogyne incognita* on tomato were studied in pots under greenhouse conditions. Efficacy in promoting plant growth and reducing disease severity and final nematode densities were evaluated for two arbuscular mycorrhizal fungi (AMF; *Funneliformis mosseae* and *Rhizophagus irregularis*), three soil bacteria with different living strategies (the endophyte *Bacillus megaterium*, a rhizospheric *Pseudomonas putida* and the hyperparasite of nematodes *Pasteuria penetrans*) and combinations of the fungi and bacteria. In *M. incognita*-infested plants, *F. mosseae* increased tomato growth more than *R. irregularis*, and plants inoculated with *B. megaterium* presented higher shoot fresh weight than with *P. putida* or *P. penetrans*, but dual inoculation did not improve tomato growth more than single inoculations. Disease severity and final nematode densities were reduced by *F. mosseae* compared to non-mycorrhizal plants. *B. megaterium* and *P. penetrans* reduced both the root galling and the final nematode densities compared to treatments without bacteria. *P. penetrans* reduced final nematode densities more than *B. megaterium* or *P. putida*. Dual inoculation of AMF and *P. penetrans* showed the highest efficacy in reducing the final nematode densities in tomato.

Keywords: AMF; biocontrol; *Bacillus*; *Meloidogyne*; *Pasteuria*; *Pseudomonas*

1. Introduction

Root-knot nematodes (RKN) of the genus *Meloidogyne* are sedentary endoparasites that induce root-knot symptoms and cause serious agricultural damage (Trudgill & Blok, 2001). Several sources suggest that plant parasitic nematodes reduce agricultural production 12–20% worldwide (Oka et al., 2000), and losses can reach 30–60%, particularly under protected cultivation (Talavera et al., 2012). Research trends are directing efforts towards biological control and bio-protection for environmentally friendly management of RKN (Timper, 2011), but the scientific knowledge on the efficacy of biocontrol agents for RKN lags behind that for other pests and diseases.

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Biocontrol technologies for RKN include arbuscular mycorrhizal fungi (AMF), since most studies have reported a protective mycorrhizal effect against nematodes, reducing damage or improving plant growth in RKN-infested plants (Azcón-Aguilar & Barea, 1997). The mechanisms suggested for the AMF effect on RKN are spatial and nutrient competition, changes in the biological and chemical status of the rhizosphere or induced resistance in the host plant (Gera-Hol & Cook, 2005).

Plant growth-promoting rhizobacteria (PGPR) can also protect plants against nematodes (El-Hadad et al., 2010; Oliveira et al., 2007). Approximately, 7–10% of all rhizobacteria display antagonistic potential against nematodes by various modes of action, including competition, antibiosis and induced resistance (Burkett-Cadena, Kokalis-Burelle, Lawrence, Van Santen, & Kloepper, 2008). Several *Bacillus* and *Pseudomonas* species are effective in managing RKN diseases in pot experiments (Siddiqui, Qureshi, & Akhtar, 2009; Singh & Siddiqui, 2010), and *Bacillus megaterium* de Bary and *Pseudomonas putida* Trevisan have been cited previously as RKN suppressors (El-Hadad et al., 2010; Huang et al., 2010; Siddiqui & Akhtar, 2008).

The bacterial hyperparasite *Pasteuria penetrans* (Thorne) Sayre & Starr reduces RKN populations through a highly specific parasitic interaction. Bacterial multiplication occurs within the pseudoceloma of infected nematodes, causing adult mortality and loss of fertility in survivors. Furthermore, mobility of juveniles and their penetration into the roots are also reduced when a high number of spores adhere to second-stage juveniles (J2) in the soil (Chen & Dickson, 1998; Davies, 2009).

Under natural conditions, these soil microorganisms interact simultaneously and continuously with the plant host and the environment in the rhizosphere. Therefore, the study of their interactions under controlled conditions and the search for positive microbial interactions in the rhizosphere that enhance their antagonistic effects on soil pathogens should be encouraged, since they could improve the efficacy of biological control (Barea, Azcón, & Azcón-Aguilar, 2002). The effects of AMF and soil bacteria on RKN on tomato has been investigated in several studies, e.g., AMF and *P. penetrans* (Talavera, Itou, & Mizukubo, 2002) and AMF and PGPR (Liu, Dai, Wu, Li, & Liu, 2012; Siddiqui & Akhtar, 2009), but there is little information available in comparative studies on their efficacy in reducing RKN disease.

The aim of the present study was to evaluate a strategy that combines the use of AMF with other soil microorganisms with different action mechanisms on the control of *Meloidogyne incognita* (Kofoid & White) Chitwood in tomato. The use of mycorrhisation by *Funneliformis mosseae* (Nicol. & Gerd.) Walker & Schüßler or *Rhizophagus irregularis* (Blaszk, Wubet, Renker and Busco) Walker & Schüßler combined with the endophyte *B. megaterium*, a rhizospheric *P. putida* or the hyperparasite of nematodes *P. penetrans* was tested.

