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Effect of the fertilization and growth promoting microrganisms on *Schizolobium parahyba*

Efeito da adubação e microrganismos promotores de crescimento sobre *Schizolobium parahyba*

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Abstract

The objective of this work was to evaluate the effect of the chemical and biological fertilizing on the development of two varieties of *Schizolobium parahyba*, paricá and guapuruvú. The chemical fertilizer NPK 20-05-20 and the plant growth promoting microorganisms, *Rhizobium* sp. (Rhi) and *Rhizophagus clarus* (Arbuscular Mycorrhizal Fungus - AMF). The experimental design was a randomized complete block with five replicates and eight treatments. Forty-six day old seedlings of each variety were planted in pits containing hydroretent gel. Then the treatments were added: 1. Control; 2. Rhi; 3. AMF; 4. NPK; 5. Rhi + NPK; 6. Rhi + AMF; 7. AMF + NPK; 8. Rhi + AMF + NPK. Plant length, stem diameter and survival in the environment were evaluated. The ANOVA followed by the Duncan test ($\alpha = 0{,}05$) was used to compare the means of the treatments. In the four evaluated periods (37, 111, 250 and 360 days), both varieties had a positive effect on height, diameter and survival when applied to NPK treatments. The interaction AMF and *Rhizobium* sp. favored the growth in diameter of the guapuruvú. For paricá variety that same treatment provided higher growth in height, however, AMF impaired development in diameter of this variety. Thus, it was observed that under low fertility conditions, the interaction AMF + Rhi favored a greater resistance of guapuruvú to abiotic stress compared to treatment 3, and the presence of grasses in the experimental area may have contributed to the reduction on the development of *S. parahyba*.
Key words: Guapuruvú. Paricá. *Rhizobium sp. Rhizophagus clarus*. Survival.

Resumo

O objetivo deste trabalho foi avaliar o efeito da adubação química e biológica no desenvolvimento de duas variedades de *Schizolobium parahyba*, paricá e guapuruvú. O adubo químico NPK 20-05-20 e os microrganismos promotores de crescimento de plantas, Rhizobium sp. (Rhi) e Rhizophagus clarus (fungo micorrízico arbuscular - FMA). O delineamento experimental utilizado foi de blocos casualizados, com cinco repetições e oito tratamentos. Plântulas com 46 dias de idade de cada variedade foram plantadas

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em covas contendo gel hidratante. Em seguida, os tratamentos foram adicionados: 1. Controle; 2. Rhi; 3. FMA; 4. NPK; 5. FMA + Rhi; 6. Rhi + NPK; 7. FMA + NPK; 8. FMA + Rhi + NPK. O comprimento das plantas, o diâmetro do caule e a sobrevivência no ambiente foram avaliados. A ANOVA seguida do teste de Duncan (α = 0,05) foi utilizada para comparar as médias dos tratamentos. Nos quatro períodos avaliados (37, 111, 250 e 360 dias), ambas as variedades tiveram efeito positivo na altura, diâmetro e sobrevivência quando aplicadas aos tratamentos com NPK. A interação FMA e Rhizobium sp. favoreceu o crescimento em diâmetro do guapuruvú. Para a variedade paricá, o mesmo tratamento proporcionou maior crescimento em altura, entretanto, o FMA prejudicou o desenvolvimento em diâmetro dessa variedade. Assim, observou-se que sob condições de baixa fertilidade, a interação FMA + Rhi favoreceu uma maior resistência do guapuruvú ao estresse abiótico comparado ao tratamento 3, e a presença de gramíneas na área experimental pode ter contribuído para a redução no desenvolvimento de *S parahyba*.
Palavras-chave: Guapuruvú. Paricá. *Rhizobium sp. Rhizophagus clarus*. Sobrevivência.

Introduction

The decrease of the forest areas in Brazil has been aggravated in the last decades due to commercial exploitation, such as the forests clearing for both the timber industry and creation of new areas for the agricultural system (ARRAES et al., 2012). In recent years, the Legal Amazon has been heavily deforested for economic purposes. The restoration of these ecosystems is of extreme importance, because in a deforestation there is a decrease of the essential nutrients stock, which endangers the ecological region balance (GOMES; LUIZÃO, 2012). Concerning the Atlantic Forest, the situation is more worrisome, the deforestation rates indicate a drastic loss of forest cover in the last decades, leaving only 11.6 % of the natural vegetation cover (RIBEIRO et al., 2009) and in an intensely fragmented state: 83.4% of the fragments are less than 50 ha (RIBEIRO et al., 2011). Thus, the need for restoration of the Atlantic Forest is extreme, as it is one of the richest and most threatened areas of the planet (LIMA et al., 2015).

