GROWTH RESPONSE OF BANANA TO THREE MYCORRHIZAL FUNGI IN *PRATYLENCHUS GOODEYI* INFESTED SOIL

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ABSTRACT

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The effects of the interaction between three arbuscular mycorrhizal (AM) fungi and the lesion nematode *Pratylenchus goodeyi* on the growth of banana were studied under greenhouse conditions in the Canary Islands. Banana cv 'Grand Naine' inoculated with *Glomus mosseae* and *G. agregatum* showed significantly increased plant development for most growth parameters in *P. goodeyi* infested soil 10 months after inoculation with the AM fungi and 8 months after nematode exposure. Nitrogen levels in mycorrhizal plants were significantly higher in *P. goodeyi* infested soils. Early mycorrhizal inoculation appears to increase host tolerance by enhancing plant nutrition and by reducing the nematode induced lesions in the roots.

Key words: Arbuscular mycorrhizal fungi, banana, *Glomus agregatum, G. intraradices, G. mosseae,* interaction, lesion nematodes, *Pratylenchus goodeyi,* tolerance.

RESUMEN

Jaizme-Vega, M. C. y J. Pinochet. 1997. Respuesta en crecimiento del banano a tres hongos formadores de micorrizas arbusculares en suelo infestado con *Pratylenchus goodeyi.* Nematrópica 27:69-76.

Se estudiaron los efectos del la interacción entre tres hongos formadores de micorrizas arbusculares y el nematodo de las lesiones *Pratylenchus goodeyi* sobre el crecimiento del banano bajo condiciones de invernadero en las Islas Canarias. Plantas de banano 'Grand Naine' inoculadas con *Glomus mosseae* y *G. agregatum* en suelo infestado con *P. goodeyi* mostraron un mayor desarrollo para la mayoría de los parámetros de crecimiento 10 meses después de la inoculación con ambos hongos y 8 meses después de la inoculación con el nematodo. Los niveles de nitrógeno en la planta fueron significativamente más altos cuando éstas estaban micorrizadas con *G. agregatum* y *G. mosseae* en suelos infestados de *P. goodeyi.* La micorrización temprana parece aumentar la tolerancia de la planta estimulando la nutrición y reduciendo el porcentaje de lesiones causadas por el nematodo en las raíces.

Palabras clave: Banano, *Glomus agregatum, G. intraradices, G. mosseae,* hongos formadores de micorrizas arbusculares, interacción, nematodos lesionadores, *Pratylenchus goodeyi,* tolerancia.

INTRODUCTION

The root-lesion nematode *Pratylenchus goodeyi* Sher & Allen is considered an important nematode pest of commercial bananas in the Canary Islands (Rodríguez, 1990). The nematode is widespread in the three major producing islands, Gran Canaria, Tenerife and La Palma where the main areas of nematode infestation occur over 150 meters above sea level where cooler conditions prevail. All banana culti-

vars of *Musa* AAA used in the Canary Islands are highly susceptible to *P. goodeyi* (Rodríguez and Rodríguez, 1976). In Central and East African countries sharing similar environmental conditions with the Canary Islands, this species was found to be damaging, especially in highland bananas and plantains (Bridge *et al.*, 1994; Gowen and Quénéhervé, 1990; Price and Bridge, 1995).

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that colonize the roots of most cultivated plant species. This favors plant development by increasing nutrient uptake, growth rates and hormonal activity (Abbot and Robson, 1984; Linderman, 1992). Mycorrhizae may also increase plant tolerance to biotic and abiotic stress conditions, such as transplanting, soil salinity, drought and attack by soilborne pathogens (Gerdemann, 1968; Smith, 1987). Several micropropagated tropical plant species, like avocado (Vidal *et al.*, 1992), pineapple (Jaizme-Vega and Azcón, 1991) and banana (Declerck *et al.*, 1994; Jaizme and Azcón, 1995; Rizzardi, 1990) have a high mycorrhizal dependency for promoting growth.