2. Materials and methods

The experiment consisted of 24 treatments arranged in a 2 × 3 × 4 factorial design [three factors: nematode, AMF and bacteria, with two, three and four levels, respectively: (non-nematode vs. *M. incognita*), (non-AMF vs. *F. mosseae* vs. *R. irregularis*) and (non-bacteria vs. *B. megaterium* vs. *P. putida* vs. *P. penetrans;* Table 1). Each treatment was replicated five times and pots were arranged in five blocks within a greenhouse in a complete randomised-block design. Experiments were repeated once in

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Table 1. Combinations of treatments tested to determine the effects of mycorrhisation (AMF) by *F. mosseae* or *R. irregularis* singly and by dual inoculation with *B. megaterium*, *P. putida* or *P. penetrans* on *M. incognita* on tomato cv Durinta.

Treatment code	AMF inoculum	Bacterial inoculum	RKN inoculum
ØØØ	–	–	–
IØØ	*R. irregularis*	–	–
MØØ	*F. mosseae*	–	–
ØBØ	–	*B. megaterium*	–
IBØ	*R. irregularis*	*B. megaterium*	–
MBØ	*F. mosseae*	*B. megaterium*	–
ØSØ	–	*P. putida*	–
ISØ	*R. irregularis*	*P. putida*	–
MSØ	*F. mosseae*	*P. putida*	–
ØPØ	–	*P. penetrans*	–
IPØ	*R. irregularis*	*P. penetrans*	–
MPØ	*F. mosseae*	*P. penetrans*	–
ØØN	–	–	*M. incognita*
IØN	*R. irregularis*	–	*M. incognita*
MØN	*F. mosseae*	–	*M. incognita*
ØBN	–	*B. megaterium*	*M. incognita*
IBN	*R. irregularis*	*B. megaterium*	*M. incognita*
MBN	*F. mosseae*	*B. megaterium*	*M. incognita*
ØSN	–	*P. putida*	*M. incognita*
ISN	*R. irregularis*	*P. putida*	*M. incognita*
MSN	*F. mosseae*	*P. putida*	*M. incognita*
ØPN	–	*P. penetrans*	*M. incognita*
IPN	*R. irregularis*	*P. penetrans*	*M. incognita*
MPN	*F. mosseae*	*P. penetrans*	*M. incognita*

Note: Treatments are encoded with three letters, Ø, not inoculated; I, plants mycorrhised by *R. irregularis*; M, plants mycorrhised by *F. mosseae*; B, plants inoculated with *B. megaterium*; S, plants inoculated with *P. putida*; P, plants inoculated with *P. penetrans*; N, soil infested with *M. incognita*.

consecutive years and were conducted at 'IFAPA Camino de Purchil' (N37°10′16.5″–W3°38′8.62″) in Granada, Spain.

Seeds of tomato (*Solanum lycopersicum* L.) cv Durinta, susceptible to *Meloidogyne*, were surface sterilised by soaking them in 5% NaOCl for 10 min and subsequently washed several times with distilled water. Tomato seeds were sown in polystyrene trays with 25 cm^3 cells containing a previously sterilised sphagnum peat (80%) and perlite (20%) mixture. Tomato seedlings were grown in a climatic chamber at 24 ± 3°C for three weeks. Groups of homogeneous seedlings were used for planting in polypropylene pots (11 cm tall, 12 cm diameter at the top and 10 cm at the bottom) containing 500 cm^3 of sterile silt–sandy soil (84% sand: 10% silt: 6% clay) with 0.5% organic matter, pH 7.7 and 3.8 dS m^{-1} electrical conductivity.

Two species of AMF, *F. mosseae* BEG119 and *R. irregularis* BEG123, from the collection maintained at the Estación Experimental del Zaidín, CSIC (Granada, Spain), were tested. The inocula consisted of rhizospheric soil containing spores, hyphae and mycorrhizal root fragments with 75% of colonisation that was adjusted to contain 125 mycorrhizal propagules per gram. Three grams of AMF inoculum

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was added to the soil in each cell of the germination trays and plants were again inoculated at planting with 15 g of inoculum. Non-mycorrhizal plants received the same amount of sterilised rhizospheric soil (60 min, 121°C, 1 atm). Root colonisation by AMF was checked under the microscope in additional plants, three weeks later, by selective staining of chitin, a major constituent of fungal structures (Koske & Gemma, 1989).

Native isolates of *B. megaterium* and *P. putida* (Marulanda-Aguirre, 2006), from the collection of Estación Experimental del Zaidín (CSIC) were used. The bacterial inoculum was prepared in liquid media following Marulanda-Aguirre, Azcón, Ruíz-Lozano, and Aroca (2008). At planting, bacterial inoculum, at a rate of 1 ml (10^8 colony forming units, cfu) per plant, was poured using a pipette on lateral roots, at a distance of 1 cm from the primary root meristem (López-Bucio et al., 2007). The same amount of sterilised medium (20 min, 121°C, 1 atm) was added to the plants without bacterial inoculum.

