APPLIED SOIL ECOLOGY 39 (2008) 144–152

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/apsoil

Interaction among N-fixing bacteria and AM fungi in Amazonian legume tree (*Schizolobium amazonicum*) in field conditions

Marco Antonio Siviero [b], *Alessandra Marega Motta* [a], *Dáfila dos Santos Lima* [a], *Renato Rosselli Birolli* [b], *Samuel Yun Huh* [b], *Ivana Abonizio Santinoni* [a], *Letícia Sayuri Murate* [a], *Cícera Maria Antonia de Castro* [a], *Marina Yumi Horta Miyauchi* [a], *Waldemar Zangaro* [c], *Marco Antonio Nogueira* [a], *Galdino Andrade* [a,*]

[a] *Laboratório de Ecologia Microbiana, Departamento de Microbiologia, CCB, Universidade Estadual de Londrina, Caixa Postal 6001, 86051-990 Londrina, PR, Brazil*

[b] *Grupo Arboris, Rua Gonçalves Dias, 919 Centro, 68633-000 Dom Eliseu, PA, Brazil*

[c] *Departamento de Biologia Animal e Vegetal, CCB, Universidade Estadual de Londrina, Caixa Postal 6001, 86051-990 Londrina, PR, Brazil*

ARTICLE INFO

Article history:
Received 5 March 2007
Received in revised form
9 November 2007
Accepted 5 December 2007

Keywords:
Schizolobium amazonicum
Agroforestry
Amazon
Mycorrhiza
Nitrogen fixation

ABSTRACT

The Amazon forest has suffered an accelerated degradation process due to cutting to implant agricultural systems, pasture and electricity generation projects and disorganized mining and wood exploitations. The challenge is to develop new technologies for wood production in agroforestry systems. *Schizolobium amazonicum* is a legume tree, with fast growth and its wood is employed to make furniture. More and more areas have been sowed with *S. amazonicum*, but this production system is very poor technologically. In the present paper we proposed to evaluate the effect on the plant growth and plant survival of three arbuscular mycorrhizal fungi (*Glomus clarum*, *Glomus intraradices* and *Glomus etunicatum*) associated with three N-fixing bacteria strains (two *Rhizobium* sp. and one *Burkholderia* sp.). Two methods of planting were used: direct sowing or transplantation of seedlings after initial growth in nursery. *G. intrarradices* was more effective in plant growth when inoculated in seed, and the bacteria strains had no effect when inoculated alone or with AM fungi. However, in seedlings the dual inoculation was more effective. At 210 days Rhi1 and Rhi2 associated with *G. clarum* or *G. etunicatum* increase plant growth. At 390 days *G. clarum* associated with LEM6 or Rhi1 increased most of the parameters evaluated, including biomass and wood production. Direct sowing is the traditional method largely used in the non-tillage areas and was more ineffective. The presence of microorganisms showed significant differences when compared with non-inoculated plant. The results suggested that some microbial combinations were effective in stimulating plant growth, but further experiments need to be carried out to evaluate which N-fixing bacteria and AM fungi is more effective for each planting systems for *S. amazonicum*.

* *Corresponding author*. Tel.: +55 4333714791; fax: +55 4333714791.
E-mail address: andradeg@uel.br (G. Andrade).

0929-1393/$ – see front matter
doi:10.1016/j.apsoil.2007.12.004

1. Introduction

The Amazonian forest has suffered an accelerate degradation process due to cutting to implant agricultural systems, pasture and electricity generation projects, disorganized mining, and wood exploitations (Rodrigues et al., 2004). This process began in the 1960s and has been more intense in recent years, with the destruction of large areas of primary forest creating a landscape characterized by forest fragments at different successive stages, under strong pressure from man and with serious problems in preserving biodiversity (Vieira et al., 1998; Gascon et al., 2001).

Schizolobium amazonicum Huber ex. Ducke is a native woody legume from the Amazonian forest belonging to the Fabaceae family (subfamily Caesalpinoideae) (APG II, 2003) and is a non-nodulating tree. *S. amazonicum* grow fast and it is a big tree (20–30 m high and 1 m of diameter), occurring in dry land forest (Ducke, 1949). This legume tree has been replanted in agroforestry systems that were cleared from primary rain forest in the 1970s and 1980s in favor of pasture. Today it is an important source of wood to supply the local industry of wood sheet for the furniture industry in many countries. The cultivated areas with *S. amazonicum* have increased each year, due to the excellent plant characteristics such as good seed source in quality and amount, fast growth, wood density and low disease and pest incidence. In addition, *S. amazonicum* is a good C sequester. The dynamic of total above ground biomass sequestration potential for *S. amazonicum* plantations ranged from 134 to 366 tonnes ha^{-1}, considering two different stand densities (300 and 450 trees ha^{-1}) and using selected biomass equations and an average biomass expansion factor (BEF) of 1.3 suggested by Brow (1997) for tropical broadleaf plantations. An average total biomass of about 209 tonnes ha^{-1} is reached after 15 years.

The planting system is low tech and needs to be developed to improve the wood production by *S. amazonicum* and its quality. Currently, a few papers are found in the literature related with wood production. Rondon (2002) studied the influence of different population density and spacing on plant growth, and found that the spacing of 4 m × 4 m produced more biomass. Lima et al. (2003) evaluated different doses of boron and found that 0.15 mg dm^{-3} showed more effect on plant growth. *S. amazonicum* is largely regenerated by planting seedling into the soil. Seeds are also used in reforestation but in small areas when compared with to seedlings areas. Despite the low fertility conditions of soil, and the small number of native arbuscular mycorrhiza fungi (AM) propagules, could influence the survival of seedlings and plant growth in the field conditions.

