

Interactions of *Meloidogyne javanica* and *Glomus* sp. on Growth and N₂ Fixation of *Acacia seyal*

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Abstract. Recently biological agents have gained importance in controlling the diseases caused by plant parasitic nematodes. It has been shown that vesicular and arbuscular mycorrhizal fungi (VAM) have potential as biocontrol agents. This study investigated the interactions between *Meloidogyne javanica* (Treub) Chitwood commonly found in Senegal and an endomycorrhizal fungus *Glomus* sp., isolated in Senegal on a sahelian acacia, *Acacia seyal*. In pots, the following treatments were applied: (1) Non inoculated, (2) infection with a rhizobial strain ORS 1088, (3) infection with VAM and (4) infection with VAM + ORS 1088. All these treatments were with and without *M. javanica* (3000 J₂ per plant) inoculated after 12 week culture. We have shown that the mycorrhizal seedlings with or without *M. javanica* have a better growth than the non mycorrhizal seedlings infected or not by *M. javanica*. The root-knot nematode reduced significantly the biological nitrogen fixation but had not effect on the mycorrhizal symbiosis. The mycorrhizal application did not reduce nematode population per plant but significant differences have been measured between nematode populations from mycorrhizal and non mycorrhizal roots of each plant. In this study, it has been shown that mycorrhizae markedly influence plant health by altering plant reactions to nematodes. The mechanisms which could be involved in these interactions are discussed and the possibilities to improve this mycorrhizal effect are exposed.

Keywords: *Acacia seyal*, endomycorrhizae, forest tree, *Glomus* sp., interaction, *Meloidogyne javanica*, nematode, rhizobia, *Rhizobium* sp., vesicular-arbuscular mycorrhizal fungi.

INTRODUCTION

Mycorrhizal fungi form symbiotic associations on the roots of the vast majority of woody plants. Many trees require mycorrhizas to survive and grow in natural forest ecosystems. There are two major groups of mycorrhizas (ectomycorrhizas and vesicular-arbuscular mycorrhizas), of which vesicular-arbuscular (VA) mycorrhizas are the most widely distributed. They occur on the most vascular plants and in all terrestrial habitats, from deserts to tropical forests. Many N₂-fixing shrubs are very dependent on mycorrhizas to improve nutrient uptake required for plant growth and efficient N₂ fixation.

In savannas and arid regions of Australia, Africa, South and North America and India, shrubs and trees of the legume genus *Acacia* (Mimosaceae) are abundant. In the Sahel region of Africa, *Acacia* is often the dominant tree species. Some of them serve to prevent wind and rain erosion, control sand dunes, are sources of wood and arabic gum and provide shade for livestock. Most *Acacia* species have *Rhizobium* nodules and fix N₂.

Mycorrhizal fungi are known to stimulate the growth of tree species. In particular, acacias have responded to inoculation with VA mycorrhizal fungi in pot experiments and under field conditions (Mosse, 1977; Munns & Mosse, 1980). Factors which influence tree response to mycorrhizal inoculation in the field are: (1) tree species, (2) the fertility of the planting site, (3) the species composition and population

level of the naturally occurring mycorrhizal fungi, (4) the persistence and competitiveness of the introduced mycorrhizal fungus and (5) the composition and population of the microflora and microfauna in the planting site.

Plant-parasitic nematodes are a cosmopolitan and important threat to production of agricultural crops. In particular, root-knot nematodes are very common in subtropical and tropical vegetables. Four species have been more particularly described on these crops: *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* (Johnson & Fassuliotis, 1984). All these species may reduce or prevent normal nodulation and N₂ fixation (Germani *et al.*, 1984; Huang & Barker, 1983) but VA mycorrhizal fungi may reduce the effects of root-knot nematodes (Thomson Cason *et al.*, 1983).

The present study has been initiated to investigate the relationships between the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood, a strain of *Rhizobium* sp. (ORS 1088) and an endomycorrhizal fungus (*Glomus* sp.) on *Acacia seyal* (Del.)

MATERIALS AND METHODS

Plant material

In order to obtain fast and regular germination, the seeds of *Acacia seyal* from province Velingara (Senegal) were pretreated with concentrated sulphuric acid for 30 minutes. After treatment, the seeds were washed 4 times in sterile



distilled water in order to remove all traces of sulphuric acid. These pretreated seeds were plated in Petri dishes on moistened paper and kept in the dark at 30°C. Dishes were checked daily and those germinated were used when rootlets were 1-2 cm long. The germinated seeds were grown in 1 dm³ polyethylene bags filled with soil that had been autoclaved (140°C, 60 min). Its physiochemical characteristics were as follows: pH H₂O 7.2; fine silt 1.6%, fine sand 1.3%, coarse sand 32.9%; total carbon 0.054%; total nitrogen 0.015%. The seedlings were maintained in a glasshouse (25°C day, 20°C night) and watered twice weekly without fertilisation.