Several cultural practices combined with the use of nematicides have traditionally been used for nematode control in the Canary Islands (Rodríguez, 1975), although chemical control has not always been effective nor economically feasible for bananas grown in subtropical conditions. Early mycorrhizal inoculation promotes plant growth and increases the tolerance of bananas to attack by root-knot nematodes (Pinochet *et al.*, 1996). This biotechnological approach applied to micropropagated plants has not been tested against root-lesion nematodes on bananas and might represent an effective nematode management strategy for commercial banana production in the Canary Islands. The aim of this research was to determine the plant growth response of 'Grand Naine' banana inoculated with three different AM fungi in *P. goodeyi* infested soil.

MATERIALS AND METHODS

Micropropagated banana *Musa* AAA cv. Grand Naine was supplied by CULTESA (Cultivos Vegetales *In vitro* de Tenerife, S. A.), Tenerife, Canary Islands, Spain. Plantlets measuring approximately 8 ± 1 cm height with 3 fully developed leaves were received in nutrient agar (Murashige and Skoog, 1962) and were transplanted to acclimatization trays (60 × 40 cm) for early mycorrhizal infection during the hardening phase.

Mycorrhizae and inoculation procedures: Three AM fungi were evaluated. *Glomus mosseae* (Nicol. & Gerd.) Gerd & Trappe, *G. intraradices* Schenck & Smith and *G. agregatum* (Schenck & Smith) Koske isolates, were provided by the Dept. de Patología Vegetal of IRTA, Cabrils, Barcelona, Spain, and maintained on clover (*Trifolium repens* L.) pot cultures at ICIA (Tenerife) in the Canary Islands. The inoculation rate was 1500 g of soil inoculum per tray. This inoculum consisted of thoroughly mixed rhizosphere soil samples containing spores, hyphae and mycorrhizal root fragments.

A total of 4 trays (one inoculated with *G. mosseae*, one with *G. intraradices*, one with *G. agregatum*, and one with non-inoculated controls) containing an average of 30 plantlets per tray were prepared. Substrate used in trays consisted of a pasteurized 1:1:3 (v/v) soil (47% sand, 31% silt, 22% clay), "picón" (porous volcanic ash soil used locally) and peat (Floratorf®, Floragard GmbH, Germany) mixture. The phosphorus (P) content of the substrate was low (16 ppm). Following transplanting of banana plantlets, trays were irrigated

with a filtrate from non-sterilized nursery soil at a rate of 9 cm^3 per plant (obtained by filtering a soil-water dilution of 250cm^3/ liter through a Whatman No. 1 filter) with the purpose of replacing soil microbiota lost in the sterilizing process. The hardening phase lasted 6 weeks. During this period plant material was acclimatized in a poly tunnel covered with a black screen. Temperature fluctuated between 27 and 31°C and relative humidity was maintained at 80%. Trays were irrigated with distilled water as needed. A foliar fertilizer with Wuxall® Super AA 8-8-6 (Argos Schering, AgrEvo, S. A., Valencia, Spain) was applied every three weeks.

At the end of the hardening phase and prior to transplant, 10 plants from each tray inoculated with the three AM fungi and non-inoculated controls were assessed for mycorrhizal infection before nematode inoculation. Remaining plants were then individually transplanted to 7-liter containers filled with a pasteurized 1:1:1 peat, picón and soil. This substrate had less than 10% organic matter and pH 6.8. An optimum P level equivalent to 0.5 g K_2HPO_3/ kg soil was established. This optimum P rate is considered adequate for young banana plants at this growth stage. Mycorrhizal and non mycorrhizal plants were then kept in a greenhouse until nematode inoculation, which occurred one week following transplant and two months after mycorrhizal inoculation. Our procedures were designed to simulate transplanting nursery AM inoculated plantlets into fields infested with nematode pathogens.