Most genera of the Leguminosae family can nodulate and fix nitrogen, although there are some important exceptions (Sprent, 2001). However, *S. amazonicum* is a non-nodulating

Table 1 – Effects of PGPR *Burkholderia* sp. strain (LEM6) and *Rhizobium* strains (Rhi1 or Rhi2) and AM fungi (*Glomus clarum*, *Glomus etunicatum* and *Glomus intrarradices*) on steam diameter on soil surface (DSS), total height (TH), height until first leaf (HFL), total number of leaves and biomass of *Schizolobium amazonicum* at 210 days after sowing

AM fungi	Bacteria strains			
	Non-bacteria	LEM6	Rhi1	Rhi2
DSS (mm)				
Non-AM	28.47 b,A	28.12 a,A	28.64 a,A	25.47 a,A
Gc	29.38 b,A	24.80 a,A	31.83 a,A	27.64 a,A
Ge	27.70 b,A	30.19 a,A	28.15 a,A	27.86 a,A
Gi	36.19 a,A	28.42 a,B	29.16 a,B	31.13 a,A,B
TH (cm)				
Non-AM	112.02 a,A	118.82 a,b,A	114.41 a,A	100.23 a,A
Gc	123.02 a,A	97.64 b,B	132.47 a,A	114.17 a,A,B
Ge	112.02 a,A	125.35 a,A	114.93 a,A	107.11 a,A
Gi	126.94 a,A	114.22 a,b,A	118.17 a,A	123.61 a,A
HFL (cm)				
Non-AM	63.95 a,A	65.32 a,A	64.12 a,A	58.88 a,A
Gc	63.45 a,A	59.32 a,A	64.05 a,A	66.75 a,A
Ge	70.55 a,A	68.40 a,A,B	61.84 a,A,B	58.63 a,B
Gi	64.81 a,A	61.25 a,A	65.05 a,A	64.89 a,A
No leaves				
Non-AM	7.72 a,A	7.85 a,A	7.59 a,A	7.02 a,A
Gc	8.21 a,A	7.25 a,A	8.23 a,A	7.78 a,A
Ge	7.19 a,A	7.78 a,A	7.80 a,A	7.44 a,A
Gi	8.24 a,A	7.80 a,A	7.28 a,A	7.68 a,A
Biomass (dm^3)				
Non-AM	0.82 b,A	0.69 a,A	0.78 a,A	0.53 a,A
Gc	0.81 b,A	0.46 a,A	0.97 a,A	0.68 a,A
Ge	0.61 b,A	0.83 a,A	0.68 a,A	0.64 a,A
Gi	1.72 a,A	0.69 a,B	0.76 a,B	0.90 a,B

Means (n = 50) sharing the same letter are not significantly different according to Tukey HSD test ($P < 0.05$). The small letters compare among AM fungi treatments and capital letters compare among bacterial treatment.

legume tree, but would benefit from microbial interaction besides *Rhizobium*. Other microorganisms may also benefit plants by means of free-living nitrogen fixation, phosphate solubilization or phytohormones production (Artusoon et al., 2006). Some of these microorganisms are bacteria so-called plant-growth promoting rhizobacteria (PGPR) (Artusoon et al., 2006; Dobbleare et al., 2003) and are able to stimulate plant growth mainly due to phytohormones production.

AM fungi are of widespread occurrence and may represent the natural status of most tropical plant species especially in the successive groups such as pioneer and early secondary (Zangaro et al., 2003). Furthermore, legume trees can establish mutual symbiosis with AM fungi, which may result in reciprocal transfer of P from the fungus to the plant in exchange for carbon from the plant to the fungus and improved growth of tropical woody leguminous plants (Zangaro et al., 2003). Dual inoculation of legume trees with rhizobia or other plant growth promoting bacteria and AM fungi can increase plant growth (Abd-Alla et al., 2000). In Brazil, Franco and Faria (1997) have studied nodulation and mycorrhizal association in legume trees to restore vegetation in poor or depleted soils with the goal of restoring their fertility. However, little information is available about symbiotic relationships of dual inoculation in native Brazilian legume trees, especially *S. amazonicum* a non-nodulating legume tree.

The aim of our study was to investigate the interaction between nitrogen fixing bacteria and AM fungi and their effects on plant growth and wood production of *S. amazonicum* in a field conditions at the Southeastern of Pará State.

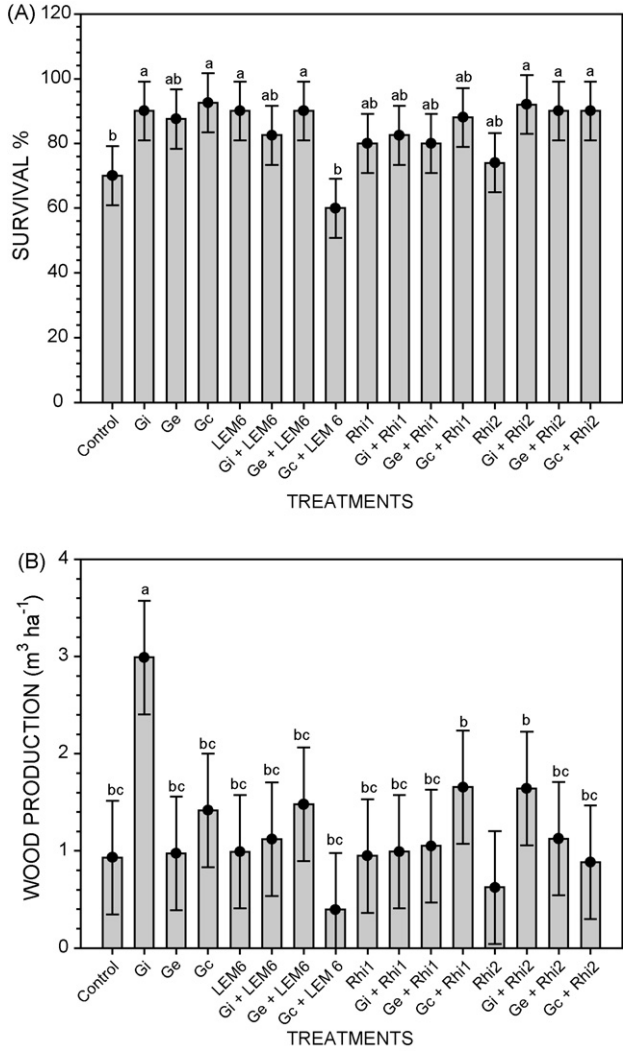

Fig. 1 – Effect of PGPR bacteria, *Burkholderia* sp. strain (LEM6) and *Rhizobium* strains (Rhi1 or Rhi2) and AM fungi (*Glomus clarum*, *Glomus etunicatum* and *Glomus intrarradices*) on *Schizolobium amazonicum* at 210 days after sowing: (A) survival and (B) wood production. Bar represent the stand error of mean values ($n = 50$). Means sharing different letters are statistically different at $P < 0.05$ by Tukey HSD test.