Cultured methods

Glomus sp. inoculum, originally isolated from *Acacia albida* in Senegal, was propagated on sudangrass (*Sorghum vulgare*) for 12 weeks in a glasshouse. Colonized sudangrass roots and adhering soil were used as inoculum.

The strain ORS 1088 of *Rhizobium* sp. isolated on *Acacia seyal* was cultured in glass flasks containing liquid yeast extract-mannitol medium (Vincent, 1970) for 8 days at 37°C on an orbital shaker. The concentrated bacterial suspensions contained approximately 10⁹ cells per ml.

Meloidogyne javanica was increased on tomato (*Lycopersicon esculentum* Mill.), cv Roma in a glasshouse. After two months culture, the tomato roots were harvested and the nematodes were extracted in a mistifier for one week (Seinhorst, 1950). The suspension of freshly hatched juveniles was thoroughly mixed and 5 ml samples used to estimate their numbers. Required quantities of nematodes were poured in one hole (5 mm by 100 mm) in one side of the seedlings and covered with soil.

Experimental details

One week after sowing, four treatments were performed : (1) non inoculated (control), (2) ORS 1088: one ml bacterial suspension ORS 1088 was injected in each bag with a syringe, (3) *Glomus* sp.: the sudangrass plants were harvested after 12 weeks of growth. The roots were washed from soil with tap water, cut into segments 1 cm long, and mixed in water. A sample of 1 g root fresh weight was put into one hole (1 cm by 3 cm) to one side of each seedlings and covered with soil. To help standardize the soil microflora, nonmycorrhizal treatments received samples of 1 g (fresh weight) of non-mycorrhizal root, (4) *Glomus* sp.+ORS 1088. Each treatment was represented by a block containing 16 plants. Plants were kept in a glasshouse for 12 weeks before nematode inoculation. Six plants of the ORS 1088 and *Glomus* sp.+ORS 1088 treatments and 10 plants of the control and *Glomus* sp. treatments were inoculated with nematodes. Three thousand juveniles (J₂) suspended in 5 ml distilled water were poured in each bag after 12 week culture.

At five week intervals during this experiment, total stem length was measured. Nineteen weeks after seeds germinated,

6 plants of each treatment were sampled in order to quantify the mycorrhizal infection, the rhizobial symbiosis and the nematode population.

The mycorrhizal infection was determined as follows: a sample of 1 g root fresh weight of each plant was stained with acid fuchsin for measurement of the fraction of root length with visible mycorrhizal structures (Kormanik & Mc Graw, 1984). Root nodules were counted and their dry weights were determined (1 week, 60°).

In order to determine the final nematode population, the whole soil content of each bag was thoroughly suspended in water for nematode extraction using Cobb's decanting and sieving method. Galls per plant have been counted or the gall index per root seedling has been determined as follows: 0: no galls; 1: 1 to 5 galls; 2: 6 to 20 galls; 3 : more than 20 galls; 4: coalescing galls on the entire root system; 5: rotten root system. The juveniles were extracted by placing the roots in amrist chamber for three weeks (Seinhorst, 1950). Each week, nematodes number was counted.

The shoot dried weights were measured after oven-drying for one week at 60°C and the total nitrogen was quantified by the Kjeldahl method (Bergersen, 1980).

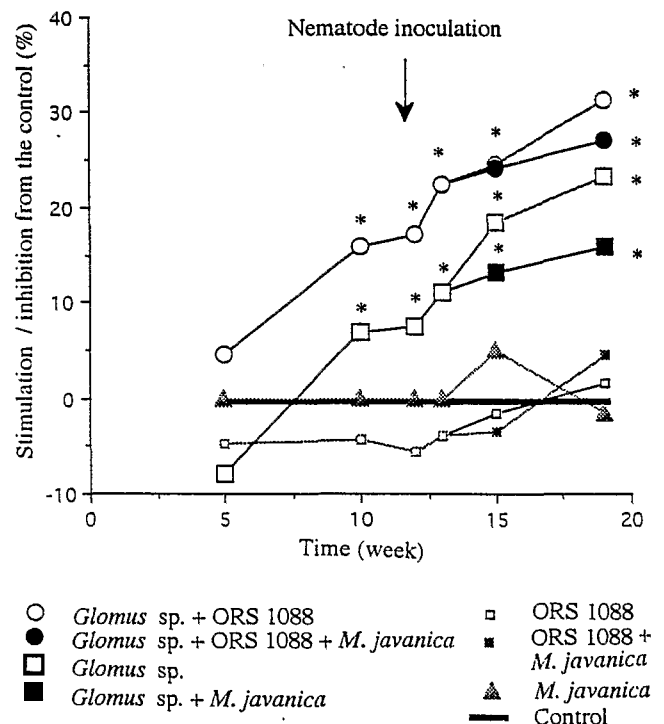


Fig. 1. Effects of the fungal and bacterial treatments with and without *Meloidogyne javanica* on the height of *Acacia seyal* seedlings grown in bags.

