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Short communication

Mycorrhizas reduce tomato root penetration by false root-knot nematode *Nacobbus aberrans*

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are considered as a possible alternative for the biological control of plant-parasitic nematodes. The false root-knot nematode *Nacobbus aberrans* is an endoparasite that induces the formation of galls in plant roots and causes severe damage to crops of economic importance. The purpose of the study was to evaluate the effect of the individual and combined application of two AMF species (*Rhizophagus intraradices* and *Funneliformis mosseae*) on the tomato root penetration of *N. aberrans* second-stage juveniles (J2). Forty-five days after the application of AMF, 300 J2 were inoculated per plant. Tomato roots were analyzed after 4, 8 and 12 days in order to quantify the nematodes that had invaded them. Plants inoculated with AMF presented a lower number of juveniles within the roots compared with non-mycorrhizal plants. No significant differences were observed between the individual and combined application of AMF regarding the number of nematode juveniles. The use of *R. intraradices* and *F. mosseae* (both individually and combined) reduced the entry of nematodes in tomato roots. The antagonistic effect of AMF on the invasion of *N. aberrans* J2 is reported for the first time.

1. Introduction

Agricultural damage caused by plant-parasitic nematodes is estimated to be 80 billion dollars annually worldwide (Nicol et al., 2011). *Nacobbus aberrans* (Thorne, 1935) Thorne and Allen, 1944 is one of the ten most relevant plant-parasitic nematodes worldwide, due to the economic losses it causes (Jones et al., 2013). In parasitized roots, it generates galls similar to those produced by *Meloidogyne* spp. (root-knot nematode); for this reason, *N. aberrans* is known as "the false root-knot nematode". It is native to the American continent and is present in Argentina, Bolivia, Chile, Ecuador, Mexico, Peru and the United States (EPPO, 2011). The main crops affected by this nematode are tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), sugar beet (*Beta vulgaris* L.) and bean (*Phaseolus vulgaris* L.) (Manzanilla-López et al., 2002).

Due to the growing concern about the effects of chemical pesticide applications on the environment and human health, different alternatives for the control of plant-parasitic nematodes are being evaluated (Akhtar and Siddiqui, 2008). Arbuscular mycorrhizal fungi (AMF) establish a symbiotic association with plants, confer them direct benefits, such as an improved absorption of nutrients (mainly phosphorus), and protect them against soil-borne pathogens (Smith and Read, 2008). The use of AMF as biostimulants and biological control agents in horticultural crops has greatly increased in the last two decades, mostly due to their ability to secure production and yield stability within a sustainable environment. The AMF inoculum can be produced in greenhouses using sterile substances, such as vermiculite, to grow host plants; additionally, there is a particular focus in the market on products based on spores obtained from the roots of plants under monoxenic conditions (*in vitro* culture system) (Rouphael et al., 2015).

The application of AMF has proved to be efficient for the biological control of plant-parasitic nematodes in the field, increasing the crop yields (Jonathan et al., 2004; Odeyemi et al., 2010; Affokpon et al., 2011) and in greenhouse, under controlled conditions (Gómez et al., 2008; Zhang et al., 2008; Liu et al., 2012; Marro et al., 2014; Schouteden et al., 2015). However, the mechanisms of antagonistic action on these parasites remain largely unknown (Vos et al., 2012a).

The nutritional status of the plant can influence its response to the

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attack of pathogens (Harrier and Watson, 2004). Since the use of a mixture of AMF species has been more beneficial for plant development than single species (Albrechtova et al., 2012; Hart et al., 2015), this may imply a greater antagonistic effect on plant-parasitic nematodes. So far, information about the effect of the combination of two or more AMF species on nematodes is scarce. On one side, the simultaneous inoculation of *Funneliformis mosseae* (Nicolson and Gerd.) Walker and Schüßler (ex *Glomus mosseae*) and *Rhizophagus fasciculatus* (Thaxt.) Walker and Schüßler (ex *G. fasciculatum*) in bean plants significantly reduced the number of cysts, eggs by cyst, as well as the final population of *Heterodera cajani* Koshy, 1967, compared to the individual effect of each of those AMF species (Pandey, 2011). On the other side, the application of fertilizers and a mixture of AMF species from different genera decreased the total number of *M. incognita* (Kofoid and White, 1919) Chitwood, 1949 in balsam (*Impatiens balsamina* L.); such effect was lower when only *F. coronatum* (Giovann.) Walker and Schüßler (ex *G. coronatum*) was inoculated (Banuelos et al., 2014).

Vos et al. (2012a) demonstrated the negative effect of *F. mosseae* on the penetration of tomato roots by *M. incognita* second-stage juveniles (J2). Even though *N. aberrans* induces galls in roots as *Meloidogyne* spp., its life cycle is completely different (Eves-van den Akker et al., 2014). For that reason, the objective of this study was to evaluate the effect of the individual and combined inoculation of two AMF species on the penetration level of *N. aberrans* juveniles in pre-mycorrhized tomato roots.

