EFFECT OF THE ARBUSCULAR MYCORRHIZAL FUNGUS *GLOMUS MANIHOTIS* ON THE ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA*, IN BANANA

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Summary. To assess the effect of arbuscular mycorrhizal fungi on the root-knot nematode *Meloidogyne javanica,* micro-propagated plants of banana 'Grande Naine' (*Musa acuminata* Colla AAA) were inoculated with *Glomus manihotis* at the beginning of the hardening phase. After the establishment of the mycorrhizal symbiosis, plants were inoculated with *Meloidogyne javanica*. The effects of the nematode/mycorrhizal interaction were assessed 120, 160 and 200 days after nematode inoculation. Results showed that the mycorrhizal symbiosis reduced root galling and the population of *M. javanica* in the roots. However, the reduction in nematode population in roots was significant only until four months after inoculation with nematodes. The nematodes did not affect negatively root colonization by the mycorrhizal fungus. Root mycorrhization in the nursery could be a useful means of limiting root-knot damage during early growth stages of banana after transplanting in the field.

Beneficial rhizospheric microbe-plant interactions have a great influence on plant health and soil quality (Lynch, 1990). Arbuscular mycorrhizal fungi are obligate symbionts that colonise the roots of most cultivated plant species. These natural associations favour plant establishment, enhancement of nutrient uptake, and protect against biotic and abiotic stresses (Barea *et al.*, 2004).

Several species of root-knot nematodes, *Meloidogyne* spp., are widespread in the banana plantations of the Canary Islands (Rodríguez, 1990) where all banana cultivars are susceptible. Species of *Meloidogyne* can be an important limitation in banana production in dry subtropical conditions (Gowen and Quénérhervé, 1990). Apart from the typical symptoms in root tissues (gall formation), nematode infection causes significant growth suppression and reductions in fruit yield and the productive life of plantations (Speijer and DeWaele, 1997). Early mycorrhization has been shown to increase tolerance of nematode infection by bananas by enhancing plant nutrition or by suppressing root-knot nematode reproduction (Jaizme-Vega *et al.*, 1997).

The aim of the present work was to study the temporal evolution of infection by the root-knot nematode *Meloidogyne javanica* (Treub) Chitw. inside root tissues of micro-propagated banana plantlets, previously inoculated with the mycorrhizal fungus *Glomus manihotis* Howeler, Sieverding *et* Schenck at the beginning of the hardening phase.

MATERIALS AND METHODS

Plant material. Micro-propagated banana, *Musa acuminata* Colla AAA, cv. Grande Naine, was provided by CULTESA (Cultivos Vegetales *in vitro* de Tenerife S.A.). Plantlets, measuring approximately 8 ± 1 cm in height with three fully developed leaves, were received in nutrient agar and subsequently transplanted into seed trays of 24 litres capacity (35 plantlets/tray) that were filled with a steam-sterilized substrate (2:2:1 = soil : volcanic ash : peat TKS·1-Instant Sphagnum-Torf Klasmann Deilmann GmbH, Germany).

Mycorrhizae inoculation and procedure. The arbuscular mycorrhizal fungus (AMF) *G. manihotis* (an isolate collected from Colombia) was cultured on alfalfa *(Medicago sativa* L.) roots, developing a root colonization of 74%. The AMF inoculum consisted of rhizospheric soil containing pieces of mycorrhizal roots, hyphae and spores. It was applied by adding 2 kg of inoculum to each seed tray at the beginning of the nursery phase. During this period, plants were grown under greenhouse conditions, covered by a black tunnel with an ambient temperature of 30 ºC and a relative humidity of 90%. They were irrigated with distilled water according to need and fertilized with Osmocote® 17-10-10 (slow release mineral fertilizer).

Experimental design and culture conditions. After the nursery phase, which lasted for 30 days, plants were individually transferred to 6-litre pots filled with a steam-sterilized substrate mixture (3:2:1 = soil:volcanic ash:peat TKS·1-Instant Sphagnum-Torf Klasmann Deilmann GmbH, Germany). This pot size was chosen because in previous tests it was found suitable for growing banana plantlets for a period of 200 days. They were arranged in a completely randomized design and kept under greenhouse conditions (27-32 ºC and 70-80% RH). Fertilization was according to standard fertilization programmes in commercial banana nurseries.