* Significantly different from the control without nematodes according to one way analysis of variance (P<0.05).

Table 1. Effect of *Meloidogyne javanica* on the rhizobial and mycorrhizal symbiosis on *Acacia seyal*.

Treatment	No. of nodules per plant	Total dry wt of nodules per plant (mg)	Total N ₂ of leaves	Mycorrhizal rate (%)
Control	17.8 a	37.2 a	1.64 a	0.0 a
Control+				
<i>M. javanica</i>	3.0 b	28.2 a	1.2 b	0.0 a
ORS 1088	7.7 b	38.2 a	1.6 a	0.0 a
ORS 1088+				
<i>M. javanica</i>	4.5 b	19.9 a	1.2 b	0.0 a
<i>Glomus</i> sp.	16.0 a	113.6 b	1.6 a	48.0 b
<i>Glomus</i> sp. +				
<i>M. javanica</i>	3.3 b	32.3 a	1.5 a	23.3 b
<i>Glomus</i> sp. +				
ORS 1088	6.8 b	41.6 a	1.7 a	37.7 b
<i>Glomus</i> sp. +				
ORS 1088 +				
<i>M. javanica</i>	8.5 b	26.5 a	1.3 b	48.0 b

The values followed by the same letter are not significantly different according to one-way analysis of variance ($P < 0.05$).

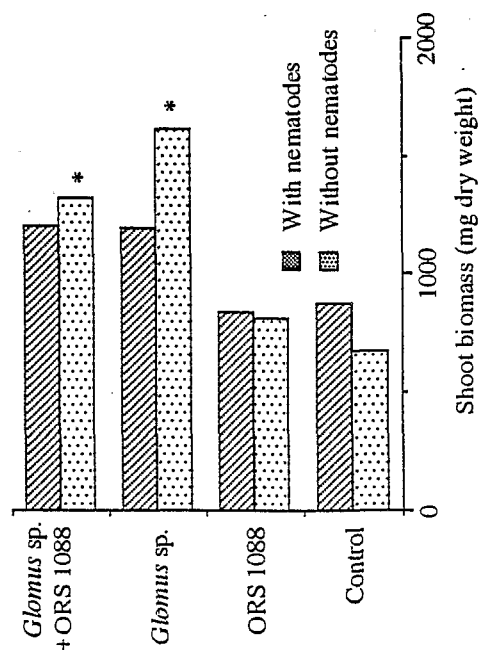


Fig. 2. Effect of the fungal and bacterial treatments with and without *Meloidogyne javanica* on the shoot biomass (mg dry weight) of *Acacia seyal* after 19 weeks culture.

* significantly different from the control without nematodes according to one way analysis of variance ($P < 0.05$).

Table 2. Development of *Meloidogyne javanica* in the presence of mycorrhizae and rhizobia.

Treatment	Gall index	Number of J ₂ s per plant	Multiplication rate*
Control	0.8 a	10900 a	3.6 a
ORS 1088	1.0 a	16200 a	5.4 a
<i>Glomus</i> sp.	2.3 b	6833 a	2.3 a
<i>Glomus</i> sp. +			
ORS 1088	1.3 a	10895 a	3.7 a

The values followed by the same letter are not significantly different according to one-way analysis of variance ($P < 0.05$).

*Multiplication rate: final population / initial population.

Four other plants were sampled from the control and *Glomus* sp. treatments. Their root systems were removed from the bags, gently washed from soil and observed with a stereomicroscope. The roots with endomycorrhizal spores were separated from ones without spores. Each type of root was placed in a mist chamber for three weeks and their oven-dried weights were measured.

Means were compared using one-way analysis of variance and least significant differences (5%). For the analysis of mycorrhizal infection, the data were transformed by arcsin(sqrt) before statistical analysis; for nematode populations, data were transformed by log (x+1).

