Effect of a mycorrhizal *Glomus* sp. on growth of plantain and on the development of *Radopholus similis* under controlled conditions

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*Nematosis* is one of the most damaging nematodes of banana and plantain in Cameroon. Yield loss due to this nematode on plantain can be more than 50% in case of severe infestation. Arbuscular mycorrhizal fungi (AMF) have been shown to reduce nematode populations in several plant species. Investigations were carried out under controlled conditions to evaluate the effect of a *Glomus* sp., isolated from plantain in Cameroon, on plant growth and on nematode infestation. Tissue-cultured plants were inoculated with the fungus during the weaning phase. Nematode infestation was done six weeks after AMF infection. Inoculation with the *Glomus* sp. significantly increased plant growth parameters. Populations of *R. similis* were significantly lower on mycorrhizal plants than on non-mycorrhizal ones. These preliminary results indicate that the above *Glomus* sp. can be used to enhance growth of plantain plantlets and as a potential biocontrol agent.

Key words: control, interactions, nematodes, *Musa*, mycorrhiza.

Nematodes are responsible for significant yield losses in banana and plantain (*Musa* spp.) (Sarah 1989). In Cameroon, several nematode species have been isolated from banana and plantain roots. The most frequently encountered species are *Radopholus similis*, *Pratylenchus goodeyi*, *Helicotylenchus multicinctus*, *Pratylenchus coffeae*, *Meloidogyne* spp. and *Hoplolaimus* spp., with *R. similis* and *P. goodeyi* being the most damaging (Bridge et al. 1995). *R. similis* is dominant at lower altitudes (<1000 m), whereas *P. goodeyi* predominates at altitudes above 1000 m (Fogain et al. 1998). Yields of dessert bananas and plantains in Cameroon have been doubled with the application of nematicides (Fogain et al. 1996; Fogain 2000).

Several control strategies combining non-chemical and chemical methods have been developed to reduce damage caused by nematodes. Among the non-chemical methods, the use of arbuscular mycorrhizal fungi (AMF) has been shown to reduce the level of damage caused by root-nematodes on plant root systems (Caron 1989; Jaizme-Vega et al. 1997). AMF have also been reported to reduce damage caused by nematodes in the genera *Rotylenchus* and *Pratylenchus* (Pinochet et al. 1995).

Plant growth enhancement is another benefit of the symbiosis between AMF and plant species. Several authors have reported that inoculation with AMF of tissue-cultured banana plantlets during the weaning phase resulted in a significant increase in growth of the plants (Declerck et al. 1995a,b; Jaizme-Vega & Azcon 1995).

The objectives of this study were to evaluate the effect of early AMF inoculation of tissue-cultured plantain plants (*Musa AAB*) on plant growth parameters and on *R. similis* infestation.

Materials and methods

Study site, biological material and experimental design

The study was carried out in a screenhouse at Njombé, located 80 m above sea level with an average annual temperature of 27 °C. A *Glomus* sp. isolated from plantain rhizosphere in Cameroon at low altitude (100 m above sea level) was cultured on leek (*Allium porrum* L.) and cowpea (*Vigna unguiculata* (L.) Walp.) for several months. Spores were then transferred to cowpea grown on quartzitic sand for mass production.

*R. similis* used in the study was a local strain isolated from plantain roots in Njombé and reared on tissue-cultured banana plants (cv. Grande Naine) for six weeks. Planting material consisted of tissue-cultured plantlets of the plantain cultivar Batard. The experiment was arranged in a 2 × 2 factorial design with five replicates.