Nematode inoculation procedure: The nematode inoculum consisted of a population of *P. goodeyi* isolated from the same banana cultivar originally collected in Buenavista, Tenerife, Canary Islands. The population was cultured monoxenically on carrot (*Daucus carota* L.) disk cultures (Moody *et al.*, 1974), and incubated at 21°C for 2 generations. Species identification was confirmed by SDS-PAGE electrophoresis (Jaumot *et al.*, 1997). For soil infestation, nematodes were collected from flooded cultures with a pipette. Inoculum was adjusted to deliver a suspension of 2 000 nematodes per plant through 8 holes, 3 cm deep and located 5 cm apart from the base of the transplanted banana.

Greenhouse experiment: An experiment with 4 treatments lasting 10 months (after AM inoculation) was established: 1) Plants established in *P. goodeyi* infested soil; 2) Plants inoculated with *G. mosseae* in *P. goodeyi* infested soil; 3) Plants inoculated with *G. intraradices* in *P. goodeyi* infested soil; 4) Plants inoculated with *G. agregatum* in *P. goodeyi* infested soil. Each treatment was replicated 15 times in a completely randomized design. Ambient greenhouse temperature fluctuated between 19 and 35°C during the course of the study. Plants were watered as needed and fertilized weekly following similar fertilization practices to those used in commercial banana nurseries or plantations for the different growth stages of the plant.

At the end of the experiment, number of leaves, fresh and dry top weight, fresh root weight, shoot diameter, plant height and leaf surface area (Area Meter LI-COR, Inc. Lincoln, Nebraska, USA Mod. Li-3100) were assessed. Also, the final nematode population density in roots, the number of nematodes per gram of root, the lesion index of the root, the percentage of AM colonization, and the macronutrient (N, P, K) content in leaves and pseudostem were determined.

Nematode extraction from roots and lesion indices were made according to the method described by Pinochet (1988), modified by using a 25-µm-pore-size screen (500 mesh) in the sieving process. Five percent fresh weight of the root system was used to estimate the percentage of AM

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). The percentage of root colonization was determined using the grid line intersect method (Giovanetti and Mosse, 1980). Mycorrhizal root samples were excised after clarifying and staining the root, mounted on millimetric slides and observed with a light microscope.

To analyze macronutrients, the pseudostems and leaves (avoiding senescent or necrotic tissue) were thoroughly washed with mild detergent, and rinsed three times in distilled water. Samples were dehydrated in a fan-ventilated oven at 60 ± 1°C during 24 hours, ground in a ball mill and digested in nitric and perchloric acid (Jones *et al.*, 1991). Analysis for P and K was made with a F586-587 Varian Liberty 220 inductively coupled plasma (ICP) emission spectrometer (Munter and Grande, 1981). Two readings were made per sample. Nitrogen content was determined according to the Kjeldahl procedure (Rund, 1984).

All data were analyzed by ANOVA. Data on nematode reproduction were $\log_{10}$ (x + 1) transformed for analysis. Means were compared by Tukey's Honestly Significant Difference Test ($P \leq 0.05$). Data for lesion indices and percentage of AM root colonization were transformed to arcsin$\sqrt{x}$ for analysis.

RESULTS

Six weeks after mycorrhizal inoculation with the three *Glomus* species and prior to nematode inoculation at the end of the hardening phase, banana plantlets in trays inoculated with the *G. mosseae* isolate grew significantly more (increased total fresh weight and number of leaves) than those inoculated with *G. intraradices* or *G. agregatum*, and non-mycorrhizal controls (Table 1). The percentage of root colonization by *G. intraradices* (100%) and *G. mosseae* (94%) was higher than that of *G. agregatum* (49%).

Table 1. Growth response and mycorrhizal colonization of three AM fungi on banana plantlets cv. Grand Naine six weeks after inoculation with *Glomus intraradices, G. agregatum* and *G. mosseae,* and prior to exposure with *Pratylenchus goodeyi.*

Treatment[y]	Total fresh weight (g)	No. of leaves	Percentage of mycorrhizal infection[z]
Control	7.09 b	7 b	0
Glomus intraradices	6.04 b	6.5 b	100 a
G. agregatum	7.22 b	6.7 b	49 b
G. mosseae	10.21 a	8.6 a	94 a

[y]Data are means of 10 replications. Means in the same columns followed by the same letter do not differ according to Tukey's Honestly Significant Difference Test ($P \leq 0.05$).
[z]Percentage of AM colonization based on arcsin$\sqrt{x}$ transformed values for analysis.