RESULTS

Plant growth

After 10 weeks, the seedlings were taller in the treatments with the endomycorrhizal fungus with or without the rhizobial strain ORS 1088 than in the control or the treatment with ORS 1088 alone (Fig. 1). After nematode inoculation, this difference was maintained between the treatments with and without *Glomus* sp. throughout the experiment (Fig. 1). Without *M. javanica*, the shoot biomass of the mycorrhizal seedlings was greater than in the control and ORS 1088 treatments (Fig. 2). However no differences were recorded between the treatments with *M. javanica* (Fig. 2). The biomass of the lateral roots of the mycorrhizal plants was greater than that of the non-mycorrhizal plants (Fig. 3). No differences in the biomass of the tap roots were noted between treatments (Fig. 3).

Rhizobial and mycorrhizal symbiosis

The seedlings of the *Glomus* sp. and control treatments were contaminated by indigenous rhizobial strains (Table 1). The endomycorrhizal fungus did not effect the nodulation of these indigenous bacteria or the inoculated bacteria ORS 1088. *Meloidogyne javanica* reduced the number of nodules per plant in the treatments without ORS 1088. These differences were not observed when ORS 1088 was inoculated (Table 1). The total dry weight of nodules per plant was only depressed

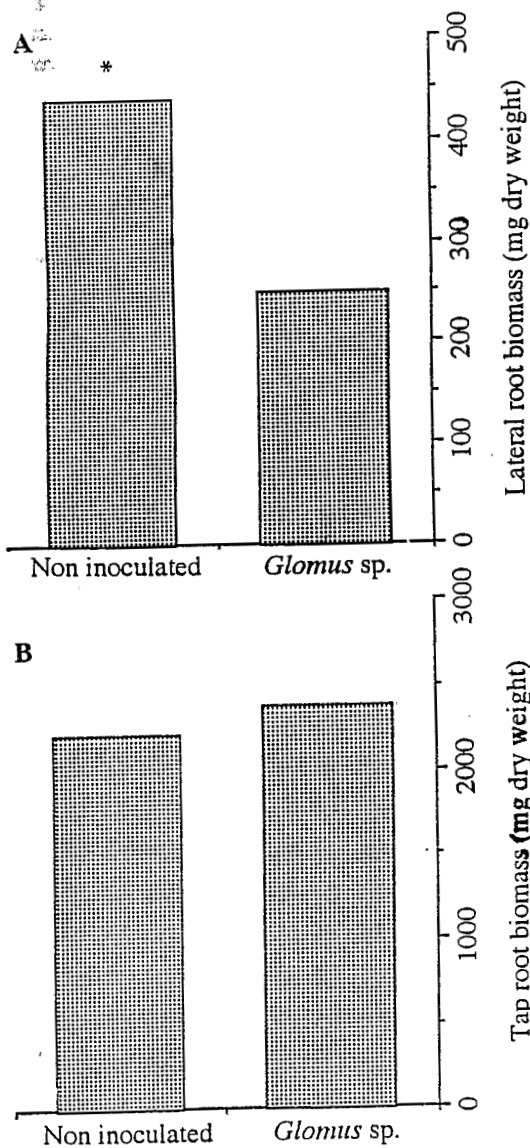


Fig. 3. Effect of *Glomus* sp. on the growth of lateral roots (A) and tap roots (B) infested with *Meloidogyne javanica*. *: significantly different from the control without *Glomus* sp. according to one way analysis of variance ($P < 0.05$).