2. Materials and methods

2.1. Nematode inoculum

A population of *N. aberrans* from the locality of Lules (province of Tucumán, Argentina) was used. The nematodes were multiplied on tomato (cv. Platense) under greenhouse conditions. Egg masses were extracted from infected roots and placed in Petri dishes containing sterile water. They were maintained at room temperature (25 ± 2 °C) until eggs hatched. For inoculation, mobile J2 were recovered after three days of hatching.

2.2. AMF inoculum

Rhizophagus intraradices (Schenck and Smith) Walker and Schüßler (origin: La Plata, Culture Collection, LPS, culture Tierra del Fuego N° 28) and *F. mosseae* (origin: La Plata, Culture Collection, LPS, culture San Bernardo N° 1) were propagated on leek plants (*Allium porrum* L.) using a mixture of perlite, vermiculite and sterile soil (1:1:1) as a substrate. The AMF inocula used in this experiment consisted of leek roots. They were cut into 1-cm pieces and stained using the methodologies developed by Phillips and Hayman (1970), and Grace and Stribley (1991). The percentage of arbuscular mycorrhizal colonization (%AMC) was calculated according to the technique described by McGonigle et al. (1990).

2.3. Plant material, treatments and inoculation

Tomato seeds cv. Platense were surface sterilized for 5 min in 10% sodium hypochlorite (NaClO). They were washed with sterile water and sown in trays containing sterile soil and sand (3:1) to promote germination. Soil physicochemical properties were as follows: organic matter = 4.06%; organic carbon = 2.36%; N = 0.22%; P = 116.7 ppm; pH = 6.6. One hundred and twenty seedlings, which had two true leaves, were individually transplanted to pots (20-cm long × 4-cm diameter; capacity of 190 g of soil). Before J2 inoculation, plants were treated as follows: 1) AMF-free (control); 2) *R. intraradices* (RI); 3) *F. mosseae* (FM); and 4) *R. intraradices* and *F. mosseae* (RI + FM). In general, the whole experiment was based on the methodology developed by Vos et al. (2012b); however, some changes were made as described below. At the time of transplanting, the pot was filled with 3/4 of the aforementioned substrate, and 0.30 g of leek roots colonized with each AMF species were added in a layer. The%AMCs were 83% and 68% for RI and FM, respectively. For the combined treatment (RI + FM), 0.15 g of leek roots colonized with each fungus species was inoculated, whereas, for the control treatment, 0.30 g of non-colonized leek roots was added. Finally, the AMF inoculum and tomato roots were then covered with the sterile substrate. The plants were arranged in a completely randomized design and grown under greenhouse conditions (24/18 °C day/night, 16/8 h day/night photoperiod, watered daily). The plants were not fertilized. After 45 days, six plants from each treatment were randomly uprooted in order to evaluate the%AMC and the presence of fungal structures (arbuscules, vesicles, hyphae and entry points), as mentioned above. Afterwards, at the same time, 24 plants from each treatment were inoculated with 300 J2 contained in 2.5 ml of sterile water by making three holes near the roots. Eight plants from each treatment were uprooted at 4, 8 and 12 days after inoculation (DAI), respectively. The roots were stained following the technique described by Byrd et al. (1983) and they were macerated with a blender once during 10 s (INIBAP, 1997). Maceration of each root plant was suspended in a final volume of 40 ml and later homogenized, and three 1.5 ml aliquots were taken to quantify *N. aberrans* juveniles in a counting chamber under an optical microscope. The whole experiment was repeated twice (October-November 2014 and March-April 2015).

2.4. Data analysis

The number of *N. aberrans* juveniles in roots was analyzed using a General Linear and Mixed Model; the treatments (control, RI, FM and RI + FM), time (4, 8 and 12 DAI) and their interaction (treatment*-time) were considered as fixed effects and the repetition as a random effect. The best fitted model for heterogeneous variances was chosen based on the Akaike Information Criterion and the Bayesian Information Criterion (Zar, 1999). The Di Rienzo, Guzmán and Casanoves (DGC) test ($p \leq 0.05$) was carried out to compare means *a posteriori*. The statistical analysis was performed using the software Infostat (Di Rienzo et al., 2013) and its interface with the software R (R Core Team, 2009).

3. Results and discussion

Plants inoculated with AMF and analyzed 45 days after transplanting presented arbuscules, vesicles, hyphae and entry points. The %AMCs (mean ± standard error) were as follows: RI = 32.3 ± 9%; FM = 38 ± 6%; and RI + FM = 28.5 ± 9%. No mycorrhization was observed in the control plants.