2. Materials and methods

2.1. Experimental design

The experiment was carried out at Dom Eliseu County—Pará State from January 2005 to February 2006. The climate in the region is characterized as hot and wet with a dry period in the winter and a rainy summer (Köeppen climatic type is AM). The mean rainfall varies from 1800 to 2300 mm. The soil in the experimental area is a Xanthic Ferralsol according to the FAO (1994) classification, 'Latossolo Amarelo', according to Brazilian classification. The original vegetation in the study area was cleared from primary rain forest in 1970s until 1980s in favor of pasture, and this area was abandoned, and secondary growth began to develop. In 2003, the secondary growth was cut and burned and the area has since then been used for experiments and planting of *S. amazonicum*.

Two plantation systems (seed and seedlings) were assessed. In each system, three N-fixing bacteria [*Rhizobium* sp. strain BR4406 (Rhi1), *Rhizobium* sp. strain BR4407 (Rhi2) and *Burkholderia* sp. strain (LEM6)] and three AM fungi [*Glomus clarum* (Gc), *Glomus etunicatum* (Ge) and *Glomus intrarradices* (Gi)] with five blocks (16 m × 160 m) were assessed. The treatments in each block were arranged in a completely random design (3 N-fixing bacteria strains × 3 AM fungi) and their respective controls. Each block was composed by four rows and each rows had forty plants that corresponded to four treatments per row with ten plants. The plants were arranged in spaces of 4 m × 4 m among plants and rows; between block the space was 8 m.

In this experiment the parameters evaluated were diameter at soil surface (DSS), total height (TH), height of until the first leaf (HFL), number of leaves, biomass (area. breast height. 0.80), survival (%) and wood production (biomass. % death. 2.5).

Data were evaluated by analyses of variance (ANOVA). The Tukey's Honest significant difference (HSD) tests were performed at $P \leq 0.05$ level of probability.

2.2. Substrate and plant

The soil in the experimental area is Xanthic Ferralsol with the following chemical composition: pH ($CaCl_2$) 4.8; H + Al 2.9 $cmol_c\ dm^{-3}$, Al^{3+} 0.2 $cmol_c\ dm^{-3}$; Ca^{2+} 3.3 $cmol_c\ dm^{-3}$, Mg^{2+} 1.0 $cmol_c\ dm^{-3}$, K^+ 0.24 $cmol_c\ dm^{-3}$; P 10.0 mg dm^{-3}, C

19.0 g dm^{-3}; S-SO_4^{2-} 4.2 mg dm^{-3}, Na^+ 4.0 mg dm^{-3}, B 0.3 mg dm^{-3}, Fe^{2+} 99.0 mg dm^{-3}, Mn 7.3 mg dm^{-3}, Cu 0.2 mg dm^{-3} and Zn 3.0 mg dm^{-3}.

The seeds of *S. amazonicum* were collected at the native forest at the Pará state where the tree occurs naturally. Before sowing the seeds were scarified in one extremity by mechanical worn. In the seed system, two seeds were sown in each well and thinned one seedling after 1 month. For the seedling system one 30 days-old plantlet (cultivated in a nursery in plastic bags with 1000 mL with non-sterile soil) was planted before taken out of the plastic bag.

2.3. *Bacteria inoculum*

The bacterial strains used as inoculum were two *Rhizobium* sp. isolated from nodules of legume tree (BR4406 and BR4407) supplied by EMBRAPA—Agrobiologia collection and one free living N-fixing *Burkholderia* sp. strain from our own collection named LEM6 (Albino et al., 2006).

The strains of *Rhizobium* sp. were grown in Petri dishes with TY media (Stanghelli et al., 1977) and the *Burkholderia* sp. strain in Nfb media (Döbereiner and Day, 1976). The bacterial strains were inoculated and incubated at 28 °C 72 h^{-1}. In the field, bacteria were re-suspended in sterile saline solution (NaCl 0.85%) plus carboximethylcellulose (0.5%). The final cell concentration for each bacteria inoculum was approximately 10^{10} colony forming unit (CFU mL^{-1}) adjusted by visual comparison between $CaCO_3$ solution standard and cells suspension for each strain. The seeds were inoculated before sowing by immersion in a bacterial suspension and seedlings were inoculated with 10 mL of the same bacterial suspension around the plant after planted.

2.4. *AM fungi inoculum*

Three AM fungi, were used as inoculum *G. clarum*, *G. etunicatum* and *G. intrarradices*. All inocula are from our own collection and are maintained in pots with *Brachiaria decumbens* as host plants. Ten grams of inoculum were added in the wells containing colonized roots, spores and mycelia before seed and plantlet were planted. After adding the inoculum, it was covered with a soil layer (approximately 2 cm) and then the sowing began.

3. Results

In spite of the fact that the measures were made monthly, we are showing the last two evaluations which correspond to 210 and 390 days after the experiment's installation.