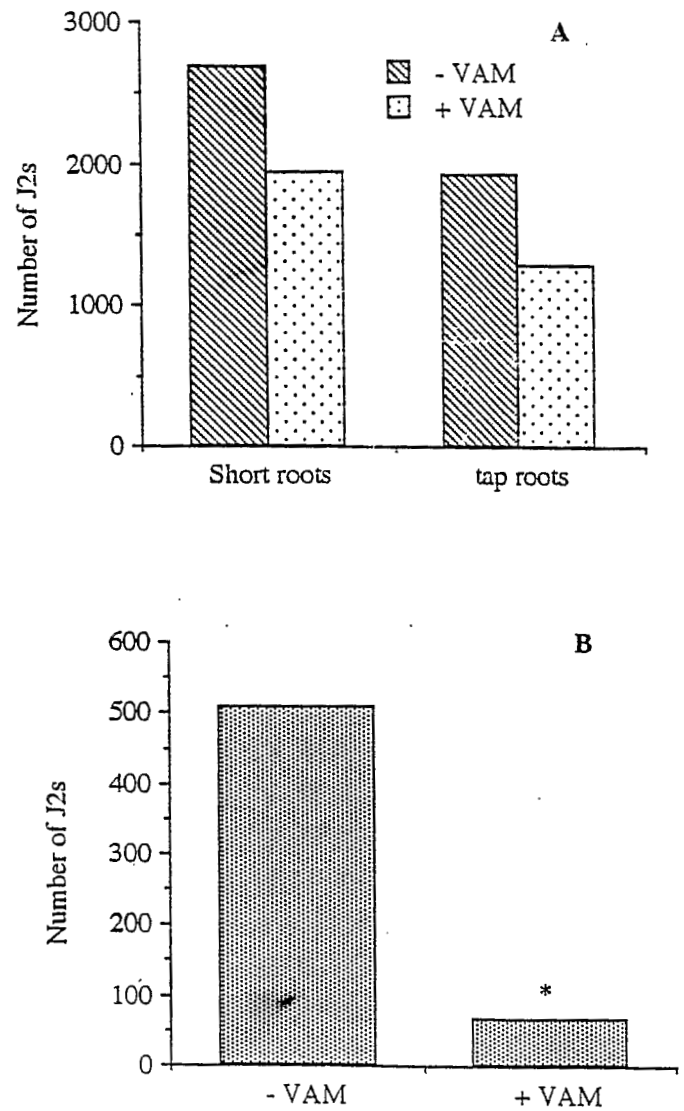


Fig. 4. Distribution of juveniles (J_2) of *M. javanica* in the lateral and tap roots in the mycorrhizal and non mycorrhizal plants (A) and distribution of the juveniles in the root system of the mycorrhizal plants between mycorrhizal and non mycorrhizal lateral roots (B).

*: significantly different according to one way analysis of variance ($P < 0.05$).

by *M. javanica* in the treatment with *Glomus* sp. alone (Table 1). In contrast, the total nitrogen content of leaves was significantly less in all the treatments except where *Glomus* sp. was present (Table 1). *Meloidogyne javanica* had no effect on mycorrhizal infection (Table 1).

Nematode development

No differences were observed between treatments in the number of J_2 s per plant or the multiplication rate (Table 2). However, the seedlings inoculated with *Glomus* sp. alone had a significantly larger gall index. The distribution of the juveniles was the same between the lateral roots and tap roots in the plants treated or not with *Glomus* sp. (Fig. 4). But, for the seedlings treated with mycorrhizae, the number of nematode juveniles in the lateral roots without mycorrhizal spores was significantly greater than in the lateral roots with spores (Fig. 4).

DISCUSSION

The endomycorrhizal fungus stimulated the growth of the seedlings but had no positive effect on nodule development of rhizobia. The nodulation with indigenous rhizobial strains was inhibited by *M. javanica*, but the nematode did not effect the rhizobial strain ORS 1088. Consequently, these two rhizobia have a different interaction with *M. javanica* and further research is necessary to explain this result.

The multiplication of *M. javanica* is not altered by the endomycorrhizal fungus. The number of J_2 s per plant and the multiplication rate were similar on mycorrhizal and non mycorrhizal plants. However, distribution of juveniles was very different in the root systems. With *Glomus* sp., only the lateral roots without spores and the tap roots were infested by *M. javanica*. In order to measure the juvenile hatching, the lateral roots were not stained to observe the mycorrhizal structures as staining with acid fuchsin kills the nematode. It is known that, when the mycorrhiza matures, the external mycelium usually produces large resting spores and smaller secondary spores, or external vesicles (Barea & Azcon-Aguilar, 1985). Consequently, the mycorrhizae which are well established in the lateral roots has an antagonistic effect against *M. javanica*. The mechanisms are unknown but they may have a physical or physiological basis; mycorrhizae may alter or reduce root exudates which attract nematodes, or inhibit nematode development or their reproduction within root tissues.

Several *Acacia* species which are used in agroforestry programmes are very susceptible to *Meloidogyne* sp. (e.g. *A. holosericea*, *A. mangium*). Generally these trees grow in nurseries during 4 months before the forest plantations. At this step, a controlled mycorrhization can improve the seedling growth and after the endomycorrhizal symbiosis can help these trees to establish in infested soils. The fungal symbiont which

have colonised the root systems during the nursery period can protect the roots against root-knot nematodes. Consequently, a part of the root system can ensure the mineral nutrition of the acacia. It will be necessary to enhance this mycorrhizal effect with a screening of more effective fungal strains and to explain the mechanisms involved in this antagonistic effect.

However the mycorrhizal symbiosis cannot totally suppress the nematode development. This problem can prevent the soil rehabilitation process in order to restore agricultural systems. Indeed in a agroforestry system with trees and crop vegetables, these mycorrhizal fungi associated with the trees can also infect the crop vegetables and consequently enhance their growth and their resistance against nematodes.

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