

Original article

**Effect of endomycorrhizas and nematodes
on the growth of seedlings of *Dicorynia guianensis*
Amshoff, a tree species of the tropical rain forest
in French Guiana**

M Béreau^{1*}, E Louisanna¹, J Garbaye²

¹Station de recherches forestières, Inra, BP 709, 97387 Kourou;

²Centre de recherches forestières de Nancy, Inra, 54280 Champenoux, France

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Summary – Seedlings of *Dicorynia guianensis* Amshoff, an economically important timber tree species of the primary tropical rain forest of French Guiana, were grown in pots containing a disinfected soil inoculated or not with forest soil, mycorrhizal roots of *D guianensis* or nematodes. Plant growth parameters and root endomycorrhizal colonization were measured after 200 days. The results are inconclusive about the role of nematodes but clearly show that *D guianensis* is dependent on endomycorrhizal symbiosis for its development: the top dry weights of the seedlings inoculated with soil or roots (with 87–84% endomycorrhizal roots) are 77 and 54% higher, respectively, than that of the uninoculated seedlings (with no mycorrhizas observed). In relation with previous observations in the forest, these results support the hypothesis that endomycorrhizas play a major role in the regeneration of *D guianensis*.

tropical rain forest / *Dicorynia guianensis* / seedlings / endomycorrhizas / experimental approach

Résumé – Effet des endomycorhizes et des nématodes sur la croissance des semis de *Dicorynia guianensis* Amshoff, une essence de la forêt tropicale humide primaire de Guyane française. Des semis de *D guianensis* ont été cultivés en pots sur un sol désinfecté inoculé ou non avec un sol forestier, des racines mycorhizées de *D guianensis* ou des nématodes. Des paramètres de croissance des plantes et la colonisation endomycorhizienne des racines ont été mesurés au bout de 200 j. Les résultats ne sont pas concluants en ce qui concerne le rôle des nématodes, mais montrent clairement que *D guianensis* est dépendant de la symbiose endomycorhizienne pour son développement : les poids de matière sèche des parties aériennes des semis inoculés avec sol ou racines (aux racines endomy-

* Correspondence and reprints

Tel: (594) 32 92 82; fax: (594) 32 69 14

corhizées à 87–84 %) sont respectivement 77 et 54 % supérieurs à celui des semis témoins non inoculés (non mycorhizés). En relation avec des observations antérieures en forêt, ces résultats confortent l'hypothèse que les endomycorhizes jouent un rôle important dans la régénération de *D guianensis*.

forêt tropicale humide / *Dicorynia guianensis* / semis / endomycorhizes / approche expérimentale

INTRODUCTION

Mycorrhizal symbioses play a major role in the mineral nutrition of plants in most terrestrial ecosystems. However, most of our knowledge on the dependency of plants on symbiotic fungi is based on studies in temperate regions, and relatively little mycorrhizal research has been carried out in neotropical rain forests (Janos, 1980, 1984): to what extent do mycorrhizas play a role in the regeneration of the forest (survival and growth of tree seedlings)? Alexander et al (1992), discussing the role of mycorrhizas in the regeneration of some Malaysian forests trees, suggest that mycorrhizas are obligate for the establishment of tree seedlings.

In a previous work, Béreau and Garbaye (1994) observed that endomycorrhizal symbioses were dominant in this type of forest. Root galls due to endoparasitic nematodes were also observed on two Caesalpinioideae, including *Dicorynia guianensis* Amshoff (local names: Angélique, Basralocus, Angelica do Para, Tapiuna), an economically important timber species.

Thus, the aim of the present work was to examine experimentally the relationship between endomycorrhizas, nematodes and growth of *D guianensis* seedlings.

MATERIALS AND METHODS

The experiment was set up according to a fully randomized block design, with four treatments, four blocks and ten plants within each block-treatment combination.

The seeds of *D guianensis* were extracted from pods collected on the forest floor at the experimental site of Paracou (Bariteau and Geoffroy, 1989) at the end of the wet season (May–June 1994), air-dried and kept for 6 months at room temperature. They were treated with pure sulfuric acid for 10 min and washed five times with sterile distilled water in order to break their dormancy. They were then surface-sterilized with a 0.1% mercury chloride solution (HgCl_2) for 5 min and rinsed four times with sterile water. The seeds were aseptically germinated on sterile filter paper humidified with distilled water and used after root emergence (7 days).