At harvest, fresh top weights were higher in *G. agregatum* and in *G. mosseae* inoculated plants than in *G. intraradices* and nonmycorrhizal plants (Table 2). Plants from all mycorrhizal treatments had greater dry top weights than plants inoculated with the nematode alone. The same trend occurred for plant height. The *Glomus agregatum* treatment had highest fresh root weights and differed from *G. mosseae.* Pseudostem diameter was also higher in the *G. agregatum* treatment than in all others. Foliar surface was highest in *G. mosseae* inoculated plants. Plants from the *G. mosseae* treatment differed only from those of *G. intraradices* in having significantly more leaves.

Banana plants without mycorrhiza had a higher root lesion index than plants with the three mycorrhizal treatments (Table 3). *Glomus mosseae* inoculated bananas had the lowest lesion index and differed from

Table 2. Effect of *Glomus intraradices*, *G. agregatum* and *G. mosseae* on plant growth in combination with *Pratylenchus goodeyi* on 'Grand Naine' banana 10 months after inoculation with the AM fungi and 8 months after inoculation with 2 000 nematodes per plant.

Treatment[z]	Fresh top weight (g)	Dry top weight (g)	Fresh root weight (g)	Pseudostem diam. (mm)	Foliar surface (cm)	Plant height (cm)	No of leaves
Pratylenchus goodeyi (*Pg*)	1 060 b	21.7 b	421 ab	78 b	3 270 b	123 b	10 ab
Glomus intraradices + *Pg*	1 120 b	23.5 a	417 ab	77 b	3 320 b	132 a	9 b
G. agregatum + *Pg*	1 230 a	24.7 a	500 a	96 a	3 530 b	131 a	10 ab
G. mosseae + *Pg*	1 240 a	25.1 a	385 b	83 b	3 890 a	138 a	11 a

[z]Data are means of 15 replications. Means in the same columns followed by the same letter do not differ according to Tukey's HSD test ($P \leq 0.05$).

the other two *Glomus* treatments. No differences were found between the three mycorrhizal and *P. goodeyi* inoculated plants for final nematode population and nematodes per gram of root. In treatments with joint inoculations of pathogen and symbiont, *G. mosseae* achieved a higher percentage of AM colonization.

No macroelement deficiencies (NPK) were detected by foliar analysis in any of the treatments (Table 4), although N levels were significantly higher in plants colonized by *G. agregatum* and *G. mosseae* than in nonmycorrhizal plants. No differences between treatments were recorded for P or K, although AM plants tended to have higher levels of both elements.

DISCUSSION

In this study, nematode free plants or treatments with the three AM fungi without the nematode were not established for reasons of practicality. These treatments

Table 3. Reproduction of *Pratylenchus goodeyi* and mycorrhizal root colonization by *Glomus intraradices*, *G. agregatum* and *G. mosseae* isolates in combination with the nematode on 'Grand Naine' banana 10 months after inoculation with the AM fungi and 8 months after inoculation with 2 000 nematodes per plant.

Treatment[y]	Lesion index of roots (%)[z]	Final nematode population in roots	Nematodes per gram of root	Percentage of AM colonization
Pratylenchus goodeyi (*Pg*)	23 a	347 370	830	—
Glomus intraradices + *Pg*	13 b	214 610	510	32 b
G. agregatum + *Pg*	15 b	319 830	640	34 b
G. mosseae + *Pg*	4 c	285 900	740	47 a
		NS	NS	

[y]Data are means of 15 replications. Means in the same columns followed by the same letter do not differ according to Tukey's HSD test ($P \leq 0.05$). Actual data is presented for nematode reproduction based on $\log_{10}(x + 1)$ transformed values for analysis. Percentage of Lesion index and AM colonization based on $\arcsin\sqrt{x}$ transformed values for analysis. NS = non significant.

[z]Lesion index of the root based on percentage of lesioned root tissue (Pinochet, 1988).