Table 2 – Effects of PGPR *Burkholderia* sp. strain (LEM6) and *Rhizobium* strains (Rhi1 or Rhi2) and AM fungi (*G. clarum*, *G. etunicatum* and *G. intrarradices*) on steam diameter on soil surface (DSS), total height (TH), height until first leaf (HFL), total number of leaves and biomass of *S. amazonicum* at 390 days after sowing

AM fungi	Bacteria strains			
	Non-bacteria	LEM6	Rhi1	Rhi2
DSS				
Non-AM	57.22 b,A,B	62.64 a,b,A	61.97 a,A	52.97 b,B
Gc	64.30 a,b,A,B,	56.89 b,B	68.82 a,A	64.05 a,A,B
Ge	63.23 a,b,A	64.74 a,A	64.57 a,A	61.86 a,A
Gi	66.38 a,A	66.38 a,A	64.32 a,A	65.09 a,A
TH (cm)				
Non-AM	292.51 b,A,B	318.76 a,b,A,B	337.53 b,A	273.23 a,B
Gc	353.70 a,A	302.03 b,A	395.38 a,A	340.61 a,A
Ge	349.18 a,A	358.02 a,A	357.03 a,b,A	312.92 a,A
Gi	364.97 a,A	357.21 a,A	352.92 a,b,A	368.23 a,A
HFL (cm)				
Non-AM	134.00 a,A,B	124.28 b,A	146.46 a,A	112.50 b,B
Gc	147.03 a,A	143.52 a,b,B	171.10 a,A	148.35 a,A,B
Ge	149.70 a,A	158.70 a,A	152.07 a,A	141.44 a,A
Gi	155.44 a,A	156.70 a,A	150.69 a,A	159.71 a,A
N leaves				
Non-AM	17.28 b,B	21.33 a,A	21.11 a,A	17.46 b,A
Gc	22.72 a,A	20.10 a,A	23.30 a,A	21.55 a,A
Ge	22.12 a,A	21.94 a,A	21.96 a,A	21.04 a,A
Gi	22.95 a,B	22.67 a,A	21.48 a,A	21.80 a,A
Biomass (dm^3)				
Non-AM	7.57 b,A,B	8.61 a,b,A,B	9.44 a,A	6.38 b,B
Gc	10.03 a,b,A,B	7.06 b,B	12.67 a,A	9.70 a,b,A,B
Ge	9.42 a,b,A	10.12 a,b,A	10.13 a,A	8.62 a,b,A
Gi	11.08 a,A	10.70 a,A	10.04 a,A	10.91 a,A

Means ($n = 50$) sharing the same letter are not significantly different according to Tukey HSD test ($P < 0.05$). The small letters compare among AM fungi treatments and capital letters compare among bacterial treatment.

After 210 days of sowing, plants inoculated with *G. intrarradices* without bacteria showed greater DSS when compared with others treatments and no significant differences were observed in TH. On the other hand, plants inoculated with *G. etunicatum* without bacteria showed higher HFL when compared with *G. etunicatum* associated with Rhi2 strain, but no differences were observed among AM fungi treatments (Table 1). There were no differences on the number of leaves, but plants inoculated only with *G. intrarradices* had the biggest biomass when compared with other treatments (Table 1). All treatments had more survival when compared with control, except for plants inoculated with *G. clarum* plus LEM6, which did not differ from the control (Fig. 1A). Plants inoculated with *G. intrarradices* and with an absence of bacteria showed the best wood production when compared with the other treatments (Fig. 1B).

At 390 days after sowing the DSS, only *G. intrarradices* without bacteria differs to the control, and in the others treatments with dual inoculation, LEM6 strain plus *G. etunicatum* and *G. intrarradices* did not differed to the control but was bigger than *G. intrarradices* with LEM6. The strain Rhi2 plus AM fungi showed more DSS when compared with control. AM fungi alone increased TH when compared with control plants. When the bacteria and AM fungi were present, *G. clarum* plus LEM6 decreased TH when compared with other AM and LEM6. With Rhi1 strain, the interaction with AM fungi increased TH. On the other hand the interaction with AM fungi and Rhi2 did not show any effect on TH. When bacteria were inoculated, with or without AM fungi, no differences were found when compared with the control, except for Rhi1 strains which stimulated total height when co-inoculated with *G. clarum*. HFL was stimulated when the AM fungi and LEM6 or Rhi2 were co-inoculated. The interaction between bacteria and AM species did not affect the number of leaves. However, all species of *Glomus* and all bacterial strains showed effect on leaves numbers when they were inoculated alone. Biomass was still increased by *G. intrarradices* inoculated alone, but at 390 days this AM species also improved biomass when interacting with Rhi2 (Table 2). Plant survival showed the same results observed at 210 days, where non-inoculated plants and *G. clarum* plus LEM6 treatment showed the lowest level of survival when compared with other treatments (Fig. 2A). Also, the same tendency was observed in wood productivity, where the three treatments caused less wood production at 210 days, with the same effect at 390 days (Fig. 2B).

In the areas where seedlings were employed, the treatments showed different effects when compared with seeds. At 210 days, the DSS of seedlings was not influenced by AM fungi, but when plants where inoculated with *G intrarradices* plus Rhi1, they showed significant differences when compared with the non-AM plants. In TH and HFL no differences were observed at this time and no differences were observed in the number of leaves. In relation to Biomass, only the dual inoculation *G. intrarradices* plus Rhi1 increased biomass when compared with *G. intrarradices* plusLEM6 (Table 3). Plant survival was increased by *G. etunicatum* plus Rhi1 and *G. intrarradices* plus Rhi2 when compared with non-inoculated plants. Seedlings inoculated with *G. clarum* plus LEM6 strain showed lower plant survival than was observed when using seeds (Fig. 3A). At 210 days the wood productivity did not show significant differences among treatments when compared with control in seedlings planted area (Fig. 3B).

In contrast to what occurred in the sowed area, where the treatment with *G. clarum* plus LEM6 did not show any stimulatory effect, and in some cases had suppressive effects, in plantlet at 390 days the DSS of plants inoculated with *G. clarum* plus LEM6 showed significant differences when compared with bacteria control. Also, the same treatment had significant differences in TH and HFL when compared with the bacteria control. Again, *G. etunicatum*, when co-inoculated with LEM6, increased the number of leaves, when comparing with the non-AM plants. The plant biomass was also increased by *G. clarum* plus LEM6 (Table 4). The seedlings survival had not significant differences (Fig. 4A) and wood production was increased only in plants inoculated with *G. clarum* and Rhi1 (Fig. 4B), in composition to plants inoculated with *G. clarum* or Rhi1 separately.