A ferrallitic forest soil was collected at the experimental site of Paracou, sieved through a 1 cm screen and steam-disinfected at 90 °C three times for 2 h each, with 1 day intervals. Table I

Table I. Chemical properties of the soils used in the experiment.

	A	B
pH (H_2O)	4.7	4.3
pH (KCl)	4.2	4.0
Organic C (mg g^{-1})*	16	45
Total N (mg g^{-1})**	1.0	1.4
Extractible P (mg kg^{-1} ***)	0.2	0.3
Exchangeable K^+ (meq/100 g)	1.8	3.2
Exchangeable Ca^+ (meq/100 g)	0.7	0.4
Exchangeable Mg^+ (meq/100 g)	1.1	2.2
Exchangeable Na^+ (meq/100 g)	0.6	0.7

A: steam-disinfected soil used as growth substrate in all treatments; B: fresh forest soil used as inoculum in the soil-inoculated (Si) and nematode-inoculated (Ni) treatments. * Walkley and Black method (1934); ** Bremner method (1960); *** Olsen et al method (1954).

gives the chemical properties of the steamed soil. Two weeks later, this soil was filled into 1.3 L black plastic pots according to the following four treatments:

- i) Control (disinfected soil without any addition).
- ii) Soil-inoculated (disinfected soil mixed with 30% v/v of a fresh forest soil extracted from a nearby plot).
- iii) Root-inoculated (disinfected soil plus ca 10% w/w roots of *D guianensis*). The roots were washed with 6% Ethoprophos to eliminate the nematodes, abundantly rinsed with tap water, cut into 5–10 cm pieces and thoroughly mixed with the disinfected soil. In addition, part of these roots were blended in water and 70 mL of the suspension was added on top of each pot.
- iv) Nematode-inoculated (disinfected soil plus 90 mL per pot of nematode suspension). This suspension was prepared by wet-sieving 30 kg of forest soil with tap water through a 125 µm screen. Nematode galls from seedlings of *D guianensis* in the forest were crushed in a mortar and added to the suspension. Live nematodes were present in the final suspension used for inoculation.

Two germinated seeds were introduced into each pot but only one plantlet was kept after 2 weeks. The seedlings were grown in a shade tunnel intercepting 85% of the incident light (in order to simulate the light intensity at the level of forest floor in closed stands) in Kourou (on the coast of French Guiana), for 204 days. The temperature fluctuated between 27 and 35 °C. The atmospheric relative humidity, generally higher than 90%, was dependent on the season and on the time of day. The pots were automatically drip-watered for 2 min every day with about 50 mL tap water per pot.

The leaflets were counted every week from week 3 (*Dicorynia* leaves are pinnate composite). From week 14, the height of the seedlings (terminal bud above ground) was also measured weekly.

The experiment was terminated on week 29; at that time, the following operations were performed:

- The total leaf area per plant was measured with a portable area meter (LI-COR 3000). Leaves and stems were separately oven-dried at 80 °C for 48 h and weighed.
- The root systems were washed free of soil and individually rated for their development according to a scale from 1 (the smallest root systems)

to 3 (the largest). The ten root systems of each treatment in each block were cut into 4–5 cm pieces pooled together and thoroughly mixed. A random subsample was cut into 1 cm pieces, then cleared and stained (according to Kormanik and McGraw, 1982) for quantifying endomycorrhizal colonization by the technique of Trouvelot et al (1986), which consists in evenly spreading root segments on a microscope slide and observing 100 successive fields. Fields containing intracellular vesicles and/or hyphal coils were recorded as being colonized. The results were expressed as percent of fields with colonized roots and transformed by arcsin square root before being subjected to the analysis of variance. Arbuscules were not found, which is consistent with field observations on both seedlings and mature trees of *D guianensis* in French Guiana (Béreau and Garbaye, 1994).

The analysis of variance of the data was first performed two-ways (four blocks and four treatments) for detecting general effects, and then one-way (four treatments and 40 replicates) for detecting significant differences between individual treatments (except for mycorrhizal colonization because the roots of the ten seedlings were pooled in each block-treatment combination).

