

## Co-inoculation of rhizobium strains and one of the arbuscular micorrhizal fungus on *Stylosanthes guianensis* cv. CIAT-184

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The effect of the co-inoculation of three rhizobium strains and one of the arbuscular micorrhizal fungus (AMF) on *Stylosanthes guianensis* cv. CIAT-184 was evaluated. The experiment was carried out in the Pastures and Forages Station of Cascajal, Villa Clara province, in a ferruginous nodular Gley soil. Eight treatments were studied: the rhizobium strains S1, S2 and S3 alone and combined with the AMF species *Funneliformes mosseae*, a control with *F. mosseae* without rhizobium and another without rhizobium or *F. mosseae*. Treatments were distributed in a random block design with four replications. Results of the first two cuttings are presented. S2 and S3 strains increased significantly the nodulation (31-40 nodules/plant) and the nitrogen concentration in the aerial biomass (3.55-4.00 %) regarding S1 and the control treatments. *F. mosseae* increased percentages of frequency and colonization intensity and the spore density in the rhizosphere, as well as the P concentrations in the aerial biomass (0.25 %). The co-inoculation of both microorganisms improved the effectiveness of the nodulation and increased the micorrhizal structures and *Stylosanthes* yield. Best results were obtained with S2 and S3 strains in presence of *F. mosseae*. It was confirmed under the conditions of this study the synergic effect of the nitrogenfixing bacteria and the arbuscular micorrhizal fungi, as well as the advantages of the co-inoculation of both microorganisms for improving yield and nutritive value of *Stylosanthes*, at least during its establishment.

Key words: *biological nitrogen fixation, arbuscular micorrhiza, forage legumes, nutrition*

For agricultural and livestock production systems, tropical forage legumes are an excellent option due to their agronomical and nutritional characteristics. This plant family can contribute to the improvement of the quality of animal diets when managed efficiently in integral systems (Ruiz *et al.* 2005). However, for guaranteeing a better establishment and persistency, the adequate supply of nutrients is required, especially when these plants are cultivated in association with grasses (Urbano *et al.* 2005).

The effective management of the rhizospheric microorganisms can constitute an agricultural model, ecologically and economically viable for increasing the productivity, persistency and nutritive value of forage legumes and, in turn, the reduction of the use of conventional fertilizers (Nascimento *et al.* 2008 and González *et al.* 2012). The symbiotic associations established between these plants, the arbuscular mycorrhizal fungi (AMF) and the bacteria of the Rhizobium genus, together with other microorganisms of the rhizosphere, contribute to improve the nutritional status and yields of the forage legumes, besides preserving soil fertility (Toro *et al.* 2008).

From these premises, this study was conducted for evaluating the effect of the co-inoculation of three rhizobium strains and one of the arbuscular mycorrhizal fungus (AMF) on *Stylosanthes guianensis* cv. CIAT-184 during its establishment.

### Materials and Methods

The experiment was conducted at the Pastures and

Forages Station of Cascajal, located in Santo Domingo, Villa Clara province, in a ferruginous nodular Gley soil (Anon 1999), characterized by strongly acid pH, low organic matter (OM) and assimilable phosphorus content and low base interchange capacity (BIC) (Paneque *et al.* 2010). Table 1 shows its main chemical characteristics.

Eight treatments were evaluated conformed by the rhizobium strains S1, S2 and S3 alone and combined with the inoculation of the AMF INCAM-2 strain, an ecotype of the species *Funneliformes mosseae* (Nicol. & Gerd.) (Schüßler and Walker 2010). There was also a control with AMF without rhizobium and another without inoculation. These treatments were distributed in a random block design with four replications. No fertilizer or soil fertility improver was applied. Plot had a total area of 21 m<sup>2</sup> and a calculation area of 14 m<sup>2</sup> (six rows 5 m long). Soil preparation was made conventionally by ploughing up, slight harrowing, crossing, harrowing and furrowing. *Stylosanthes* was sown in rows 70 cm apart and using slight drill sowing with dosages of 8 kg of total seed ha<sup>-1</sup> (2 kg of pure germinable seed ha<sup>-1</sup>). Sowing was realized in June 2011. Inoculation of the rhizobium strains and of the AMF strain was made by the method of seed covering at sowing in amounts equivalents to 50 and 100 g of rhizobium inocula and AMF, respectively. For each kilogram of seed, inoculants contained in the case of the rhizobium, 10 x 10<sup>10</sup> CFU g, and the micorrhizal inoculum 33 spores/g. The strains of rhizobacteria came from plant nodules of *Stylosanthes guianensis* without inoculation that grew spontaneously in a *Brachiaria brizantha* cv.