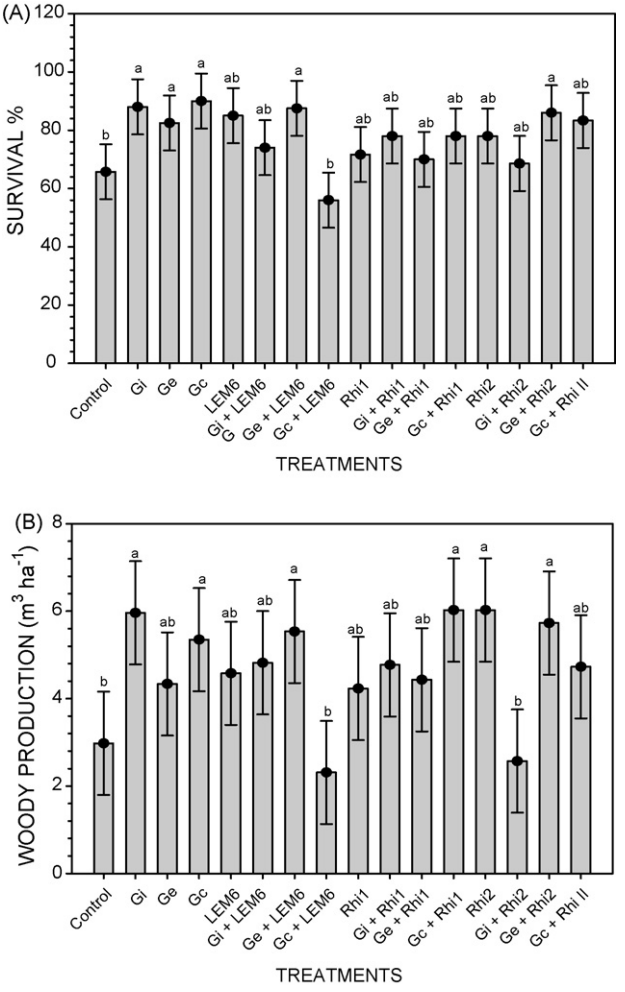

Fig. 2 – Effect of PGPR bacteria, *Burkholderia* sp strain (LEM6) and *Rhizobium* strains (Rhi1 or Rhi2) and AM fungi (*G. clarum*, *G. etunicatum* and *G. intrarradices*) on *S. amazonicum* at 390 days after sowing: (A) survival and (B) wood production. Bar represent the stand error of mean values ($n = 50$). Means sharing different letters are statistically different at $P < 0.05$ by Tukey HSD test.

Table 3 – Effects of PGPR *Burkholderia* sp strain (LEM6) and *Rhizobium* strains (Rhi1 or Rhi2) and AM fungi (*G. clarum*, *G. etunicatum* and *G. intrarradices*) on steam diameter on soil surface (DSS), total height (TH), height until first leaf (HFL), total number of leaves and biomass of *S. amazonicum* at 210 days after seedling planting

	Bacteria strains			
AM fungi	Non-Bacteria	LEM6	Rhi1	Rhi2
DSS (mm)				
Non-AM	30.68 a,A	26.71 a,A	26.75 b,A	29.30 a,A
Gc	26.63 a,A	30.61 a,A	32.24 a,b,A	29.21 a,A
Ge	31.06 a,A	28.81 a,A	31.29 a,b,A	30.64 a,A
Gi	28.86 a,B	25.54 a,B	36.58 a,A	28.74 a,B
TH (cm)				
Non-AM	132.55 a,A	106.37 a,b,A	113.06 a,A	110.41 a,A
Gc	111.52 a,A	132.87 a,A	135.02 a,A	119.16 a,A
Ge	136.57 a,A	123.30 a,b,A	128.24 a,A	118.86 a,A
Gi	114.43 a,A	101.83 b,A	115.55 a,A	117.76 a,A
HFL (cm)				
Non-AM	71.74 a,A	64.27 a,A	71.36 a,A	64.41 a,A
Gc	67.65 a,A	75.68 a,A	72.78 a,A	72.67 a,A
Ge	71.34 a,A	68.21 a,A	74.79 a,A	68.50 a,A
Gi	66.13 a,A	66.25 a,A	76.08 a,A	69.64 a,A
No leaves				
Non-AM	8.16 a,A	7.78 a,A	7.57 a,A	8.05 a,A
Gc	7.13 a,B	8.06 a,A,B	8.89 a,A	7.90 a,A,B
Ge	8.34 a,A	9.04 a,A	8.28 a,A	8.44 a,A
Gi	7.94 a,A	7.38 a,A	7.27 a,A	8.64 a,A
Biomass (dm^3)				
Non-AM	0.99 a,A	0.60 a,A	0.67 a,A	0.81 a,A
Gc	0.63 a,A	1.08 a,A	1.09 a,A	0.78 a,A
Ge	1.00 a,A	0.74 a,A	1.01 a,A	0.88 a,A
Gi	0.79 a,A,B	0.55 a,B	1.17 a,A	0.74 a,A,B

Means ($n = 50$) sharing the same letter are not significantly different according to Tukey HSD test ($P < 0.05$). The small letters compare among AM fungi treatments and capital letters compare among bacterial treatment.

4. Discussion

Soil erosion and disturbance results in reduction of microbial community including AM propagules which can be critical for plant growth because AM symbiosis and others microorganisms are key biological components in a tropical rainforest ecosystem (Matsumoto et al., 2005). A low density of AM propagules normally limits the establishment of native plants and plant growth. Regarding to *S. amazonicum*, it was necessary to use effective and ineffective AM fungi and N-fixing bacteria, in order to enhance the ability of the plant to become established and to cope with stress situations such as nutrient limitation and/or imbalance.

Specific rhizospheric microorganisms are also important and can play a relevant role in promoting root growth and mycorrhizal development, facilitating plant performance in a tropical agroforestry system. This could be critical for optimal establishment of plants in these areas (Patreze and Cordeiro, 2004; Zangaro et al., 2003). Nevertheless, there are few reports describing the beneficial effects of PGPR on performance of woody legumes in tropical agroforesty systems.