The roots of all plants inoculated with *N. aberrans* were parasitized. The numbers of juveniles in root tissues increased over time for all the four treatments ($F = 120.81$; $p < 0.0001$) (Fig. 1). In plants with RI, FM and RI + FM, the penetration at 4, 8 and 12 DAI was significantly lower than for the control treatment ($F = 11.04$; $p < 0.0001$), whereas no significant differences were observed among the three AMF treatments. In comparison to the control, the reduction was 20%, 26% and 27% for RI + FM, RI and FM, respectively, at 12 DAI. Vos et al. (2012a) observed that the penetration of *M. incognita* J2 in tomato roots pre-mycorrhized with *F. mosseae* was reduced by 32% compared with AMF-free plants. Even though the reduction in penetration of *N. aberrans* was similar than that of *M. incognita* in plants with FM (27% *vs.* 32%, respectively), it is important to note that there were differences in the %CMA of the inoculum: 38% (present work) *vs*. 75% (Vos et al., 2012a). These results may be explained by differences in the life cycles of the two nematode species (Inserra et al., 1984), and/or differences between the AMF isolates (Gianinazzi et al., 2002), among other factors.

Some of the described antagonistic effects of AMF on plant-parasitic nematodes are the induced systemic resistance (Elsen et al., 2008) and

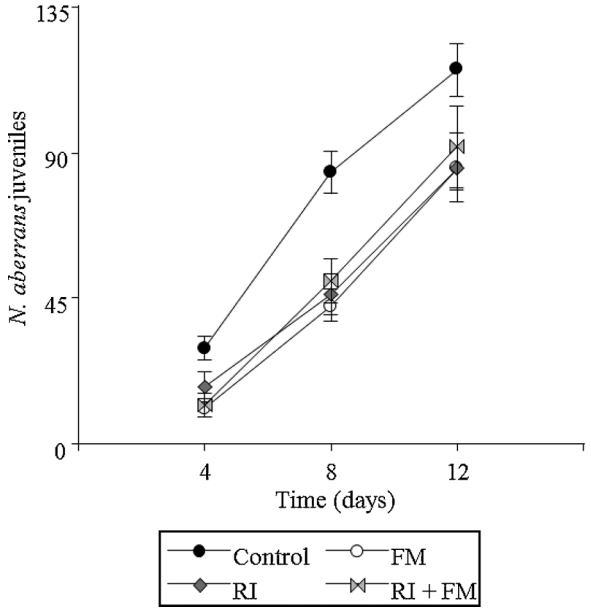

Fig. 1. Number of *Nacobbus aberrans* juveniles (mean value and standard error) that penetrated at 4, 8 and 12 days after inoculation on tomato roots colonized individually or in combination by *Rhizophagus intraradices* and *Funneliformis mosseae*.

the alteration of root metabolism, along with modified molecular composition of secretions released (Hol and Cook, 2005; Vos et al., 2013). The addition of root exudates (obtained from mycorrhized plants) to tomato roots showed a negative effect on *M. incognita* J2 mobility in the soil, and by consequence a lower root penetration (Vos et al., 2012b). As the root exudates guide plant-parasitic nematodes to the host (Curtis et al., 2009), their alteration by AMFs may negatively affect *N. aberrans* juveniles invasion of the roots.

Banuelos et al. (2014) applied a mixture of AMF species from different families (Glomeraceae, Acaulosporaceae and Gigasporaceae) of Glomeromycota to fertilized soil and observed a significant decrease in the number of *M. incognita* in balsam roots compared with plants inoculated with only one AMF species. In the present work, no differences were observed between plants inoculated individually and combined with AMF regarding the penetration of *N. aberrans*. This may be due to the fact that in our study we used fungi belonging to the same functional group (Glomeraceae), with similar physiological and morphological characteristics (Chagnon et al., 2013).

There are few previous works that observed a reduction in numbers of galls (Gardezi et al., 1995), egg masses and reproduction factor of *N. aberrans* in mycorrhized tomato plants (Lax et al., 2011; Marro et al., 2014). In those studies, only the individual effect of AMF was tested on such parameters. The present study showed for the first time the antagonistic effect of the individual and combined application of two AMF species on the infection level of *N. aberrans* J2 in pre-mycorrhized host roots. These results suggest the importance of transplanting pre-mycorrhized tomato plants in soils infested with nematodes to reduce the infection levels of the crop. Since *N. aberrans* has other infective stages in its life cycle (third and fourth stage juveniles and immature females), it would be important to evaluate the effect of AMF on those stages, as well as through the development of the crop, where the plant is subjected to multiple infections.

4. Conclusion

The effect of individual and combined AMF inoculation on the penetration of *N. aberrans* J2 in host roots was evaluated for the first time. The results showed the antagonic effect of AMF against the nematode; plants inoculated individually with *R. intraradices*, *F. mosseae* or a combination of both presented the same effect.

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