The commercial product Pasteuria (Nematec®, Japan), consisting of tomato root powder containing 10^9 endospores of *P. penetrans* per gram, was used as the inoculum. An aqueous suspension was prepared (5×10^5 endospores per ml) and 50 ml of the suspension were poured over the soil in each pot at planting time. Plants without *P. penetrans* treatment received tomato root powder previously autoclaved twice (20 min, 121°C, 1 atm).

M. incognita eggs were extracted from susceptible tomato roots through maceration in NaOCl 0.5% solution (Hussey & Barker, 1973). Hatched juveniles were obtained from the egg suspensions by the Whitehead tray method (Whitehead & Hemming, 1965) and 48-h-old J2 were used as the inoculum. One week after planting, the plants were inoculated with 1500 J2s per plant.

The chlorophyll content was measured in tomato plants using a portable Soil Plant Analysis Development (SPAD) metre 502 Minolta®, since SPAD values are correlated with plant productivity (Rharrabti, Royo, Villegas, Aparicio, & García del Moral, 2003). Five readings, from 30 to 45 days after planting, were made at midpoint of the last completely developed leaf per plant and averaged.

Plants were grown at 27 ± 3°C and 50–60% relative humidity and harvested 60 days after planting. The main shoots were cut 2 cm above the soil base in pots and the roots were separated and washed to remove adhering soil particles. The shoot and root fresh weights were determined. The disease severity (root galling) was assessed using a gall index on a 0–10 rating scale (0: no galls; 7: 100% of the roots with galls; and 10: dead plant; Bridge & Page, 1980).

For the determination of the nematode population in the roots, 10 g root subsamples were cut into fragments 1–2 cm long and macerated by blending in a 1% solution of NaOCl (Hussey & Barker, 1973). The suspension was poured into 20-μm mesh sieves, to collect eggs, juveniles and adults of *Meloidogyne*. Nematodes were counted in counting dishes, and the total number of nematodes in the whole root system was calculated by multiplying the number of nematodes per gram of roots by the root fresh weight.

For the assessment of the nematode population density in soil, nematodes were extracted from 500 cm^3 of soil by the sieving and decanting method followed by centrifugation in a solution of $MgSO_4$•$7H_20$ with a specific gravity of 1.18 g l^{-1} (Coolen, 1979). The nematode suspension was concentrated in 2 ml of water for subsequent counting under the microscope.

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Final nematode population densities were calculated as the sum of nematodes extracted from roots plus those extracted from soil and are expressed as nematodes per 500 cm^3 of soil. All values are expressed as mean ± standard error.

Statistix 9.0 software (Analytical Software®, USA) was used for all statistical analyses. Data from both experiments were subjected to an analysis of variance (ANOVA) and combined because no statistical differences were found between the repeated experiments (Table 2). Data compiled on plant growth variables (shoot fresh weight and chlorophyll content) were subjected to a three-way factorial ANOVA [three factors (nematode, AMF and bacteria) and 2 × 3 × 4 levels, respectively, which resulted in 24 treatments]. Data on nematode reproduction (root galling and final nematode densities), which were obtained only from those treatments inoculated with *M. incognita*, were analysed by a two-way ANOVA [two factors (AMF and bacteria) and 3 × 4 levels, which resulted in 12 treatments]. Before analysis, the Shapiro–Wilk and Levene's tests were applied to check for normality and homoscedasticity and, if significant, data were normalised by transforming them to $\log_{10}(x + 1)$.

The factorial ANOVAs conducted here do not provide information regarding whether specific means are significantly different from one another; they simply indicate that a significant interaction between factors exists. To address whether these means are significantly different from one another, several one-way ANOVAs were computed. To conduct these ANOVAs, some groups were created, according to the significant interactions obtained. One-way ANOVAs were then computed to see if the means across those groups differed. When the F values were significant, means were compared by Tukey's test ($P < 0.05$).

3. Results

The results of the factorial ANOVAs, with the significance of main factors and interactions, are presented in Table 2. The three-way ANOVA for the plant growth

Table 2. Statistical significance of the ANOVA for comparison of the two experiments on tomato cv Durinta inoculated with nematodes, AMF and bacteria. Statistical significance of the three-way ANOVA on the combined data of the two experiments for the variables: shoot fresh weight and chlorophyll content and the two-way ANOVA for the variables: root galling and final nematode densities.

	Chlorophyll content	Shoot fresh weight	Root galling	Final nematode densities
Experiment (1 vs. 2)	NS	NS	NS	NS
Nematode (main effect)	**	***	NC	NC
AMF (main effect)	***	NS	**	***
Bacteria (main effect)	NS	NS	***	***
Nematode × AMF	NS	**	NC	NC
Nematode × bacteria	NS	**	NC	NC
AMF × bacteria	NS	NS	***	***
Nematode × AMF × bacteria	NS	NS	NC	NC

NS, not statistically different ($P > 0.05$); NC, not calculable.
**$P < 0.01$; ***$P < 0.001$.