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Nematode inoculation procedure. The nematode inoculum consisted of a population of *M. javanica* isolated from banana in the south-east of Tenerife and reared on tomato. When large egg masses had formed, nematode inoculum was prepared by macerating infected roots in a blender for 15 seconds at 14,500 rpm in a 0.12-0.15% sodium hypochlorite solution (Hussey and Barker, 1973). To collect eggs and juveniles (J2), the suspension was sieved through 150, 74 and 25 µm-pore sieves (100, 200 and 500 mesh respectively). Eggs and juveniles remaining on the 25 µm-pore were rinsed with tap water. A suspension containing 2,800 eggs and juveniles in 20 ml of water was delivered to each plant through four holes, each 2 cm deep and located 3 cm from the base of the plant. Nematodes were inoculated 30 days after transplanting. The following treatments were used: control, AMF, *M. javanica* and AMF + *M. javanica*, with a total of 24 plants per treatment. Variables related to nematode and mycorrhizal fungus infection were measured 120, 160 and 200 days after nematode inoculation by analysing eight plants per treatment at each sampling.

Assessment of variables. The nematode variables measured were: percentage of galled root (Barker, 1985), number of eggs and juveniles per gram of root, and the reproduction rate of the nematodes in the roots (final population in root/initial population inoculated). For each plant, a root sample (20% of fresh weight) was used to determine the nematode population. The procedure for extracting eggs and juveniles of the nematodes from roots was similar to that for the preparation of the inoculum. The nematode suspensions were diluted and 1-ml aliquots were observed under a stereo microscope using Hawksley slides.

Assessment of the mycorrhizal infection. A small root sample (5% of fresh weight) of the whole root system was used to estimate the percentage of AMF root infection. Samples were stained with 0.05% trypan blue in lactic acid (Phillips and Hayman, 1970) modified by the procedure described by Koske and Gemma (1989). Mycorrhizal root samples, inoculated or non-inoculated with *M. javanica*, were taken after clarifying and staining the roots, mounted on slides marked with millimetre grids, and observed under a light microscope. The percentage of root colonization was determined by the method described by Brundett *et al.* (1985).

Statistical analysis. All data were analysed by ANOVA. Data on nematode reproduction were $\log_{10}(x + 1)$ transformed for analyses. Means were compared by Tukey's multiple range test ($P \leq 0.050$). The analysis was performed by using Systat® 7.0.1. (SPSS. Inc© Chicago, USA, 1997).

RESULTS

The growth of bananas was good throughout the duration of the experiment and no obvious stress was ob-

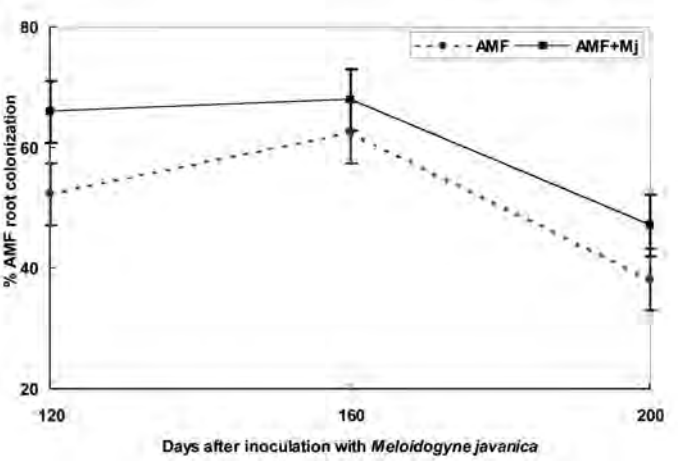

Fig. 1. Percentage of roots of banana colonized by the arbuscular mycorrrhizal fungus (AMF) *Glomus manihotis* in the presence and absence of the root-knot nematode *Meloidogyne javanica* (Mj).

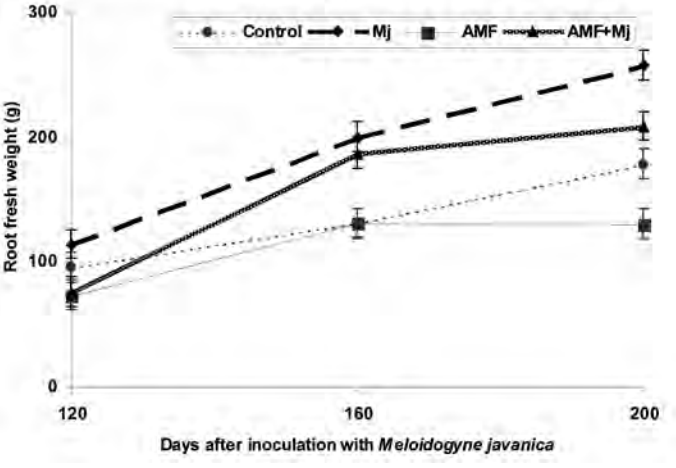

Fig. 2. Effect of the arbuscular mycorrhizal fungus (AMF) *G. manihotis* and the root-knot nematode *M. javanica* (Mj) on the fresh weight of banana roots.