RESULTS

The results concerning growth parameters and mycorrhizal colonization at the end of the experiment are presented in figure 1. The two-way analysis of variance indicates that the treatment factor was statistically significant at the 0.05 probability level for all parameters; there was no significant block effect. The size of the root systems was not subjected to statistics; however, figure 1 shows that roots were more developed in the inoculated treatments than in the control.

Mycorrhizal colonization was high in the soil-inoculated and root-inoculated treatments (87 and 84%, respectively), low in the nematode-inoculated treatment (19%) and absent from the control. Neither nematode galls nor bacterial nodules were observed on any root in the experiment.

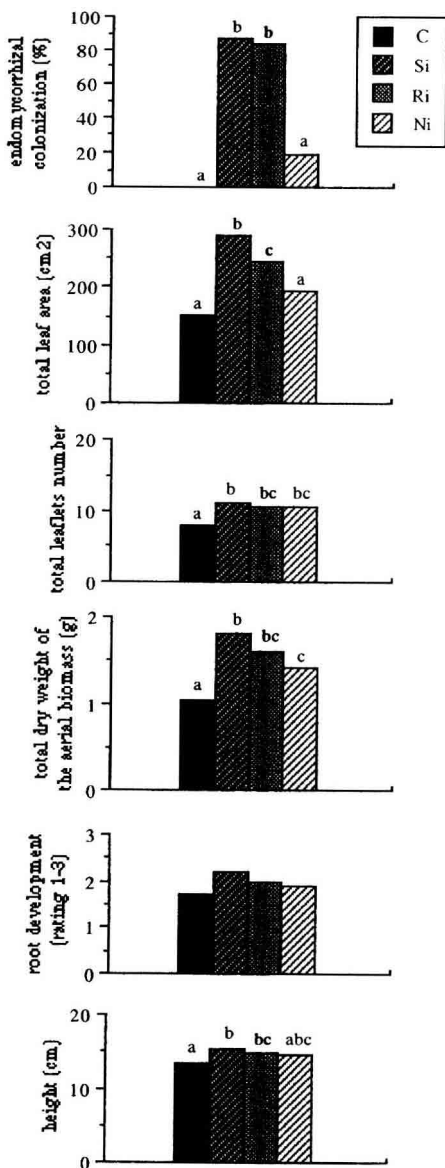


Fig 1. Growth parameters and mycorrhizal colonization per *Dicorynia guianensis* seedling after 29 weeks experimentation. C: control treatment; Si: soil-inoculated treatment; Ri: root-inoculated treatment; Ni: nematode-inoculated treatment. a, b, c: Values with the same letter are not significantly different (Fisher pooled least significant difference, $P \leq 0.05$, one-factor ANOVA).

(Field survey has shown that *D guianensis* is very seldom nodulated in French Guiana; Béreau and Garbaye, 1994.) The root systems were smaller in the control than in the inoculated treatments, but roots were free of any sign of pathogens in all treatments.

Figure 1 shows that the seedlings in both the soil-inoculated (Si) and root-inoculated (Ri) treatments had greater leaf area, more leaflets, greater shoot biomass and were taller than those in the control treatment (C); they also had greater leaf area than those in the nematode-inoculated (Ni) treatment. The Si treatment increases leaf area more than the Ri treatment.

The curves in figure 2 show leaflet number and plant height against time. The mean number of leaflets per plant was higher at all times in the three inoculated treatments than in the control. This difference increased markedly from day 150 because of reduced leaflet formation in the control. The difference in height between the inoculation treatments and the control was already notable before day 90. Later on, as for the number of leaflets, the height increment in the control treatment tended to slow down from day 160. There was no significant difference between treatments for the leaf weight per unit of surface area.