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variables revealed significant effects of the main factors nematode and AMF on the chlorophyll content (Table 2). The chlorophyll content was reduced by *M. incognita* infestation (28.44 ± 0.56 SPAD units) compared to nematode-free plants (30.99 ± 0.46 SPAD units; Figure 1A) and was increased by *F. mosseae* mycorrhisation (32.04 ± 0.64 SPAD units) compared to that by *R. irregularis* (29.39 ± 0.59 SPAD units) or non-mycorrhizal plants (27.71 ± 0.55 SPAD units; Figure 1B). Bacterial inoculation did not affect the chlorophyll content (data not shown).

Similarly, there was a significant main effect of nematode presence vs. absence on the tomato shoot fresh weight. The average shoot fresh weight of all plants infested by *M. incognita* (40.2 ± 1.01 g) was reduced in comparison to that of uninfested plants (47.6 ± 0.78 g), but no significant main effects of mycorrhisation or bacterial inoculation were detected on shoot weight. However, significant interactions were found between nematodes and AMF or nematodes and bacteria (Table 2). The nematode × AMF interaction showed that in *M. incognita*-infested plants, the shoot fresh weight was lower in plants mycorrhised by *R. irregularis* (37.0 ± 2.12 g) than in those mycorrhised by *F. mosseae* (42.1 ± 1.34 g; Figure 2). The nematode × bacteria interaction showed that reductions in shoot fresh weight in nematode-infested plants were lower in plants inoculated with *B. megaterium* (44.5 ± 1.46 g) than in plants treated with *P. putida* (38.4 ± 2.64 g) or *P. penetrans* (36.0 ± 1.85 g; Figure 3).

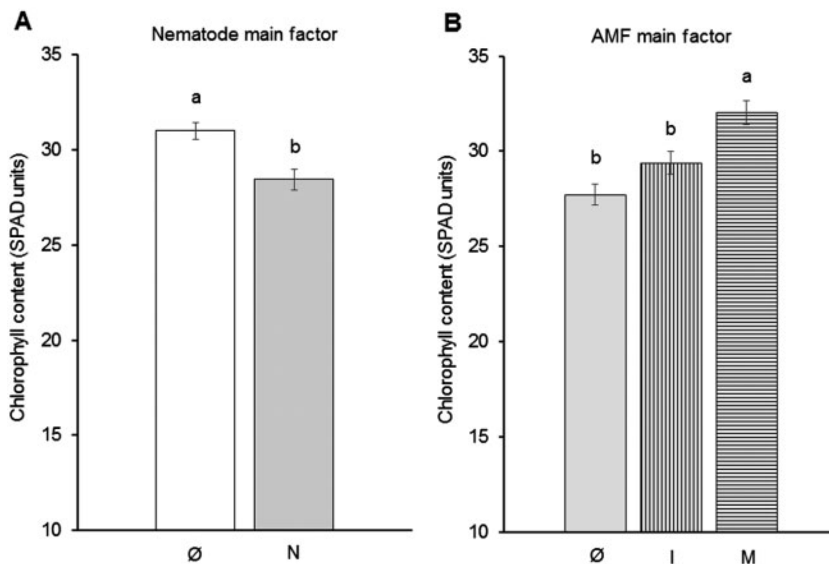

Figure 1. Main effects of nematode (A) and AMF inoculation (B) on the chlorophyll content of tomato cv Durinta.

Note: Columns represent the mean of a combination of 12 (A) or 8 (B) treatments with 10 replicates each. Bars represent standard error. Different letters in each column designate significant differences ($P < 0.05$) between treatments after Tukey's test. Treatments are encoded with three letters, Ø, not inoculated; I, plants mycorrhised by *R. irregularis*; M, plants mycorrhised by *F. mosseae*; N, soil infested with *M. incognita*.

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The two-way ANOVA on root galling and final nematode densities revealed significant main effects of AMF and bacterial inoculation on root galling and final nematode densities. In addition, there were significant interactions between AMF and bacteria in both variables (Table 2). Root galling was reduced on *F. mosseae*-mycorrhised plants (3.35 ± 0.13) in comparison to non-mycorrhizal plants (3.93 ± 0.12) (Figure 4A). Root galling was also reduced by *P. penetrans* (3.27 ± 0.21) and *B. megaterium* (3.23 ± 0.13) in comparison to *P. putida* (4.00 ± 0.14) or without bacterial inoculation (3.97 ± 0.13; Figure 4B). The interaction effect between AMF and bacteria indicated that combinations of AMF with *B. megaterium* (2.80 ± 0.20 and 3.10 ± 0.18 for *R. irregularis* and *F. mosseae*, respectively) or *P. penetrans* (2.60 ± 0.22 and 3.10 ± 0.41) reduced root galling in comparison to combinations with *P. putida* (4.20 ± 0.25 and 3.60 ± 0.22) or without bacterial inoculation (4.70 ± 0.15 and 3.60 ± 0.16; Figure 5).