In view of this reality, for the recovery of large areas that have been deforested and degraded, legumes have been an efficient alternative, since they provide numerous advantages for this practice, such as rapid growth and efficiency in the nutrients solubilization along the vertical and horizontal soil profile, allowing them to be absorbed, recycled and made available to other plants (COSTA et al., 2014; WANG et al., 2017).

Schizolobium parahyba var. *parahyba* (Vell.) S. F. Blake, popularly known as guapuruvú, is endemic to the Atlantic Forest, and *S. parahyba* var. *amazonicum* Huber ex. Ducke, is endemic to the Amazon region, also called paricá, both of them are varieties of fast growing tree legume species (subfamily Caesalpinioideae) that have been used in recovery projects in degraded areas. In addition, these varieties, since that they present good quality timber, have motivated their extensive cultivation by the timber sector (PIETROBOM; OLIVEIRA, 2004; ROSA, 2006).

The use of chemical fertilizers is a common practice in forest nurseries, functioning as a valuable tool in controlling the size and vigor of seedlings (SOUSA et al., 2012). However, the information that is found on the current literature about the use of fertilizers with nitrogen, phosphorus and potassium (NPK) in the growth of *S. parahyba* (VIEIRA et al., 2013) in field is rare.

Inoculation of growth promoting microorganisms such as Arbuscular Mycorrhizal Fungi (AMF) and nitrogen fixing bacteria in silviculture has also been considered as an alternative to improve the forest production process, since in the soil there are several species of microorganisms that act in the biogeochemical cycles and are determinant to improve soil fertility and plant development (MARIN et al., 2010; ALORI et al., 2017).

The present work has as objective to evaluate the chemical (with NPK) and biological inoculations with the fungus *Rhizophagus clarus* and with the diazotrophic bacterium *Rhizobium* sp. fertilization effect on the development of *S. parahyba* var. *parahyba* and *S. parahyba* var. *amazonicum* in field conditions.

Material and Methods

Experimental conditions

The experiments were carried out at the experimental station of the Agronomic Institute of Paraná, in Xambrê – PR, Brazil (23° 44' S and 53° 29' W) from October 2012 to October 2013. The climate of the region is subtropical (Cfa) (IAPAR, 2000), and the soil is a typical Dystrophic Red Latosol (Santos et al., 2006). In the study area of guapuruvú (120 m x 60 m) and paricá (156 m x 30 m) there was the presence of *Urochloa* sp., which was controlled before planting by the application of herbicide (glyphosate).The seedlings were planted with 250 mL of hydroretting gel per pit for better plant glue.

The soil chemical attributes of the area where the guapuruvú was planted: pH ($CaCl_2$) 4,7; H^{+}+Al^{3+} 4,43 $cmol_c$ dm^{-3}; Al^{3+} 0,12 $cmol_c$ dm^{-3}; Ca^{2+} 0,89 $cmol_c$ dm^{-3}; Mg^{2+} 0,47 $cmol_c$ dm^{-3}; K^{+} 0,21 $cmol_c$ dm^{-3}; P 11,95 mg dm^{-3}; C 5,25 g dm^{-3}. Regarding the paricá experimental area, the soil chemical characterization was: pH ($CaCl_2$) 4,8; H^{+}+Al^{3+} 3,82 $cmol_c$ dm^{-3}; Al^{3+} 0,05 $cmol_c$ dm^{-3}; Ca^{2+} 1,1 $cmol_c$ dm^{-3}; Mg^{2+} 0,51 $cmol_c$ dm^{-3}; K^{+} 0,19 $cmol_c$ dm^{-3}; P 5,95 mg dm^{-3}; C 5,60 g dm^{-3}.