Table 4. Effect of three arbuscular mycorrhizal fungi on macroelement nutrition (NPK) of micropropagated banana plants cv Grand Naine in *Pratylenchus goodeyi* infested soil 10 months after inoculation with the AM fungi and 8 months after inoculation with 2 000 nematodes per plant.

	Nutrient content (mg/plant)		
Treatment[a]	N	P	K
Pratylenchus goodeyi (*Pg*)	375 b	29.8	790
Glomus intraradices + *Pg*	409 ab	32.7	836
G. agregatum + *Pg*	460 a	31.2	835
G. mosseae + *Pg*	449 a	31.4	833
		NS	NS

[a]Data are means of 15 replications. Means in the same columns followed by the same letter do not differ according to Tukey's HSD test ($P \leq 0.05$).

occur rarely in nature, since *P. goodeyi* is present in 80% to 90% of the area dedicated to banana production in Gran Canaria, Tenerife and La Palma, the primary islands which export bananas (Rodríguez, 1990).

Following the hardening phase, banana plantlets inoculated with *G. mosseae* had better growth responses to early mycorrhizal infection than *G. intraradices* and *G. agregatum*. Both *G. mosseae* and *G. intraradices*, achieved a high percentage of mycorrhizal root colonization during this initial phase (94 and 100%, respectively), but differences in growth response indicate that *G. mosseae* is a more efficient AM fungus. However, 10 months after mycorrhizal inoculation, the percentage of mycorrhizal infection was 47% for *G. mosseae*, the highest among the three mycorrhizal fungi, suggesting that nematode feeding and migration in the roots could have reduced fungal colonization, since both pathogen and symbiont compete for food and space in the cortical tissues (Pinochet *et al.*, 1996). Alternatively, the rate of AM infection in the root tissues may to be lower 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.*, 1986; Swennen *et al.*, 1988). This could cause a natural reduction of the percentage of mycorrhizal root colonization in the later stages of growth, and underscore the benefits and need of early mycorrhizal inoculation.

Mycorrhizae did not appear to affect nematode reproduction in the roots. However, final population densities and nematodes per gram of root tended to be lower in mycorrhizal plants than in plants with the nematode alone. Perhaps a longer time exposure is needed to detect a suppressive effect of the AM fungi on *P. goodeyi*.

Information regarding nematode-mycorrhiza interactions on bananas is scarce. Umesh *et al.* (1988) reported the beneficial effects of mycorrhizal bananas (*Musa acuminata*) infected with the migratory endoparasitic nematode *Radopholus similis*. In that study, the antagonistic effect between pathogen and symbiont resulted in a decrease in root colonization of both *R. similis* and *Glomus fasciculatum* in banana roots. Although we detected no significant antagonistic effect on nematode population densities, mycorrhizal infection did decrease the lesion index of the root in all the cases. Discrepancies in the interaction between both studies may be due to different experimental conditions, the AM fungus involved and the different species of migratory endoparasitic nematodes studied.

Mycorrhizae mainly increase the uptake of P in P deficient soils (Gerdemann 1968) and several microelements linked to P nutrition. However, P absorption was not affected by mycorrhizal root colonization in relation to plants without

the symbiont. In contrast, N absorption was enhanced in *G. agregatum* and *G. mosseae* inoculated bananas. Bananas are known to demand high amounts of N and especially K (Lahav and Turner, 1983).

We conclude from our study that early mycorrhizal inoculation with an efficient AM isolate, such as *G. mosseae* on micropropagated banana plantlets, is beneficial for plant growth, compensating for the damage caused by the root lesion nematode by enhancing plant nutrition (mainly N), and to a lesser extent, by reducing the nematode induced lesions in the roots. A clear suppressive effect of the mycorrhizae over *P. goodeyi* on the banana host was not evident. From the practical standpoint, early mycorrhizal inoculation should be further investigated as a new nematode management approach to increase host tolerance, while being complementary to existing nematode control measures and fairly easy to implement in the current banana production systems used in the Canary Islands.

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