F. mosseae was more effective than *R. irregularis* in reducing final nematode densities (3064 ± 432 nematodes per 500 cm^3 of soil vs. 4191 ± 494 and 5602 ± 656 in plants without mycorrhiza). Regarding bacterial inoculation, final nematode densities were lower in plants inoculated with *P. penetrans* (1554 ± 214) than those with *B. megaterium* (3482 ± 344), which were also lower than those with *P. putida* (5835 ± 685) or without bacterial inoculation (6271 ± 750; Figure 6). The interaction effect between AMF and bacteria showed that all treatments reduced the final

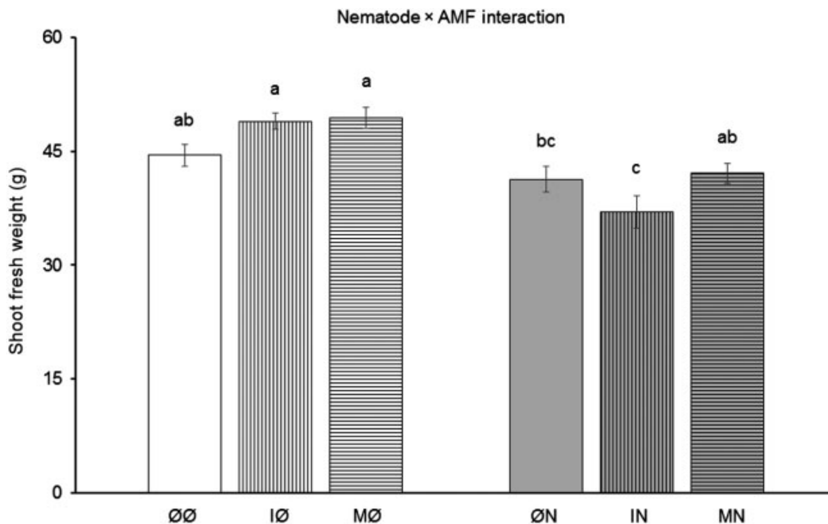

Figure 2. Interaction effects between nematode and AMF inoculation on the shoot fresh weight of tomato cv Durinta.

Note: Columns represent the mean of a combination of 4 treatments with 10 replicates each. Bars represent standard error. Different letters in each column designate significant differences ($P < 0.05$) between treatments after Tukey's test. Treatments are encoded with three letters, Ø, not inoculated; I, plants mycorrhised by *R. irregularis*; M, plants mycorrhised by *F. mosseae*; N, soil infested with *M. incognita*.

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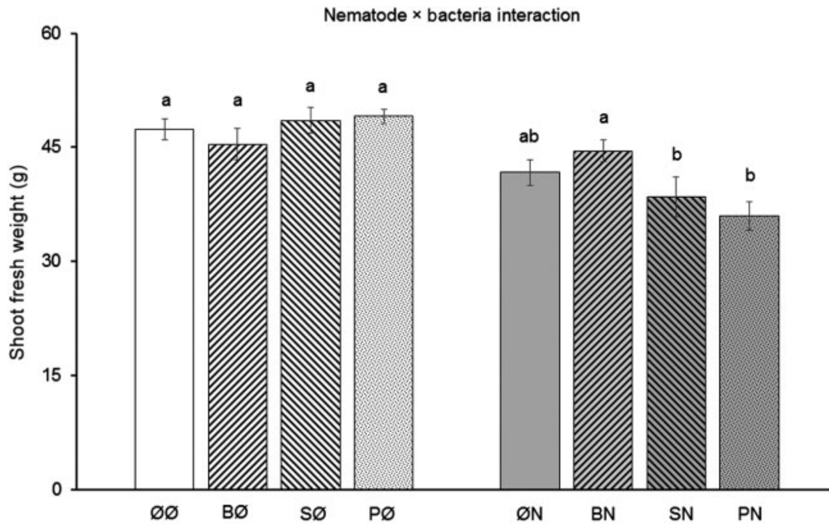

Figure 3. Interaction effects between nematode and bacterial inoculation on the shoot fresh weight of tomato cv Durinta.

Note: Columns represent the mean of a combination of 3 treatments with 10 replicates each. Bars represent standard error. Different letters on each column designate significant differences (P < 0.05) between treatments after Tukey's test. Treatments are encoded with three letters, Ø, not inoculated; B, soils inoculated with *B. megaterium*; S, soils inoculated with *P. putida*; P, soils inoculated with *P. penetrans*; N, soil infested with *M. incognita*.

nematode densities, when compared to *M. incognita*-infested plants without mycorrhizal or bacterial inoculation (Figure 7). Dual inoculation of AMF and *P. penetrans* showed a positive interaction, as the final nematode density was significantly reduced by as much as 91% compared to untreated plants and by 81%, 63% and 64% compared to the single treatments with *R. irregularis*, *F. mosseae* or *P. penetrans*, respectively.