Table 1. Chemical characteristics of the soil (0-20 cm)

pH	MO H <sub>2</sub> O (%)	P <sub>2</sub> O <sub>5</sub> (mg.100 g <sup>-1</sup> )	Ca	Mg	Na	K	BIC
			(cmol.kg <sup>-1</sup> )				
4.7	2.43	2.2	3.5	1.2	0.10	0.11	4.91

Marandú grassland cultivated under similar conditions to those of the area where this experiment was carried out. They belong to the collection of rhizobium strains of the Bacteriology Laboratory of the Physiology and Plant Biochemistry Department of the National Institute of Agricultural Sciences (INCA). The AMF strain came from the collection of the Micorrhiza Laboratory also of INCA. It was chosen due to its high efficacy for increasing yields of cultivated pastures under similar edaphoclimatic conditions to those of this experiment (Ramírez *et al.* 2006).

Two cuttings were practiced, the first 120 d after sowing. The second was performed, 60 d after the first cutting. In both cases, dry matter (DM) yield was determined. Plants were cut at 10 cm height, regarding soil surface. Samples of 200 g of fresh matter were taken for determining DM percentage and N, P and K concentrations in the biomass (AOAC 1995). DM yield was estimated from fresh matter yield (FM) and DM percentage.

Before the second cutting, root samples of 10 plants of each plot were taken for establishing the number of nodules and their coloration (FAO 1985) and evaluating the micorrhizal colonizing frequency (Giovanetti and Mosse 1980) and the colonization intensity (Trouvelot *et al.* 1986). Soil samples of the rhizosphere were taken for determining the spore density of AMF (Gerdemann and Nicolson 1963, modified by Herrera *et al.* 1995).

Results were evaluated through the analysis of variance. Duncan's (1955) multiple range test was used in the cases where there were significant differences between treatments for establishing the significant differences between means.

### Results and Discussion

There was significant difference ( $P < 0.05$ ) between treatments for the micorrhizal structures of *Stylosanthes* (table 2). Inoculation of *F. mosseae* increased the frequency and intensity of the colonization and density of spores, corroborating the effectiveness of this strain for attaining radical occupation levels higher than those of resident AMF (Ramírez *et al.* 2006), at least under the conditions of this experiment. Also, in these variables, on combining this AMF strain with the S2 and S3 rhizobium strains, significant differences were found regarding the inoculated strains by separate.

Miranda *et al.* (2005) stated that the efficiency of AMF inoculation for promoting plant growth depends on its capacity for competing with native AMF. This is related to the inefficiency of the strain, its capacity for producing external hyphae, the speed of hypha for root

colonization and its ability for maintaining colonization levels under competitive condition.

The S2 and S3 rhizobium strains supplied greater nodulation to *Stylosanthes*, although the highest effectiveness of its nodules was attained when combined with *F. mosseae*. Both strains exhibited values higher than 95 %. Rhizobium strains equally increased N concentrations in the aerial biomass, but the highest content were also attained with S2 and S3 strains, in presence of *F. mosseae* (table 3). Similar results obtained Crespo-Flores *et al.* (2012) and González *et al.* (2012) on using rhizobium strains and AMF inoculated independently and combined with other legumes as *Teramnus labialis* and *Pueraria phaseoloides*. It seems evident that the concentrations of this element found in the foliar mass of the *Stylosanthes* are directly related to the values obtained in the nodulation variables. Treatments inoculated with *F. mosseae* reflected the highest phosphorus concentrations in the aerial biomass. This confirms the function of the AMF in the improvement of the phosphoric nutrition of the forage legumes, an aspect also verified by Miranda *et al.* (2008) and Carneiro *et al.* (2010) on inoculating HMF strains in these cultures. The rhizobium strains and of AMF by themselves alone increased DM yield regarding the controls without inoculation. However, the highest yields were obtained when S2 and S3 were combined with *F. mosseae*. These results coincide with those reported by Karti (2009) who observed that forage species *Pueraria phaseoloides* and *Centrosema pubescens* produced higher biomass yields with combined utilization of biological fertilizers based on AMF and bacteria of Rhizobium genus, regarding the independent utilization of each one of them.