DISCUSSION

As shown in table I, the chemical properties of the steamed soil used as a growth substrate (A) and of the fresh forest soil used as an inoculum (B) only markedly differ in organic carbon content. Because only 30% of the forest soil used as an inoculum was added to the steamed soil in the Si treatment, we may consider that the physico-chemical properties of the substrate were not significantly modified. This is supported by the results of other experiments performed under the same environmental conditions (data not shown): the growth of

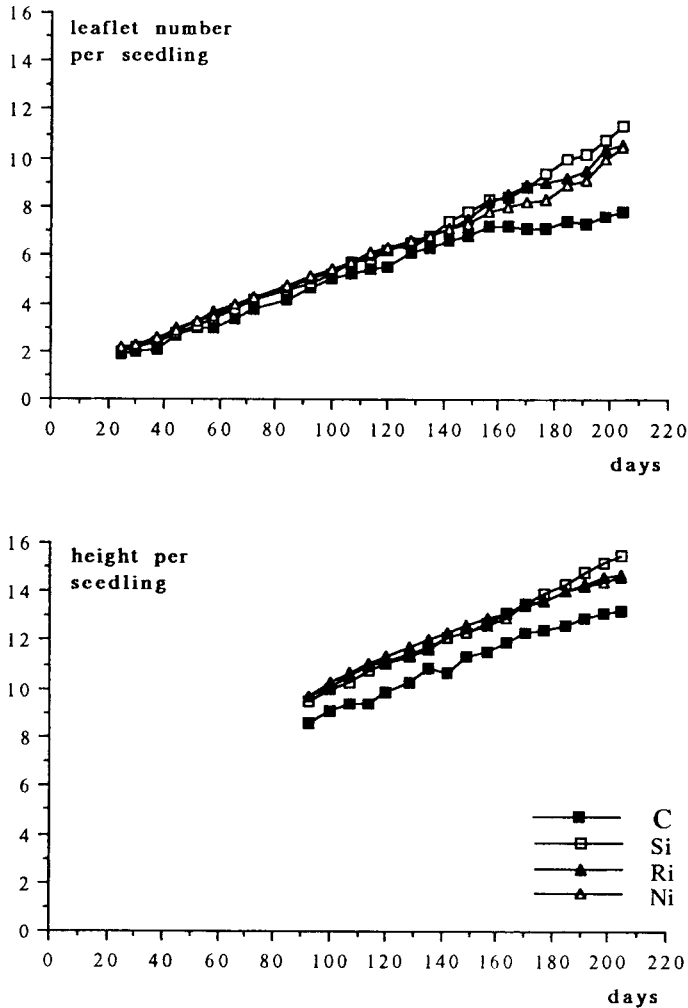


Fig 2. Leaflet number and seedling height against time. C: control treatment; Si: soil-inoculated treatment; Ri: root-inoculated treatment; Ni: nematode-inoculated treatment.

seedlings of two other endomycorrhizal species of Caesalpiniaceae from the rain forest (*Eperua falcata* Aublet and *Recordoxylon speciosum* Benoist Norm and Mar) was the same in the non-disinfected soils A and B. Moreover, the similar mycorrhizal colonization and biomass production recorded with root and soil inoculum also

indicates that the effect of the Si treatment was not due to modifications in the chemical properties of the substrate. Concerning soil bacteria, which were introduced in the three inoculated treatments but not in the control, we have already mentioned that *D guianensis* seedlings had no bacterial nodules neither in the forest nor in the inocu-

lated treatments of the experiment. Therefore, the growth difference between the control (non-mycorrhizal) and the Si treatment (heavily mycorrhizal as a consequence of the inoculation) can be attributed to mycorrhizas. The conclusion is the same with the root inoculum. It is thus established that seedlings of *D guianensis* are dependent on endomycorrhizal infection for optimal growth under our experimental conditions.

When considering the growth kinetics of the seedlings, it appears that mycorrhizal inoculation was effective as early as 20 days for leaflet number and that the seedlings' height was already markedly stimulated at 90 days. This suggests that mycorrhizal colonization occurred early and that the nutrients stored in the seeds were rapidly depleted (mean weight of a dry seed: 0.37 g). In addition, the slowing down of the growth of the control seedlings from day 160 suggests that they were less able than mycorrhizal seedlings to use depleted soil nutrients in the limited volume of the pots, presumably because of their reduced root development.