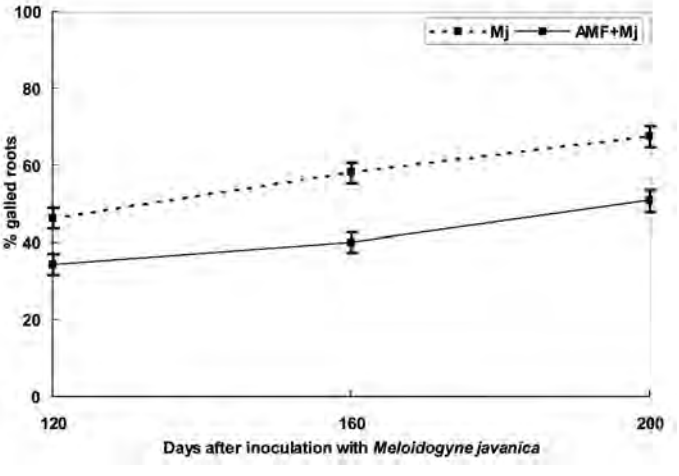

Fig. 3. Effect of *M. javanica* (Mj) on percentage of galled banana roots in the presence and absence of the arbuscular mycorrhizal fungus (AMF) *G. manihotis*.

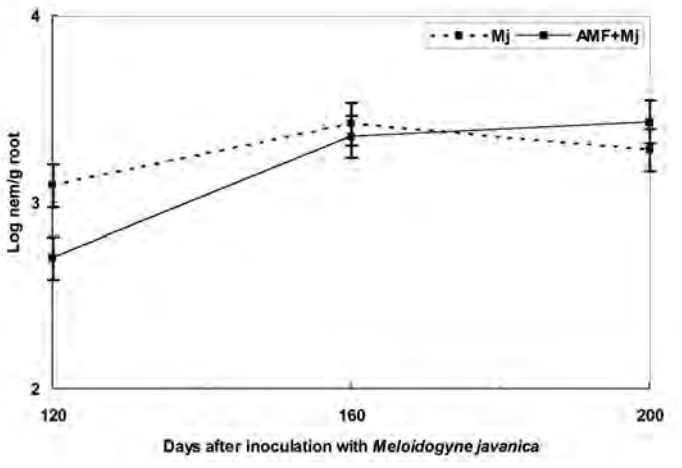

Fig. 4. Eggs and juveniles of *M. javanica* (Mj) in the roots of banana in the presence and absence of the arbuscular mycorrhizal fungus (AMF) *G. manihotis*.

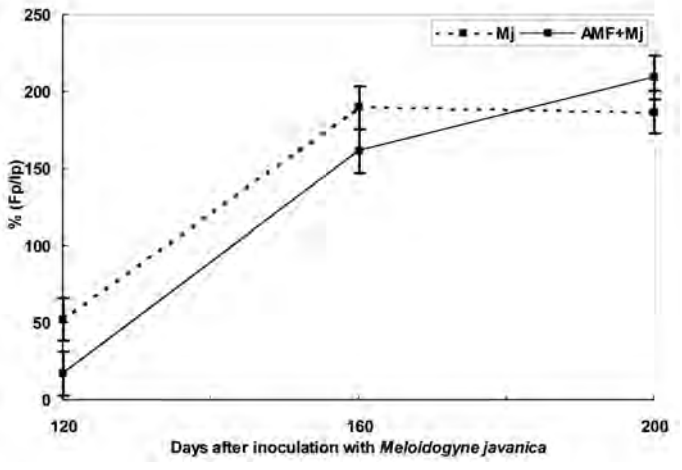

Fig. 5. Reproduction rate of *M. javanica* (Mj) in the presence and absence of the arbuscular mycorrhizal fungus (AMF) *G. manihotis*.

served. Mycorrhizal colonization of the banana roots in pots inoculated and non-inoculated with *M. javanica* was 66% and 52%, respectively, 120 days after inoculation of the nematode. It had increased further 40 days later but declined by the end of the experiment (Fig. 1). Root colonization by the AMF was always higher in pots inoculated with *M. javanica*, but the observed differences were only significant 120 days after the inoculation of the nematode.

The largest increase in root fresh weight of banana was recorded in pots inoculated with *M. javanica*, probably due to gall formation (Fig. 2). Moreover, significant differences in root fresh weight were observed in pots inoculated with the nematode and in the presence or absence of the AMF, by the end of the experiment. Root fresh weight in control pots and in those inoculated with AMF but not with the nematodes was the same until 160 days after nematode inoculation but by the end of the experiment it was less in the AMF inoculated pots.

The per cent of galled root increased with time in all plants inoculated with nematodes (Fig. 3). However, mycorrhized plants showed percentages of galled root significantly lower throughout the experiment and, at the end of the trial, mycorrhized bananas had 20% less galled root.