The different methods used for sowing showed differences on plant growth when we compared the treatments. When seeds were inoculated the AM fungi was more efficient to improve plant growth than N-fixing bacteria, similar results were also observed when dual inoculation was employed. In nursery conditions, Patreze and Cordeiro (2004) found different responses to the inoculation of AM fungi and *Rhizobium* in three species of woody tropical legume. In the same study, the authors suggested that specific species of AM fungi should be selected to use in a dual inoculation with *Rhizobium* to stimulate plant growth. In the area in which seeds were employed for reforestation, the most effective AM fungi inoculum in all parameters evaluated was *G. intrarradices* inoculated alone.

In the area reforested with seedlings, the treatment showed different effects when compared with our observations in the sowing method. In this system plantation, the dual inoculation showed to be more effective. In this case, *G. intrarradices* increased DSS and plant survival, only when co-inoculated with Rhi1 and Rhi2, respectively. In seedling area, *G. clarum* and *G. etunicatum* also improved plant growth, but always associated with the *Rhizobium* strains at 210 days and LEM 6 and Rhi1 at 390 days.

The differences found between planting systems is very interesting, because the results indicated that effectiveness of bacterial and/or AM fungi inoculum depends on how the system is used. Despite the fact that *S. amazonicum* is a non-nodulating legume and, *Rhizobium* is a symbiotic bacterium, that in many cases may act as PGPR (Galleguillos et al., 2000; Valdenegro et al., 2001). On the other hand, the free living N-fixing bacteria LEM 6 strain (*Burkholderia* sp.) increased tree

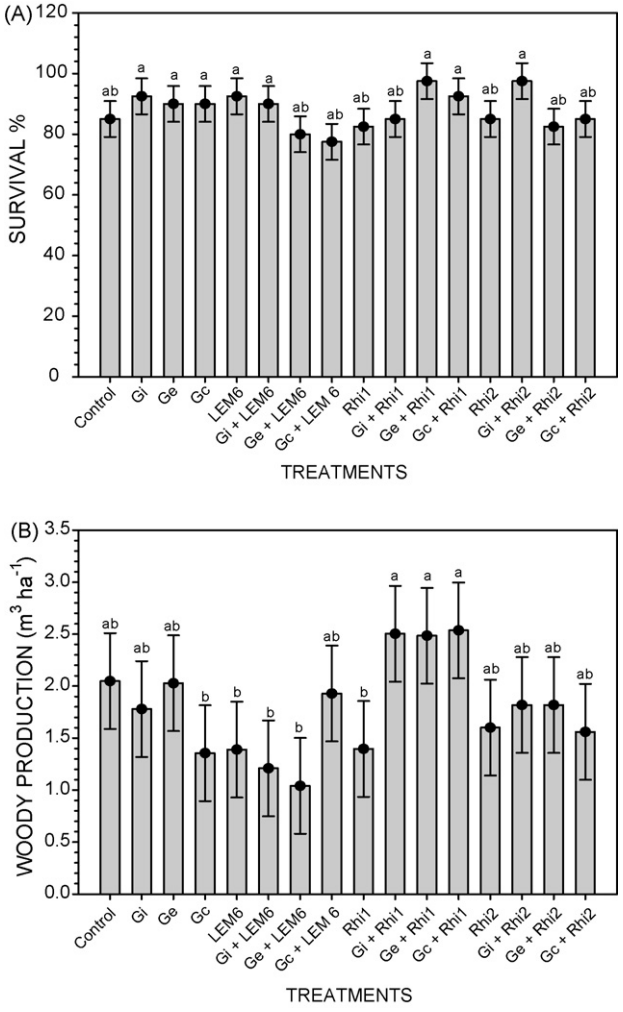

Fig. 3 – Effect of PGPR bacteria, *Burkholderia* sp. strain (LEM6) and *Rhizobium* strains (Rhi1 or Rhi2) and AM fungi (*G. clarum*, *G. etunicatum* and *G. intrarradices*) on *S. amazonicum* at 210 days after seedlings planting: (A) survival and (B) woody production. Bar represent the stand error of mean values ($n = 50$). Means sharing different letters are statisticaly different at $P < 0.05$ by Tukey HSD test.

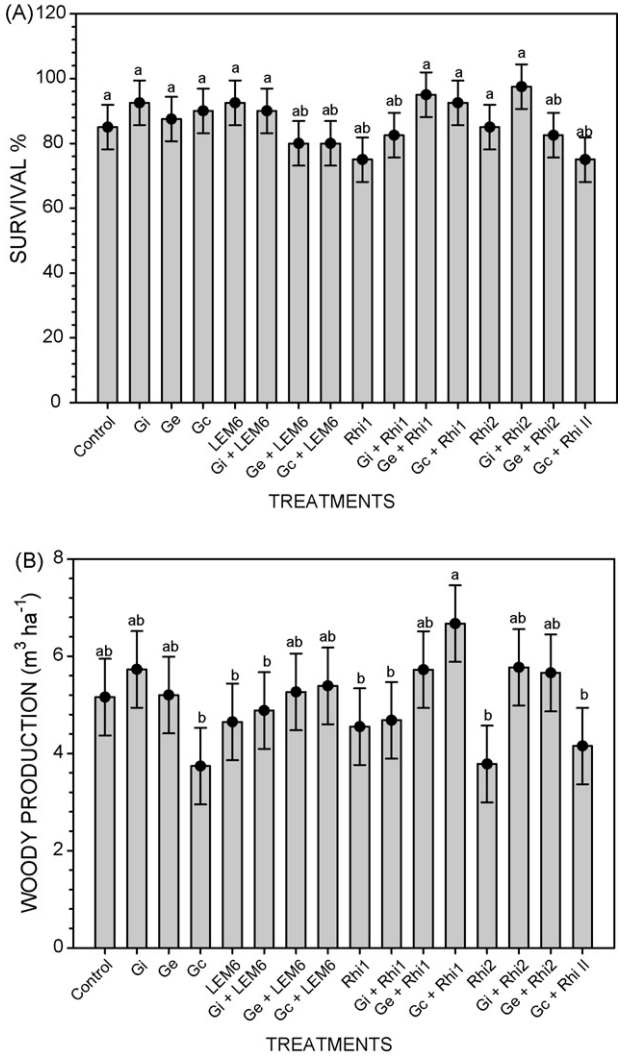

Fig. 4 – Effect of PGPR bacteria, *Burkholderia* sp. strain (LEM6) and *Rhizobium* strains (Rhi1 or Rhi2) and AM fungi (*G. clarum*, *G. etunicatum* and *G. intrarradices*) on *S. amazonicum* at 390 days after seedlings planting: (A) survival and (B) woody production. Bar represent the stand error of mean values ($n = 50$). Means sharing different letters are statisticaly different at $P < 0.05$ by Tukey HSD test.