Experimental design

The experimental design was a randomized complete block containing eight treatments with five replicates, spaced 3 m x 3 m for both areas, totaling ten samples per plot in the paricá experiment and six per plot with guapuruvú. The biological and chemical fertilizers used were *R. clarus* (Arbuscular Mycorrhizal Fungus - AMF), *Rhizobium* sp. (Rhi) and fertilizer (NPK 20-05-20). These were distributed as follows:

Treatment 1. Control;

Treatment 2. Rhi;

Treatment 3. AMF;

Treatment 4. NPK;

Treatment 5. AMF + Rhi;

Treatment 6. Rhi + NPK;

Treatment 7. AMF + NPK;

Treatment 8. AMF + Rhi + NPK.

Production of the guapuruvú and paricá varieties seedlings

The seeds of the guapuruvú variety were collected on the campus of Londrina State University (UEL), Londrina – PR, Brazil, and those of the paricá variety came from Pará – PA, Brazil, provided by Arboris group. Before sowing, the breakdown of the seed dormancy was performed by the mechanical scarification method. Seedling production was carried out in greenhouse, in 180 cm^3 tubes containing both commercial Plantmax® substrate and soil in 1:1 ratio. The seedlings were taken to the experimental station and planted after 46 days of germination.

Inoculation with nitrogen-fixing bacteria

Rhizobium sp., isolated from nodules present in *Acacia* sp. and identified by biochemical evidence, was obtained from the Microbial Ecology Laboratory (LEM), Londrina State University (UEL), cultivated in YMA medium (VINCENT, 1970) plus congo red, (25%) and incubated at 28 °C for 48 h under agitation. Cells were suspended in sterile saline (0,85% NaCl) to 3.3 x 10^7 colony forming units (CFU mL^{-1}) adjusted by visual comparison of standard $CaCO^3$ solution. The inoculation was performed with 1 mL of bacterial suspension around the seedlings when the first pair of leaves were opened.

Inoculation with AMF

The *R. clarus* inoculum was obtained from the Microbial Ecology Laboratory´s collection of UEL and propagated in pots containing 1:1 sterilized soil and sand with *Urochloa decumbens* as host plant. The pots were kept in a greenhouse from 25 to 28 ºC with natural light. Fungus spores were collected by wet sieving and decantation (GERDEMANN; NICOLSON, 1963), accounting 20 spores per gram of inoculum. Ten grams of crude inoculum (containing spores and colonized roots) were inoculated in each tube in the treatments that contained AMF, before sowing.

Chemical fertilization

In the planting areas 130 g of NPK 20-05-20 were added per well in the chemical treatments. The proportion of nutrients N, P and K was determined from the result of soil chemical analysis. The fertilization was carried out at 0-10 cm from the soil surface.

Data collection

The plants initial growth was evaluated by the height (cm), measured from the stem base to the apical bud at 37, 111, 250 and 360 days after planting, and the lap diameter (10 cm above the ground) was evaluated at 250 and 360 days after planting.

The survival analysis was done through the percentage of survival (SANTOS JUNIOR et al., 2006) obtained by means of the ratio between the total of live plants after 12 months of planting and the number of seedlings that were planted at the beginning of the experiment in each treatment.

Before planting, soil samples containing roots of *Urochloa* sp. were collected in each treatment at a depth of 0-10 cm from the soil surface. These samples were used to determine the mycorrhizal colonization rate, as well as the quantification of native spores in the experimental area. At the end of the experiment, soil samples were also collected so that they contained roots of guapuruvú and paricá for analysis of mycorrhizal root colonization by *R. clarus*, spore count of *R. clarus* and presence of native AMFs in the experimental area. The latter were collected at 0-10 cm depth from soil surface of each treatment, about 20-30 cm away from the plants stem. The spores were extracted and quantified by the wet sieving and decantation method from 100 g of soil of each sample (GERDEMANN; NICOLSON, 1963). *R. clarus* spores were separated from the other AMFs present in the area by following the morphological criteria described by Blaszkowski (1994), Schubler and Walker (2010) and International Culture Collection of Vesicular-Arbustular Mychorrhizal Fungi - INVAM (2014). Concerning the mycorrhizal colonization, the plant roots were separated from the soil samples, washed and stained by the staining and clarification method of Phillips and Hayman (1970). The colonization evaluation was performed based on the quadrants intersection technique (GIOVANNETTI; MOSSE, 1980).