4. Discussion

Several microorganisms, such as AMF, parasitic fungi, PGPR and the hyperparasite bacterium *P. penetrans* with different action mechanisms, could be simultaneously used to enhance the level of protection of the plants against nematodes, and thus provide a stable and more effective rhizosphere community over a wide range of environmental conditions (Sikora, Schäfer, & Dababat, 2007). AMF and bacteria could interact positively to improve plant nutrition and growth and protect plants against pathogens, and they are usually more effective when applied together than alone (Liu et al., 2012; Talavera et al., 2002).

Nematode and AMF inoculation had significant effects on chlorophyll content. *M. incognita* presence decreased the SPAD values whereas they were increased in *F. mosseae*-mycorrhised plants, suggesting that this AMF could partially offset the

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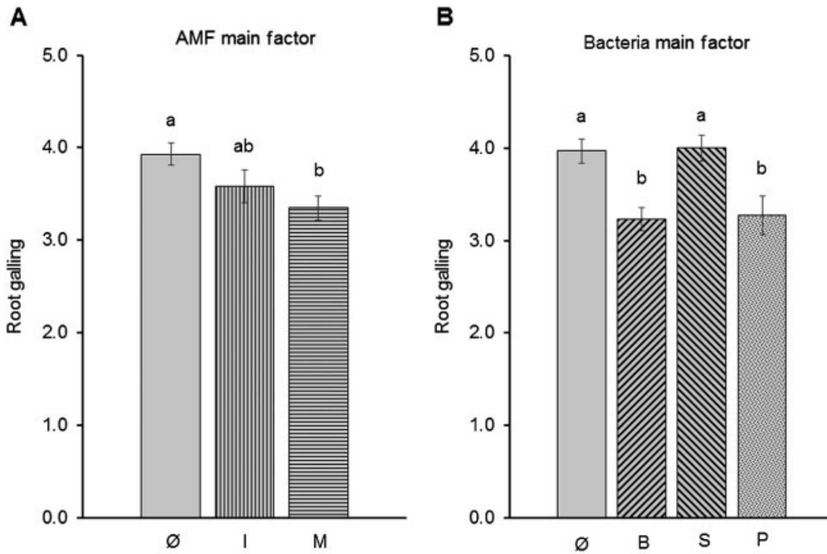

Figure 4. Main effects of AMF (A) and bacterial inoculation (B) on the root galling caused by *M. incognita* in tomato cv Durinta.
Note: Columns represent the mean of a combination of 4 (A) and 3 (B) treatments with 10 replicates each. Bars represent standard error. Different letters in each column designate significant differences ($P < 0.05$) between treatments after Tukey's test. Treatments are encoded with three letters, Ø, not inoculated; I, plants mycorrhised by *R. irregularis*; M, plants mycorrhised by *F. mosseae*; B, soils inoculated with *B. megaterium*; S, soils inoculated with *P. putida*; P, soils inoculated with *P. penetrans*.

damage caused by nematodes in this tomato cultivar, as previously reported in other tomato cultivars (Talavera, Itou, & Mizukubo, 2001). Changes in chlorophyll content are in support of the detrimental effect of the nematode as oppose to the beneficial effect of the mycorrhisation on the plant. This non-destructive measurement can be helpful for early quantification of biological stresses on the plant. The negative effect of the nematode was detected 30–45 days after planting by an 8% reduction in chlorophyll content and this effect was confirmed 60 days later by a reduction of 16% in shoot fresh weight.

In *M. incognita*-infested plants, shoot fresh weight was greater in *B. megaterium*-inoculated plants as opposed to *P. putida* or *P. penetrans*, which confirms that *Bacillus* isolates also could compensate the damage caused by nematodes in tomato plant growth (Singh & Siddiqui, 2010). However, we found no such effect on the chlorophyll content, probably because it was measured earlier than shoot fresh weight, from 30 to 45 days after planting vs. 60 days, and the effect of *B. megaterium* on the chlorophyll content was not yet strong enough to be noted. In cucumber infested by *Meloidogyne javanica*, Giné et al. (2014) showed that the relationship between the dry shoot weight at harvest and the chlorophyll content was more related when chlorophyll measurements were made at 50 days after planting than