growth in field conditions, and the similar results were also observed in other woody species (Heinonsalo et al., 2004) and rice (Raimam et al., 2007) in green-house conditions. The positive effects on plant growth observed in seedling reforested area by bacterium inoculation should be involved with the presence of roots which improve nutrients into the rhizosphere microcosm, and support the establishment of bacterial inoculum (Andrade, 2004). It is largely known that roots exude supply nutrients to the microbial community and AM fungi influence its composition both qualitative and quantitatively (Andrade et al., 1997, 1998). It is probable that the presence of roots and AM fungi improved the bacterial inoculum efficiency, as we found in the area reforested with seedlings. Also, positive impacts of dual inoculation have been demonstrated in forest tree species in Philippines (De La Cruz et al., 1988), India (Khan and Uniyal, 1999) and Kenya (Munro et al., 1999).

The bacteria strains population probably decreased or disappeared during the time of the experiment, but the effect observed in bacteria treatment every time was associated with AM inoculation. This fact suggested that AM effect is more representative than bacteria effect, and certainly the mycorrhizosphere effect was involved and stimulated indigenous bacteria population. The mycorrhizosphere effect change exudates and improve the activity of microbial community around the AM roots (Linderman, 1988), this effect could be involved on the plant growth observed in the *S. amazonicum* in a field conditions.

When plants are inoculated, the methods of cultivation, the bacterium inoculum and mycorrhizal symbiosis status can

Table 4 – Effects of PGPR *Burkholderia* sp strain (LEM 6) and *Rhizobium* strains (Rhi 1 or Rhi 2) and AM fungi (*G. clarum*, *G. etunicatum* and *G. intrarradices*) on steam diameter on soil surface (DSS), total height (TH), height until first leaf (HFL), total number of leaves and biomass of *S. amazonicum* at 390 after seedling planting

AM fungi	Bacteria strains			
	Non-bacteria	LEM6	Rhi1	Rhi2
DSS (mm)				
Non-AM	63.79 a,A	58.83 a,A	63.65 a,A	60.20 a,A
Gc	57.51 a,B	66.62 a,A	65.66 a,A,B	62.39 a,A,B
Ge	64.46 a,A	64.60 a,A	65.66 a,A	62.39 a,A
Gi	64.14 a,A	61.62 a,A	61.44 a,A	63.40 a,A
TH (cm)				
Non-AM l	358.36 a,A	320.81 b,A	354.00 a,A	305.77 a,A
Gc	303.45 b,B	382.81 a,A	369.54 a,A,B	351.00 a,A,B
Ge	370.40 a,A	367.82 a,b,A	341.91 a,A	357.96 a,A
Gi	354.60 a,b,A	327.73 b,A	341.16 a,A	342.87 a,A
HFL (cm)				
Non-AM	162.10 a,b,A	138.39 b,A	153.10 a,A	137.18 a,A
Gc	144.74 b,B	173.48 a,A	174.82 a,A	156.63 a,A,B
Ge	172.73 a,A	162.17 a,b,A	155.83 a,A	163.17 a,A
Gi	159.30 a,b,A	144.56 b,A	149.25 a,A	156.62 a,A
N leaves				
Non-AM	22.90 a,A	18.45 b,A	21.15 a,A	18.40 a,A
Gc	19.41 a,A	23.44 a,b,A	21.57 a,A	21.77 a,A
Ge	21.33 a,A	24.07 a,A	24.81 a,A	22.55 a,A
Gi	21.50 a,A	19.86 a,b,A	22.51 a,A	21.34 a,A
Biomass (dm^3)				
Non-AM	10.03 a,A	8.03b A	9.32 a,A	7.90 a,A
Gc	7.08 a,B	11.97 a,A	11.53 a,A	9.51 a,A,B
Ge	10.77 a,A	10.52 a,b,A	9.64 a,A	10.97 a,A
Gi	10.23 a,A	8.67 a,b,A	9.07 a,A	9.46 a,A

Means ($n = 50$) sharing the same letter are not significantly different according to Tukey HSD test ($P < 0.05$). The small letters compare among AM fungi treatments and capital letters compare among bacterial treatment.

affect the success of plant establishment, particularly when the trees are destined to tropical low fertility soils in agroforesty systems.

We suggest previous testing to check microbial efficiency for each woody species to select the most efficient microorganisms to improve plant growth. To achieve these aims, previous testing with selected microorganisms with specific soils and plants is needed. The growth-stimulating effect of combined microbial inoculations can be much greater than individual inoculants.

REFERENCES

Abd-Alla, M.H., Omar, S.A., Karanxha, S., 2000. The impact of pesticides on arbuscular mycorrhizal and nitrogen-fixing symbioses in legumes. Appl. Soil Ecol. 14, 191–200.

Albino, U., Saridakis, D.P., Ferreira, M.C., Hungria, M., Vinuesa, P., Andrade, G., 2006. High diversity of diazotrophic bacteria associated with the carnivorous plant *Drosera villosa* var. *villosa* growing in oligotrophic habitats in Brazil. Plant Soil 287, 199–207.

Andrade, G., 2004. Role of functional groups of microorganisms on the rhizosphere microcosm dynamics. In: Varma, A., Abbott, L., Werner, D., Hampp, R. (Eds.), Plant Surface Microbiology. Springer-Verlag, Berlin, pp. 51–69.

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular mycorrhizal fungi. Plant Soil 192, 71–79.

Andrade, G., Linderman, R.G., Bethlenfalvay, G.J., 1998. Bacterial associations with the mycorrhizosphere and hyphosphere of the arbuscular mycorrhizal fungus *Glomus mosseae*. Plant Soil 202, 79–87.