Data analysis

In order to verify if the obtained data followed a normal distribution and if they were homocedastic as well, the Shapiro Wilk test with α= 5% and the Bartlett test with α= 5%, respectively, were used. Concerning the data that did not present normal distribution and homoscedasticity of variances, a transformation was applied so that the assumptions of the variance analysis were attended. The transformation used was the neperian logarithm (ln), that is, $y^* = \ln(y)$, where y^* is the transformed data and y the original data (BOX; COX, 1964). The results were subjected to analysis of variance for all the collected variables. Treatment averages were compared by the Duncan's test using the R statistical program. In all tests, $p \leq 0,05$ was considered significant.

Results and Discussion

Chemical fertilization

In the analysis of the guapuruvú growth, it was verified that at 37, 111, 250 and 360 days, the means of the treatments with the chemical fertilization were more effective in the plants development (in height) differing from the treatments without fertilization (Table 1). A similar result was verified for plant diameter at 250 and 360 days (Table 2) providing a higher survival rate from 110 days to the end of the experiment (Table 3; Figure 1a).

Table 1. Effect of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus clarus*, N-fixing bacterium *Rhizobium* sp. (Rhi), fertilizer (NPK) and their interactions on guapuruvú total height (cm) at 37, 111, 250 and 360 days after planting.

Treatment	37 Days	111 Days	250 Days	360 Days
Control	29.73 c	41.93 b	57.97 b	74.67 b
Rhi	29.38 c	41.09 b	59.79 b	73.78 b
AMF	27.28 cd	38.04 b	56.24 b	66.55 b
NPK	37.32 b	65.93 a	93.26 a	109.47 a
AMF + Rhi	25.41 d	36.16 b	51.27 b	79.35 b
Rhi + NPK	39.14 ab	67.21 a	89.01 a	115.29 a
AMF + NPK	42.53 a	71.74 a	103.36 a	132.07 a
AMF + Rhi + NPK	36.11 b	66.42 a	89.56 a	114.35 a

Averages followed by equal letters, in the column, do not present significant statistical difference by the Duncan test ($p \geq 0{,}05$).

Table 2. Effect of the arbuscular mycorrhizal fungus (AMF) *R. clarus*, N-fixing bacterium *Rhizobium* sp. (Rhi.), fertilizer (NPK) and their interactions on the guapuruvú lap diameter (cm) guapuruvúat 250 and 360 days after planting.

Treatment	250 Days	360 Days
Control	11.50 b	19.62 cd
Rhi	12.70 b	19.01 cd
AMF	11.60 b	17.71 d
NPK	27.43 a	34.92 ab
AMF + Rhi	11.00 b	24.04 c
Rhi + NPK	25.07 a	35.66 ab
AMF + NPK	31.71 a	45.09 a
AMF + Rhi + NPK	24.76 a	33.33 b

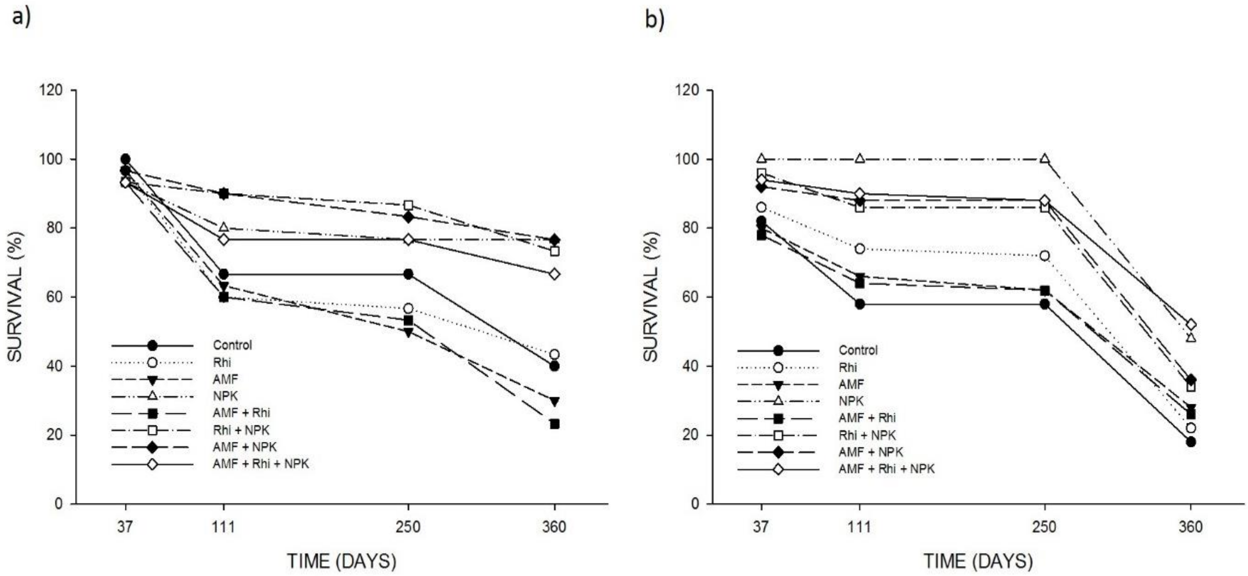
Averages followed by equal letters, in the column, do not present significant statistical difference by the Duncan test ($p \geq 0{,}05$).