On integrating the results of this experiment, the important synergic effect established between the rhizobium strains and that of AMF *F. mosseae*, was evidenced. This was manifested in the effectiveness of the nodulation and by the formation of fungic structures in the plants. Also it was demonstrated with increase of the N and P concentrations in the aerial biomass and on *Stylosanthes* yield. Rabie *et al.* (2005) and Tajini and Drevon (2012) stated that the benefits of the bacterium-AMF-legume tripartite symbiosis, related to the nodulation process of the nitrogenfixing bacteria and the establishment of arbuscular micorrhizal, could occur simultaneously and synergically so while fungi mobilize P and other nutrients from the soil, bacteria supply N not only to the plant, but also to the AMF. Also it was confirmed a direct relationship between the presence in

Table 2. Effect of the treatments on the fungic structures and nodulation of *Stylosanthes*

Treatment		Mycorrhizal structures			Nodulation	
Rhizobium strain	AMF	Colonization (%)	Visual density (%)	Spores/50 g	Nodules/plant	Effective nodules (%)
No	No	29.50 <sup>c</sup>	1.35 <sup>c</sup>	189 <sup>c</sup>	14 <sup>c</sup>	38
S1	No	31.28 <sup>c</sup>	1.35 <sup>c</sup>	220 <sup>c</sup>	19 <sup>c</sup>	41
S2	No	32.50 <sup>c</sup>	1.32 <sup>c</sup>	217 <sup>c</sup>	32 <sup>ab</sup>	66
S3	No	31.15 <sup>c</sup>	1.35 <sup>c</sup>	217 <sup>c</sup>	31 <sup>ab</sup>	89
No	Yes	49.35 <sup>b</sup>	2.46 <sup>b</sup>	410 <sup>b</sup>	15 <sup>c</sup>	40
S1	Yes	48.50 <sup>b</sup>	2.48 <sup>b</sup>	414 <sup>b</sup>	22 <sup>c</sup>	42
S2	Yes	59.00 <sup>a</sup>	2.84 <sup>a</sup>	517 <sup>a</sup>	40 <sup>a</sup>	98
S3	Yes	59.83 <sup>a</sup>	2.85 <sup>a</sup>	511 <sup>a</sup>	39 <sup>a</sup>	96
SE ±		1.35*	0.03*	3.85*	2.51*	-

<sup>abc</sup> Means with different letters in each variable differ significantly at  $P < 0.05$  (Duncan 1955) \* $P < 0.05$

Table 3. Effect of the treatments on nutrient concentrations and DM yield of the aerial biomass of *S. guianensis*

Treatment		Nutrient content (%)			DM (t ha <sup>-1</sup> )
Rhizobium strain	AMF	N	P	K	
No	No	2.62 <sup>d</sup>	0.19 <sup>b</sup>	1.28	3.76 <sup>d</sup>
S1	No	2.68 <sup>d</sup>	0.2 <sup>b</sup>	1.28	3.92 <sup>cd</sup>
S2	No	3.55 <sup>b</sup>	0.19 <sup>b</sup>	1.28	3.23 <sup>b</sup>
S3	No	3.61 <sup>b</sup>	0.2 <sup>b</sup>	1.28	6.2 <sup>b</sup>
No	Yes	3.16 <sup>c</sup>	0.25 <sup>a</sup>	1.29	4.63 <sup>c</sup>
S1	Yes	3.17 <sup>c</sup>	0.25 <sup>a</sup>	1.29	4.68 <sup>c</sup>
S2	Yes	4.00 <sup>a</sup>	0.24 <sup>a</sup>	1.29	8.76 <sup>a</sup>
S3	Yes	3.99 <sup>a</sup>	0.25 <sup>a</sup>	1.28	8.92 <sup>a</sup>
SE ±		0.08*	0.07*	0.01	0.26*

<sup>abcd</sup> Means with different letters in each variable differ significantly at  $P < 0.05$  (Duncan 1955) \* $P < 0.05$

the rhizosphere of both microorganisms and the yields of some cultures depending on their effectiveness, not only of the efficacy of both microorganisms, but also of the compatibility between symbionts and hosts (Castillo *et al.* 2008 and Sarabia *et al.* 2010). Seemingly, the benefits of the effect of the interaction of these microorganisms in the case of the S2 and S3 rhizobium strains and the HMF strain played a fundamental function in the growth and establishment of the cultivated legume by a better use of the nutrients (Sarabia *et al.* 2010).

In this experiment it was corroborated that the synergic effect of nitrogen-fixing bacteria and arbuscular mycorrhizal fungi, as well as the advantages of the co-inoculation of both microorganisms for improving the yield and nutritional status of *Stylosanthes guianensis*, at least during its establishment.

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**Received: January 16, 2014**