Weaning phase and mycorrhizal inoculation

The tissue-cultured plantlets were transferred to
200 ml pots filled with sterilised sand. Half of the plantlets each received 10 g of the quartzite sand containing spores, mycorrhizal roots and mycelium. Control plantlets received no mycorrhizal inoculum. Each plant weekly received 100 ml of nutrient solution containing the following macro-elements (mM): Ca(NO₃)₂, 0.9, CaSO₄, 0.05, CaCl₂, 0.005, KCl, 0.5, K₂SO₄, 0.25, MgCl₂, 0.05, MgSO₄, 0.05, NH₄Cl, 0.1, (NH₄)₂SO₄, 0.05, NaH₂PO₄, 0.05, and micro-elements (µM): EDTAFeNa, 80, H₃BO₃, 80, ZnSO₄, 0.8, CuSO₄, 0.8, (NH₄)₆Mo₇O₂₄, 5.6, MnCl₂, 8. The weaning phase lasted four weeks.

**Nematode inoculation**
At the end of the weaning phase, the plants were transferred to 2.5 l pots for two additional weeks before inoculation with *R. similis*. Each pot was filled with a sterilised substrate consisting of coffee husks and sand in equal proportion. Half (10 plants) of the mycorrhizal and non-mycorrhizal plants were each inoculated with 1000 *R. similis* females and larvae. Plants were watered when required and nitrogen was applied as urea at 2 g per plant every second week.

**Assessment of variables**

_Mycorrhizal evaluation:_ the frequency of AMF colonisation was assessed at the end of the weaning phase (four weeks after AMF inoculation) and at the end of the experiment (four months after AMF inoculation and six weeks after nematode inoculation). The roots were removed from plants and rinsed under running tap water to remove soil debris. Roots were then cleared in 10 % KOH, washed again and stained with 0.1 % trypan blue-lactophenol. Thirty root pieces per plant, c. 1 cm in length, were mounted on microscope slides to determine the extent of mycorrhizal infection.

**Nematode evaluation:** after removing the plants from the pots, the roots were washed and 25 g of roots were taken for nematode analysis. Roots were macerated in a blender for 20 seconds containing 150–200 ml tap water. The macerated roots were then sieved through a set of sieves with different mesh apertures (250, 125, 50 and 25 µm). Nematodes were collected from the sieves with the smallest apertures (50 and 25 µm) in a beaker and nematode densities were determined in a counting dish under a light microscope.

_Plant growth parameters:_ Plant growth parameters (height, girth and leaf surface area) were recorded every two weeks for three months. Leaf surface area was calculated using the formula length × width × 0.7 (Champion 1963).

**Statistical analysis:** nematode populations were log(x+1)-transformed before analysis was performed. Statistical analysis was done using the statistical package STAT-ITCF, and means were separated according to the Newman-Keuls test (*P* ≤ 0.05).

**Results**
No differences in growth parameters were evident the end of the weaning phase between plants inoculated and not inoculated with the *Glomus* sp. (data not shown). At the end of the experiment, plant height, girth, leaf surface area and fresh root mass were significantly greater in mycorrhizal than in non-mycorrhizal plants (Table 1). Inoculation of plants with *R. similis* resulted in a reduction in some growth parameters. Fresh root mass of non-mycorrhizal plants was significantly reduced by *R. similis*. All plant growth parameters were significantly higher in mycorrhizal plants infested with *R. similis* than in non-mycorrhizal infested plants.

### Table 1. Effect of *Glomus* sp. and *Radopholus similis* on growth parameters of plantain plants 60 days after inoculation with the nematode and 120 days after inoculation with the AMF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Girth (cm)</th>
<th>Height (cm)</th>
<th>Leaf surface area (cm²)</th>
<th>Root fresh mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.76 a</td>
<td>18.4 a</td>
<td>190.3 a</td>
<td>77.4 a</td>
</tr>
<tr>
<td>+<em>R. similis</em></td>
<td>1.52 a</td>
<td>18.0 a</td>
<td>207.0 a</td>
<td>27.4 c</td>
</tr>
<tr>
<td>+<em>Glomus</em></td>
<td>2.80 b</td>
<td>35.0 b</td>
<td>721.6 b</td>
<td>112.8 b</td>
</tr>
<tr>
<td>+<em>Glomus</em> + <em>R. similis</em></td>
<td>2.64 b</td>
<td>33.8 b</td>
<td>613.6 c</td>
<td>96.6 ab</td>
</tr>
<tr>
<td>Probability</td>
<td>0.0012</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.0006</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.40</td>
<td>5.5</td>
<td>42.8</td>
<td>23.6</td>
</tr>
</tbody>
</table>