Table 3. Effect of the nitrogen fixing bacteria *Rhizobium* sp. (Rhi), arbuscular mycorrhizal fungus (AMF) *R. clarus*, fertilizer (NPK) and their interactions on guapuruvú survival rate at 37, 111, 250 and 360 days after planting.

Treatment	Survival rate (%)			
	37 days	111 days	250 days	360 days
Control	100 A a	66.67 B b	66.67 B abc	40 C c
Rhi	96.67 A a	60 B b	56.67 B bc	43.33 B bc
AMF	96.67 A a	63.33 B b	50 B c	30 C c
NPK	93.33 A a	80 A ab	76.67 B ab	76.67 B a
AMF + Rhi	93.33 A a	60 B b	53.33 B bc	23.33 C c
Rhi + NPK	93.33 A a	90 A a	86.67 AB ab	73.33 B a
AMF + NPK	96.67 A a	90 AB a	83.33 B ab	76.66 B a
AMF + Rhi + NPK	93.33 A a	76.67 B ab	76.67 B ab	66.67 B ab

*Equal uppercase letters in the line, indicate statistically similar proportions by the Chi-square test at 5% of significance. Equal lowercase letters in the column, indicate statistically similar proportions by the Chi-square test at 5% of significance.

Figure 1. Effect of the nitrogen fixing bacteria *Rhizobium* sp. (Rhi), arbuscular mycorrhizal fungus (AMF) *R. clarus*, NPK (Fertilizer) and their interactions on the guapuruvú (a) and paricá (b) survival rates at 37, 111, 250 and 360 days after planting.



The chemical fertilization favored agreater development in height, diameter and higher survival rate also for paricá (Table 3, 4 and 5, Figure 1b). All the treatments that were fertilized showed significantly higher averages compared to the non-fertilized treatments. It was observed that 100% of the plants survived until 250 days after planting in those treatments (Table 5; Figure 1b).

Table 4. Effect of the arbuscular mycorrhizal fungus (AMF) *R. clarus*, N-fixing bacterium *Rhizobium* sp. (Rhi), fertilizer (NPK) and their interactions on the paricá total height (cm) at 37, 111, 250 and 360 days after planting.

Treatment	37 Days	111 Days	250 Days	360 Days
Control	22.34 c	33.94 b	49.30 c	54.43 b
Rhi	21.77 c	35.02 b	53.16 bc	50.87 b
AMF	21.01 c	34.50 b	51.93 bc	62.08 b
NPK	28.59 b	82.28 a	152.67 a	178.83 a
AMF + Rhi	22.57 c	37.05 b	58.24 b	63.88 b
Rhi + NPK	31.41 a	80.08 a	141.42 a	182.37 a
AMF + NPK	29.42 ab	78.72 a	137.55 a	182.74 a
AMF + Rhi + NPK	28.93 ab	78.79 a	138.95 a	156.77 a

Averages followed by equal letters, in the column, do not present significant statistical difference by the Duncan test ($p \geq 0,05$).

Table 5. Effect of the nitrogen fixing bacteria *Rhizobium* sp. (Rhi), arbuscular mycorrhizal fungus (AMF) *R. clarus*, fertilizer (NPK) and their interactions on paricá survival rates at 37, 111, 250 and 360 days after planting.