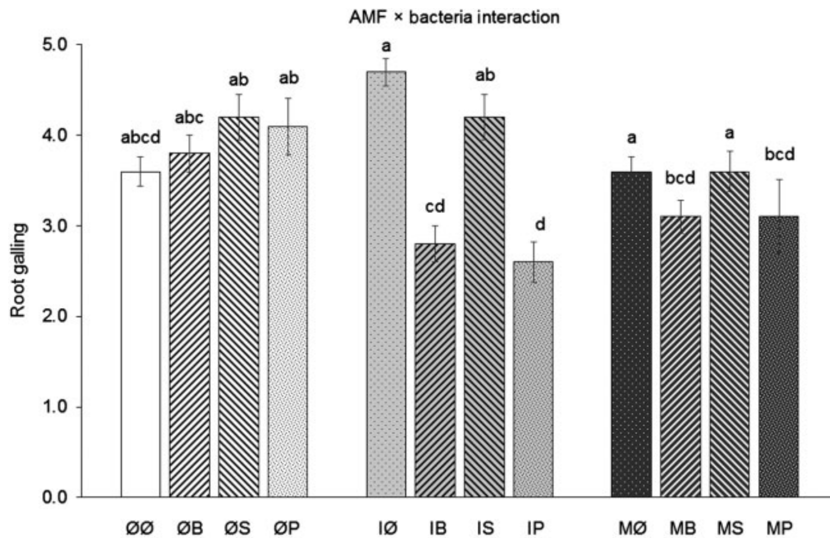

Figure 5. Interaction effects between AMF and bacterial inoculation on the root galling caused by *M. incognita* in tomato cv Durinta.

Note: Columns represent the mean of 10 replicates. Bars represent standard error. Different letters on each column designate significant differences ($P < 0.05$) between treatments after Tukey's test. Treatments are encoded with three letters, Ø, not inoculated; I, plants mycorrhised by *R. irregularis*; M, plants mycorrhised by *F. mosseae*; B, soils inoculated with *B. megaterium*; S, soils inoculated with *P. putida*; P, soils inoculated with *P. penetrans*.

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before. The endophytic nature of *B. megaterium* suggests a stronger host plant–bacterium association than other rhizospheric-inhabiting bacteria. This closer association could benefit the plant by further increasing nutrient uptake or by inducing resistance to biotic and abiotic stress in the plant (Compant, Duffy, Nowak, Clement, & Barka, 2005).

Although previous studies have reported more vigorous growth and productivity due to positive synergistic interactions between AMF and PGPR (Siddiqui & Akhtar, 2009; Zhang, Raza, Wang, Ran, & Shen, 2012), we found no stimulatory effect when combining these microorganisms, in agreement with the results of Medina, Probanza, Manero, and Azcón (2003) and Vestberg et al. (2004). These results reveal the need for a careful selection of AMF and PGPR species and strains for an effective combination to increase plant growth and reduce damage caused by plant pathogens, as the beneficial effect of these interactions will depend on specific interactions between the organisms involved.

Regarding the effects of AMF and bacterial inoculation on disease severity and nematode reproduction, *F. mosseae* reduced disease severity by 15% and final nematode densities by 45% compared to non-mycorrhizal plants, as previously reported in other tomato cultivars (Talavera et al., 2001; Vos, Tesfahun, Panis, De Waele, & Elsen, 2012). In addition, plants inoculated with *B. megaterium* or

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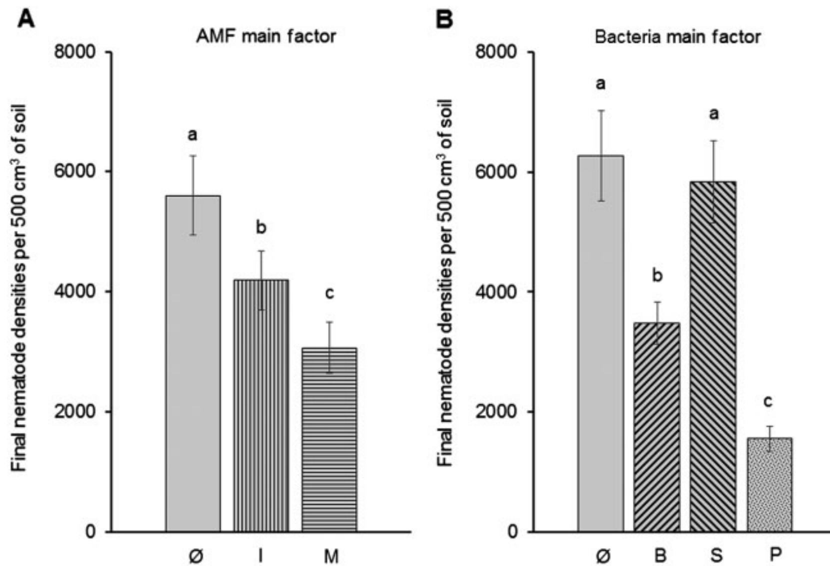

Figure 6. Main effects of AMF (A) and bacterial inoculation (B) on final *M. incognita* densities in tomato cv Durinta.
Note: Columns represent the mean of a combination of 4 (A) and 3 (B) treatments with 10 replicates each. Bars represent standard error. Different letters in each column designate significant differences ($P < 0.05$) between treatments after Tukey's test. Treatments are encoded with three letters, Ø, not inoculated; I, plants mycorrhised by *R. irregularis*; M, plants mycorrhised by *F. mosseae*; B, soils inoculated with *B. megaterium*; S, soils inoculated with *P. putida*; P, soils inoculated with *P. penetrans*.