Because both the soil and the endomycorrhizal inoculum used came from a primary forest where *D guianensis* is native, and because the climate conditions of the experiment were as close as possible to those of this forest at ground level, we may also assume that the endomycorrhizal structures observed in the pots and on the seedlings sampled in the forest (Béreau and Garbaye, 1994) are the same and that *D guianensis* seedlings are as mycorrhiza-dependant in the forest as in the experiment. This strongly supports the hypothesis that the endomycorrhizal status of the seedlings is a critical factor controlling the regeneration of *D guianensis* in the primary tropical rain forest of French Guiana.

The treatments inoculated with roots (mainly mycelium and vesicles within roots) or with soil (a more diversified inoculum with spores, mycelium and root pieces) have the same level of endomycorrhizal colo-

nization. However, the Si treatment tends to result in a better growth of the seedlings (this is statistically significant for leaf area only, but the same trend exists for leaflet number and height toward the end of the experiment). On the other hand, the Ni treatment stimulated plant growth to the same extent as the two mycorrhiza-inoculated treatments, in spite of a much lower mycorrhizal colonization of the roots; this may be due to the 125 µm screen which eliminated root fragments and large spores when preparing the nematode suspension, thus selecting a fraction of the potential symbionts. All these facts suggest that the fungal communities were not the same in the different treatments and/or that the inoculum type influenced the colonization rapidity and the efficacy of the symbiosis. According to Abbot and Gazey (1994), little is known about the impact of species diversity on the functioning of endomycorrhizal symbiosis. Alexander et al (1992) observed in Malaysian disturbed forests that mycorrhizal roots and hyphal fragments were more effective natural inocula than spores. Therefore, further research with single spore morphotype inoculation will be aimed at assessing the diversity of response of *D guianensis* to the endomycorrhizal symbiosis.

The experiment was inconclusive as far as nematodes were concerned: inoculation with roots of *D guianensis* bearing galls and living *Meloidogyne* sp did not result in any galls. However, the duration of the experiment might have been too short for galls to develop under our experimental conditions. It is known that galls due to *Meloidogyne incognita* appear on tomato roots 1 month after inoculating, but such references are lacking in the still relatively unexplored field of tropical tree seedlings. In addition, the precise age of the *D guianensis* seedlings in the forest, on which galls are commonly observed (Béreau and Garbaye, 1994), is not known.

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REFERENCES

- Abbott LK, Gazey C (1994) An ecological view of the formation of VA mycorrhizas. *Plant Soil* 159, 69-78
- Alexander IJ, Ahmad N, Susee L (1992) The role of mycorrhizas in the regeneration of some Malaysian forest trees. *Phil Trans R Soc Lond [B]* 335, 379-388
- Bariteau M, Geoffroy J (1989) Sylviculture et régénération naturelle en forêt guyanaise. *Rev For Fr* 16, 309-323
- Béreau M, Garbaye J (1994) Tree root morphology and associated organisms in the primary tropical rain forest of French Guiana. *Ann Sci For* 51, 407-416
- Bremner JM (1960) Determination of nitrogen in soil by the Kjeldahl method. *J Agric Sci* 55, 1-23
- Janos DP (1980) Vesicular-arbuscular mycorrhizas affect lowland tropical rain forest plant growth. *Ecology* 61, 151-162
- Janos DP (1984) Methods for vesicular-arbuscular mycorrhiza research in the lowland wet tropics. In: *Physiological Ecology of Plants of the Wet Tropics* (E Medina, HA Mooney, C Vazquez-Yanes, eds), Junk, the Hague, the Netherlands, 173-187
- Kormanik PP, McGraw AC (1982) Quantification of vesicular arbuscular mycorrhizae in plant roots. In: *Methods and Principles of Mycorrhizal Research* (Schenk, ed), American Pathological Society, 37-45
- Olsen SR, Cole CU, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extinction with sodium bicarbonate. *USDA Circular* 939, 19
- Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorrhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: *Mycorrhizae: Physiology and Genetics*. Ist ESM Dijon, France, 1-5 July 1985 (V Gianinazzi-Pearson, S Gianinazzi, eds), INRA, Paris, France, 217-221
- Walkley A, Black JA (1934) An examination of the Degtjareff method for determining soil organic water and a proposed modification of the chromic acid titration method. *Soil Sci* 37, 29