Treatment	Survival rate (%)			
	37 days	111 days	250 days	360 days
Control	82 A bc	58 B d	58 B c	18 C c
Rhi	86 A bc	74 A bcd	72 A bc	22 B c
AMF	80 A bc	66 AB cd	62 B c	28 C abc
NPK	100 A a	100 A a	100 A a	48 B ab
AMF + Rhi	78 A c	64 A cd	62 A c	26 B bc
Rhi + NPK	96 A ab	86 A bc	86 A b	34 B abc
AMF + NPK	92 A abc	88 A ab	88 A ab	36 B abc
AMF + Rhi + NPK	94 A abc	90 A ab	88 A ab	52 B a

*Equal uppercase letters in the line, indicate statistically similar proportions by the Chi-square test at 5% of significance. Equal lowercase letters in the column, indicate statistically similar proportions by the Chi-square test at 5% of significance.

The results suggest that both species present a critical threshold of available macronutrients in the soil for a good plant development, according to the results of Brasil et al., (2016), the application of increasing doses of phosphorus promotes the highest growth in height and DAP in paricá plants combined with nitrogen application. This study corroborates with the work of Vieira et al. (2013), in which a positive response was verified to the NPK fertilizer for both height and diameter growth of *S. amazonicum*.

From the survival analysis (Table 3 and 5, Figure 1), it can be observed that plants fertilized with NPK presented a lower mortality compared to treatments not chemically fertilized. Besides, paricá plants suffered much more with the frost compared to guapuruvú plants (Figure 1a and 1b), once paricá is endemic to the Amazon, usually supporting absolute minimum temperatures of 6 °C in the Acre region. However, it was verified that when planted in mixed plantation in Rolândia, in the north of Paraná, it was tolerant to minimum temperatures of up to -2 ºC (CARVALHO, 2007), being a factor to be studied for the establishment of this crop in colder regions.

Fertilization plus microbial interaction

At the beginning of the experiment, the treatment which was added only fertilizer, favored a development in height of guapuruvú plants similar to the treatments with fertilization plus microorganisms inoculation, or even inferior in the case of the fertilized treatment plus mycorrhizal inoculum (Table 1). Thus, at the beginning of planting the addition of FMA plus fertilizer assisted in the growth in height of this variety. This result differs from the previous study in the same area by Gonçalves, et al. (2015) when FMA efficiency was not obtained in Guapuruvú growth even with fertilizer addition, since there was a high level of P in the soil that may have inhibited the inoculant effectiveness.

At 360 days, guapuruvú plants treated with AMF plus chemical fertilization showed a larger growth in diameter compared to those receiving the same treatment plus the addition of the *Rhizobium* inoculum (Table 2). Under these conditions, there may have been competition between AMF and the bacterium in the root colonization, as observed by Biró et al. (2000), in cereal roots colonized by *Azospirillum* spp. and AMFs, both endophytes may be present in the same cortical roots area and make it feasible a direct interaction between plant-bacterium-AMF, which may result in competition for photosynthesized compounds.

Regarding the paricá's total height, it was possible to verify that at 37 days the inoculum Rhizobium sp. plus chemical fertilization was better than the other fertilized treatments (Table 4), agreeing with the work of Sivieiro et al. (2008), when a positive effect was obtained of Rhizobium sp. in the paricá initial growth. However, in the other evaluation periods this treatment provided a similar response to the other fertilized treatments.

However, at 360 days, that difference was no longer observed, probably due to the frost stress (Table 4 and 6), since temperature is a limiting factor in inoculation response. Mycorrhizal fungi present variations in the limits and optimum temperature range for both the spore germination and external mycelial extension. Many spores do not germinate or decrease germination under field conditions at lower temperature limits (BOWEN, 1987). In addition, temperature may influence the symbiotic efficiency of *Rhizobium* sp. in the field conditions, as well as other factors, such as soil acidity and nutrient concentration that may promote loss of response to inoculation, decreasing the plant efficiency in establishing a symbiotic relationship with bacteria (MORAES et al., 2010).

Table 6. Average precipitation, temperature and relative humidity data – Umuarama Weather Station/IAPAR-PR (2012-2013).