Angiosperm phylogeny group II (APG), 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APGII. Bot. J. Linn. Soc. 141, 399–436.

Artusoon, V., Finlay, R.D., Jansson, J.K., 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ. Microbial. 8, 1–10.

Brow, S., 1997. Estimation biomass and biomass change of tropical forest, a primary. FAO Forestry paper No. 134. Fao, Rome, 55 pp.

De La Cruz, R.E., Manalo, M.Q., Aggangan, N.S., Tambalo, J.D., 1988. Growth of three legume trees inoculated with VA mycorrhizal fungi and *Rhizobium*. Plant Soil 108, 111–115.

Dobbleare, S., Vanderleyden, J., Okon, Y., 2003. Plant growth-promoting effects of diazotrophs in the rhizosfere. Crit. Rev. Plant Sci. 22, 107–149.

Döbereiner, J., Day, J.M., 1976. Associative simbiose in subtropical grasses: characterization of microrganisms and dinitrogens fixing sites. In: Newton, W.E., Nyman, C.J. (Eds.), Proceedings of the 1'' International Symposium on Nitrogen Fixation II. Washington State University Press, Pullman, pp. 518–538.

Ducke, A., 1949. Notas sobre a flora neotrópica II: As leguminosas da Amazônia brasileira. Belém, IAN Boletim Técnico 18, Belém, 248 p.

FAO, 1994. Soil Map of the World. Revised Legend with Corrections. FAO/UNESCO, Rome, 140 pp.

Franco, A.A., Faria, S.M., 1997. The contribution of N_2-fixing tree legumes to land reclamation and sustainability in the tropics. Soil Biol. Biochem. 29, 897–903.

Galleguillos, C., Aguirre, C., Barea, J.M., Azcón, R., 2000. Growth promoting effect of two *Sinorhizobium meliloti* strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. Plant Sci. 159, 57–63.

Gascon, C., Bierregard Jr., R.O., Laurence, W., Rankin-De Mérona, J., 2001. Deforestation and forest fragmentation in the Amazon. In: Bierregard, Jr., R.O., Gascon, C., Lovejoy, T.E., Mesquita, R.C.G. (Eds.), Lessons from Amazonia: The Ecology and Conservation of a Fragmented Forest. Yale University Press, New Haven, London, pp. 21–30.

Heinonsalo, J., Frey-Klett, P., Pierrat, J.C., Churim, J.L., Vairelles, D., Garbaye, J., 2004. Fate tree growth effect and potential impact on soil microbial communities of mycorrhizal and bacterial inoculation in a forest plantation. Soil Biol. Biochem. 36, 211–216.

Khan, S.N., Uniyal, K., 1999. Growth response of two forest tree species to VAM and *Rhizobium* inoculations. Indian Forester 125, 1125–1128.

Lima, S.F., Cunha, R.L., Carvalho, J.G., Souza, C.A.S., Corrêas, F.L.O., 2003. Comportamento do Paricá (*Schizolobium amazonicum* Herb) submetido à aplicação de doses de boro. Cerne 9, 192–204.

Linderman, R.G., 1988. Mycorrhizal interactions with the rhizosphere microflora: The mycorhizosphere effect. Phytopathol. 78, 366–371.

Matsumoto, L.S., Martines, A.M., Avanzi, M.A., Albino, U.B., Brasil, C.B., Saridakis, D.P., Rampazo, L.G.L., Zangaro, W., Andrade, G., 2005. Interactions among functional groups in the cycling of carbon, nitrogen and phosphorus in the rhizosphere of three successional species of tropical woody trees. Appl. Soil Ecol. 28, 57–65.

Munro, R.C., Wilson, J., Jefwa, J., Mbuthia, K.W., 1999. A low-cost method of mycorrhizal inoculation improves growth of *Acacia tortilis* seedlings in the nursery. For. Ecol. Manage. 113, 51–56.

Patreze, C.M., Cordeiro, L., 2004. Nitrogen-fixing and vesicular–arbuscular mycorrhizal symbioses in some tropical legume trees of tribe Mimoseae. For. Ecol. Manage. 196, 275–285.

Raimam, M.P., Albino, U., Cruz, M.F., Lovato, G.M., Spago, F., Ferracin, T.P., Lima, D.S., Goulart, T., Bernardi, C.M., Miyauchi, M., Nogueira, M.A., Andrade, G., 2007. Interaction among free-living N-fixing bacteria isolated from *Drosera villosa* var. *villosa* and AM fungi (*Glomus clarum*) in rice (*Oryza sativa*). Appl. Soil Ecol. 345, 25–34.

Rodrigues, R.R., Martins, S.V., Barros, L.C., 2004. Tropical rain forest regeneration in an area degraded by mining in Mato Grosso State, Brazil. For. Ecol. Manage. 190, 323–333.

Rondon, E.V., 2002. Produção de biomassa e crescimento de árvores de *Schizolobium amazonicum* (Huber) Ducke sob diferentes espaçamentos na região de mata. Revista Árvore 26, 573–576.

Sprent, J.I., 2001. Nodulation in Legumes. Royal Botanic Gardens, Kew, 146 pp.

Valdenegro, M., Barea, J.M., Azcón, R., 2001. Influence of arbuscular-mycorrhizal fungi, *Rhizobium meliloti* strains and PGPR inoculation on the growth of *Medicago arborea* used as model legume for re-vegetation and biological reactivation in a semi-arid Mediterranean area. Plant Growth Regul. 34, 233–240.

Vieira, G., Brandão, M.J., Blair, C., 1998. Ecofisiologia do estabelecimento de plântulas de espécies florestais da Amazônia. In: Higuchi, N., Campos, M.A.A., Sampaio, P.T.B., Santos, J. (Eds.), Pesquisas florestais para a conservação da floresta e reabilitação de áreas degradadas da Amazônia. INPA, Manaus, pp. 153–170.

Zangaro, W., Nisizaki, S.M.A., Domingos, J.C.B., Nakano, E.M., 2003. Mycorrhizal response and successional status in 80 woody species from south Brazil. J. Trop. Ecol. 19, 315–324.