Eggs and juveniles of *M. javanica* were significantly more abundant in the roots of non-mycorrhized plants than in pots with mycorrhized bananas 120 days after transplanting, but had similar population densities thereafter (Fig. 4). The same trend was observed in the reproduction rate of the nematode in roots. At the first observation, the nematode population had increased by an average of 52% in the roots of non-mycorrhized plants and only by 17% in mycorrhized plants (Fig. 5). Thereafter, the reproduction rate of the nematode did not differ significantly between the two treatments.

DISCUSSION

Mycorrhizal inoculation of the banana cultivar 'Grande Naine', which has been described as highly susceptible to *Meloidogyne* species (Pinochet *et al.*, 1998), showed marked benefits in terms of reduction of galling of roots and nematode population in the roots for at least four months after nematode inoculation. The same effect has been observed in banana infected with several other nematode species, such as *Radopholus similis* (Cobb) Thorne (Umesh *et al.*, 1988), *M. incognita* (Jaizme-Vega *et al.*, 1997), *Pratylenchus goodeyi* Sher *et* Allen (Jaizme-Vega and Pinochet, 1997) and *P. coffeae* (Zimmermann) Filipjev *et* Schuurmas Stekhoven (Elsen, 2002). However, other authors have reported greater galling of roots in mycorrhized plants (Pinochet *et al.*, 1997). These differences may be due to edafic conditions and/or to the *Glomus* isolate employed.

The evolution of root fresh weight was affected by the presence of both organisms in our experiment. By the end of the trial, mycorrhizal colonization reduced the percentage of galled roots and, consequently, root fresh weight. The lower root weight of mycorrhized versus control bananas in pots without the nematode is not abnormal. Under standard conditions, mycorrhized plants are able to develop the same aerial mass despite having a smaller root system. The root systems of mycorrhized plants are probably more efficient at nutrient uptake so have a lower R/S (dry root weight/dry shoot weight) ratio (Smith, 1980). The growth of banana roots was reduced at later stages of our experiment, even though plants appeared vigorous throughout the experiment. However, it would be interesting to see whether this trend would also have occurred in larger pots.

The decline of the nematode population inside the roots (Fig. 4), which started after 160 days from nema-

tode inoculation, can be related to the infestation status of the roots. By 160 days after nematode inoculation, the roots of bananas that did not receive AMF were severely damaged by the nematode and, therefore, were unable to support more nematodes, which, therefore, began to decline in number. Conversely, mycorrhized nematode-inoculated plants still had a large proportion of roots not infected by the nematode (Fig. 3) and, therefore, were able to support a further slight increase of the pathogen. Moreover, the physical presence of AMF, which, once established, uses the space and nutrients sources also required by the nematode, may have limited the nematode infection (Hussey and Roncadori, 1982).

Except at the first assessment date, AMF colonization levels were identical in both of the mycorrhized treatments, thus suggesting that *M. javanica* does not adversely affect mycorrhizal colonization of banana roots, contrary to what has previously been reported (Jaizme-Vega *et al.*, 1997; Pinochet *et al.*, 1997; Elsen, 2002). The decrease in degree of root colonization by AMF 160 days after the inoculation of the nematode, probably occurred because the speed of AMF colonization of the root tissues was slower than the growth rate of the roots (Stover and Simmonds, 1991). Bananas are actively growing herbaceous plants, capable of rapidly losing and replacing old and nematode infected roots (Swennen *et al.*, 1988). This could cause an apparent reduction of the percentage of mycorrhizal root colonization in the later stages of growth and confirms the benefits of and need for early mycorrhization. Fertilizer accumulation in pots may also have adversely affected root mycorrhization at later stages of plant growth, despite the fact that the standard fertilization programme for commercial nurseries used in this experiment was adjusted to minimize this effect.

The mechanisms involved in nematode suppression by the AMF are still unknown, but it seems evident that the presence of the fungal symbiont makes it difficult for the nematode to advance inside the root because the two organisms compete for space and food resources (Hussey and Roncadori, 1982).

In summary, early mycorrhization of 'Grande Naine' banana with an efficient AMF isolate of *Glomus manihotis* is beneficial in suppressing *Meloidogyne javanica* infection. In Tenerife, micropropagated bananas are increasingly replacing conventional planting material, traditionally new suckers from the parent corm. Therefore, although root mycorrhization alone is not sufficient to protect bananas from root-knot nematode attack throughout the crop cycle, if used routinely in the nursery it could provide a useful increase in banana tolerance to the nematode, at least during the first four months of growth. In this way it would provide an extra input for the integrated management of the nematode.

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Accepted for publication on 7 November 2005.