Month/year	Precipitation (mm)	Average temperature (°C)	Relative humidity (%)
Oct/12	5.2	24.6	63.9
Nov/12	4.8	25.5	64.1
Dec/12	5.3	26.3	75
Jan/13	2.4	25.3	66
Feb/13	10.4	24.7	77.1
Mar/13	7.1	23.7	76.1
Apr/13	2.8	21.9	68.8
May/13	5.2	20.1	72.7
Jun/13	13.5	18.5	84.6
Jul/13	2.1	18	64.6
Aug/13	0.3	18.4	54
Sep/13	2.2	21.7	57.3
Oct/13	7	22.9	65.5

At the end of the planting, it was possible to observe that all treatments with chemical fertilization, to both varieties, presented more than 50% of the roots colonized by AMF (Table 7), showing that the applied fertilization in the area was not detrimental to root colonization by the fungus, contrary to what normally happens, once under conditions of high soil fertility, especially N and P, mycorrhization is generally inhibited (SIQUEIRA; FRANCO, 1988; CAVALCANTE et al., 2009). However, it is suggested that fertilization favored root colonization, which can be explained by the fact that better-nourished plants have a better condition for AMF colonization, supporting the high energy cost involved in the symbiosis, since the mechanisms of colonization regulation are functionally dependent on the carbon available for the fungus (BAREA et al., 2005).

Table 7. Mycorrhizal colonization of guapuruvú and paricá roots, number and percentage of *R. clarus* total spores in 100 g of soil sample from the two experiments at 360 days after planting. AMF: arbuscular mycorrhizal fungus; Rhi: *Rhizobium* sp.; NPK: fertilizer. Initial spore value per 100g of soil: paricá = 1300/ guapuruvú = 400.

Treatment	Colonization (%)		Spores quantification			
			Total	*R. clarus*	Total	*R. clarus*
	paricá	guapuruvú	guapuruvú		paricá	
Control	36	41	56	0	92	4
Rhi	34	46	54	2	84	4
AMF	49	54	234	64	90	40
NPK	53	59	102	2	104	8
AMF + Rhi	39	55	64	22	196	24
Rhi + NPK	55	56	68	4	66	2
AMF + NPK	63	60	118	32	162	50
AMF + Rhi + NPK	62	70	74	12	124	32

Interaction between FMA and Rizobacteria

When plants are treated with AMF and *Rhizobium* without addition of the chemical fertilizer, they have a better response in diameter development compared to AMF treatment (Table 2 and 8), being that for the paricá it was better at 250 days of planting and guapuruvú in the 360 days, suggesting a synergistic effect of AMF on rhizobacteria. According to Artursson et al. (2006), the benefits of AMF-diazotrophic bacteria interaction may occur due to the increase in the uptake of P by mycorrhizal plants, which provides better conditions for establishment of association with diazotrophs.

Table 8. Effect of the arbuscular mycorrhizal fungus (AMF) *R. clarus*, N-fixing bacterium *Rhizobium* sp. (Rhi), fertilizer (NPK) and their interactions on the paricá lap diameter (cm) at 250 and 360 days after planting.

Treatment	250 Days	360 Days
Control	9.69 d	10.74 b
Rhi	11.63 cd	9.95 b
AMF	7.14 e	12.72 b
NPK	33.73 a	34.59 a
AMF + Rhi	12.36 c	12.49 b
Rhi + NPK	26.42 b	33.93 a
AMF + NPK	31.32 ab	28.97 a
AMF + Rhi + NPK	31.50 ab	29.76 a

Averages followed by equal letters, in the column, do not present significant statistical difference by the Duncan test ($p \geq 0,05$).

After 250 days of the plants evaluation, specifically in the month of July, there was a strong frost in the region and the plants suffered an intense stress due to the low temperature (Table 6). Thus, it is also understood that co-inoculation may have favored resistance to stress periods for guapuruvú plants, a condition already identified by Janos (1996) and Biró et al. (2000) in works developed with other plants. For the growth and establishment of several tree species, the double inoculation Rhi and AMF has been shown to be an advantageous procedure, favoring, for example, the biomass production due to greater absorption and fixation of nutrients (HERRERA et al., 1993).

When it comes to Paricá, at 250 days, treatment with Rhizobium plus AMF, without chemical fertilization, was more efficient in height development than control (Table 4), presenting a positive effect for the development of this variety. In the study by Siviero et al. (2008), it was observed that the double inoculation Rhi and AMF also favored the the paricá variety development in the field.