P. penetrans exhibited a significant reduction of root galling (18–19%) and final nematode densities (39% and 75%, respectively) compared to plants without bacterial inoculation. Root galling in tomato plants caused by *M. incognita* was also palliated by inoculation with *B. megaterium* (Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012). *B. megaterium* reduced the penetration of *Meloidogyne* juveniles into rice and potato roots (Al-Rehiayani, Hafez, Thornton, & Sundararaj, 1999; Padgham & Sikora, 2007) and its secondary metabolites reduced egg hatching of *Meloidogyne graminicola* (Padgham & Sikora, 2007). On the other hand, Siddiqui et al. (2009) reported that *Pseudomonas* isolates had greater inhibitory effects than *Bacillus* spp. on emergence and penetration of *M. incognita* in pea plants. We did not study the effects of bacterial inoculation on *Meloidogyne* root penetration nor egg hatching, but the greatest suppressant effect of *B. megaterium* compared to *P. putida* on *M. incognita* could be due to the endophytic nature of *Bacillus*, which would allow more extensive colonisation of the root system than the rhizospheric bacteria, competing for space and avoiding the establishment of nematodes or through the effects of its secondary metabolites. In addition, the endospore-forming capacity of *Bacillus* species would favour the persistence of the bacterium and the durability of its effects in soil vs. the effects of other bacteria (Padgham & Sikora, 2007).

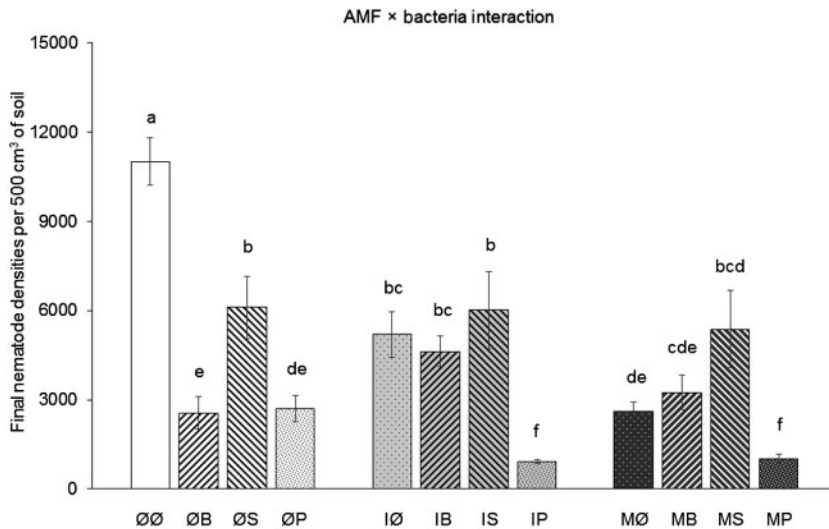

Figure 7. Interaction effects between AMF and bacterial inoculation on final *M. incognita* densities in tomato cv Durinta.

Note: Columns represent the mean of 10 replicates. Bars represent standard error. Different letters on each column designate significant differences ($P < 0.05$) between treatments after Tukey's test. Treatments are encoded with three letters, Ø, not inoculated; I, plants mycorrhised by *R. irregularis*; M, plants mycorrhised by *F. mosseae*; B, soils inoculated with *B. megaterium*; S, soils inoculated with *P. putida*; P, soils inoculated with *P. penetrans*.

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Some interaction effects on disease severity were observed depending on the combination of AMF and bacteria used. *R. irregularis* reduced disease severity (root galling) only when it was applied in combination with *B. megaterium* or *P. penetrans*, but this effect was not observed when it was applied alone or combined with *P. putida*. Treatments combining AMF and *P. penetrans* revealed the lowest root galling and final nematode densities. The treatment that combined *R. irregularis* with *P. penetrans* reduced root galling by 27% and final nematode densities by 91% compared to the untreated plants. These results agree with the hypothesis that the dual application of microorganisms with different action mechanisms is more effective against pathogens than are single inoculations (Guetsky, Shtienberg, Elad, Fischer, & Dinoor, 2002; Siddiqui & Akhtar, 2008). The application of these microorganisms together could be compatible and might be considered to be a nematode-control strategy (Talavera et al., 2002).

In summary, this study reveals that early mycorrhisation of tomato roots at the seedling stage and soil inoculation with *P. penetrans* or *B. megaterium* could reduce the final *M. incognita* densities in tomato. *P. penetrans* showed better efficacy than PGPR against *M. incognita*. Moreover, the combination of AMF and *P. penetrans* showed the highest efficacy in reducing the final nematode densities. Greater effectiveness at reducing nematode densities was not detected in treatments combining AMF and PGPR, under our experimental conditions. Future studies on the synergic effect of

PGPR with AMF species in different crops would be necessary to extend the knowledge and use of these microbial combinations for nematode control.

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