Values in columns followed by the same letter do not differ significantly according to Neuman-Keuls test (*P* ≤ 0.5).
One month after infection with the *Glomus* sp., 40 % of the root system was colonised. At the end of the experiment the frequency of mycorrhization was 59 and 41 % in plants infested and not infested with *R. similis*, respectively. Mycorrhization was not influenced by the presence or absence of nematodes.

Six weeks after inoculation with *R. similis*, the number of nematodes recovered, expressed per 100 g of roots, was significantly lower in mycorrhizal plants than in non-mycorrhizal ones (5706 vs 50 435).

**Discussion**

Considerable information has been accumulated on nematode damage on plantain (Fogain et al. 1996; Fogain 2000) and mycorrhizal benefits to plants (Declerck et al. 1995a,b; Jaizme-Vega & Azcon 1995). Although nematode–AMF interactions have been studied on banana, very little is known about the interaction between these organisms on plantain. In the present study, dense root colonisation, with typical AMF structures within roots, was obtained during the weaning phase and persisted during the experimental phase even in the presence of *R. similis*. Plant girth, height, leaf surface area and fresh root mass were also significantly increased in the mycorrhizal plantains compared to the control plants.

Mycorrhizal plants had significantly lower *R. similis* population densities six weeks after inoculation with the nematode compared to non-mycorrhizal ones. This is an indication that root colonisation by the *Glomus* sp. prior to nematode infestation resulted in a negative effect of the fungus on *R. similis*. These findings corroborate the report by Umesch et al. (1989) that *R. similis* populations were significantly lower in mycorrhizal banana plants than in non-mycorrhizal ones. Jaizme-Vega et al. (1997) also found fewer galls of the root-knot nematode *Meloidogyne incognita* on roots of banana plantlets infected with *Glomus mosseae*. Similar results were reported by Pinochet et al. (1995) in the control of *Pratylenchus vulnus* on peach (*Prunus persica* Sieb. & Zucc.) with *G. mosseae*. Protection of plants from pests and diseases has long been known as one of the benefits of the symbiosis between AMF and plants (Graham & Menge 1982; Hussey & Roncadori 1982; Krishna & Bagyaraj 1983). The real mechanism by which AMF affects the pathogens is not clearly understood. However, several mechanisms, including increased tolerance, competition for host photosynthates, and colonisation sites, anatomical changes in the root system, microbial changes in the mycorrhizosphere and the activation of plant defence mechanisms could explain the tolerance of mycorrhizal plants to soil pathogens (Azcon-Aguilar & Barea 1996). Increased P-nutrition, though controversial, has also been postulated as one of the mechanism of tolerance of plants to nematodes (Azcon-Aguilar & Barea 1996). Hussey & Roncadori (1982) reported that increased tolerance is the single most common effect of AMF on nematode-susceptible plants.

Root colonisation by *Glomus* sp. was not affected by *R. similis*. This corroborates the work of Jaizme-Vega et al. (1997), who found no effect of *M. incognita* on the percentage root colonisation of banana by *G. mosseae*. Pinochet et al. (1995) also reported no direct negative effect of *P. vulnus* on colonisation of peach roots by *G. mosseae*.

In conclusion, the study has shown that root mycorrhization of tissue-cultured plantain plantlets during the weaning phase leads to plant growth enhancement. Mycorrhization of plants prior to infection with *R. similis* resulted in a significant reduction in populations of the nematode. This suggests that *Glomus* sp. isolated in Cameroon is a potential biocontrol agent for the management of *R. similis* in banana and plantain farming systems. Use of the *Glomus* sp. might reduce the use of chemicals and consequently reduce environmental pollution. Further investigations are required to validate these results in the field and to evaluate the effect of this AMF in different environments and soil conditions.

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**References**


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