For both varieties, it was verified that treatments with inoculation of AMF and/or rhizobacteria without chemical fertilization did not favor protection against stress conditions, providing a survival rate similar to control (Table 3 and 5, Figure 1), contradicting the results of Gonçalves et al. (2015), when the effect of AMF *Glomus clarum* and *Rhizobium* sp. was tested in the development of guapuruvú. According to these authors, under stress conditions the plants that received the inoculum of *G. clarum* and *Rhizobium* sp. showed a higher survival rate compared to uninoculated plants suggesting a protective effect by these microorganisms.

Inoculant FMA

The evaluation of the presence of AMF performed in the guapuruvú experimental area before planting resulted in about 58% of the *Urochloa* sp. roots were colonized with AMF and accounted a total of 1300 spores per 100 g of soil, but at 360 days after planting, the number of total spores was much lower, being the highest value presented by the treatment with AMF (Table 7).

In relation to the paricá area, 51% of the *Urochloa* sp. roots were colonized with AMF and the soil collected before planting presented 400 spores in 100 g of soil. After one year of the experiment there was also a reduction in the amount of total spores, being the treatment AMF + Rhi with the largest number of spores. Regarding the spores of *R. clarus*, in the end of the experiment all treatments that were inoculated with this AMF, for both varieties, presented a larger number of spores of that species.

As it can be observed, in both varieties there was a reduction in the number of spores from the beginning of the experiment to the end (Table 7). Higher sporulation at the beginning of the experiment can be attributed to a high amount of pre-existing grass in the experimental area, which has a high thin roots density that contributes to a greater colonization of AMF. According to the work of Zangaro et al. (2013), AMF colonization and fungal spore density showed positive correlations with thin root morphology, suggesting that the roots characteristics strongly influence the association.

The paricá diameter at 250 days (Table 8) was lower than the control in the plants treated with AMF, suggesting that paricá may have suffered losses in the development by the sum of two factors: competition for soil resources from the interaction between grasses and mycorrhizal fungi and because this variety is vulnerable to low temperatures. This depression may have occurred due to the fact that during the experiment the return and growing of *Urochloa* sp. Occurred around the plants. In grass-dominated areas, the negative effect of underground competition on tree seedling development has been associated with the large number of roots produced by grasses (GUNARATNE et al., 2011).

First, this high soil root density reduces soil clearance by physically blocking the elongation of the initial roots of tree seedlings (MESSIER et al., 2009).

In addition, the *R. clarus* fungus has low specificity in relation to the host plants (SIVIEIRO et al., 2008), with the possibility of its hyphae simultaneously colonize the roots of more than one plant, including different species, forming in the soil a hyphae network called Common Mycorrhizal Network - CMN (FITTER et al., 1998). According to Olson et al. (2010), using molecules labeled with isotopes of radioactive carbon, the carbon can be transferred through the CMN or also remain in the fungal structures within the roots, not occurring its transfer to the aerial part of the plant.

According to Lescano et al. (2014), tree plants colonized by AMFs, in early stages of succession, when cultivated in competition with *U. brizantha*, showed limited growth, resulting in a reduction of photosynthetic capacity, but a high intensity of root colonization by AMF. This high root colonization by AMF may have been aided by the carbon transferred by *U. brizantha*, since the AMF also depressed the growth of that grass. This depression can be explained by the fact that CMN formation occurs by binding the grass roots to the tree plant, and the carbon flux from both plants was eventually drained to the AMF.

Thus, it is suggested that the large number of roots (Urochloa sp.) in the experimental area, through the CMN, may have interfered in the mycorrhizal colonization of paricá, affecting the plant development. Unlike Guapuruvú, which is more tolerant to cold, because it is endemic to the Atlantic Forest, was able to maintain the resources for its development. The outcome of bioticinteractions along productivity-based stress gradients may vary depending on the stress tolerance and competitive ability of the interacting species (WANG et al., 2008; MAESTRE et al., 2009).

Conclusions

The fertilizer either in presence or absence of inoculants provided good results in the development of both varieties of *S. parahyba* in relation to height growth, lap diameter, and higher survival rates. The 20-05-20 dosage of NPK fertilizer proved to be effective in the development of both varieties in the study areas.

The presence of grasses in the experimental area may have contributed to the reduction of growth and development of *S. parahyba*. Therefore, studies without the grass interference, are necessary to prove the AMF action AMF in field in the development of that species. Evaluating the effect of chemical fertilization and inoculation with growth promoting micro-organisms on wood quality would be future proposals that would contribute to forestry.

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