Co-inoculation of ryzobium strains and one of arbuscular mycorrhizial fungi (*Glomus cubense*) and its effect on kusdzú (*Pueraria phaseoloides*). Technical note

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The effect of co-inoculating two strains of ryzobium and one of arbuscular mycorrhizia fungi (AMF) *Glomus cubense* on the nutritional stage and yield of kusdzú (*Pueraria phaseoloides* vc. CIAT-9900), cultivated on a red lixiviated ferralitic soil, was studied. The experiment included eight treatments, distributing the ryzobium strains K1 and K2, the application of 50 kg of N ha^{-1}, a control without ryzobium or nitrogen fertilizer, ryzobium alone or combined with *G. cubense*, in a random block design with four replicates. The results of the first cut are presented. In absence of the mycorrhizia inoculation, K2 produced N contents in the biomass (2.53 %) and yields of dry matter (2.73 t DM ha^{-1}) significantly higher than K1, and similar to those reached with the nitrogen fertilization. However, the co-inoculation of *G. cubense* with any of the ryzobium strains increased significantly the nodulation (46 nodules plant^{-1}) and produced yields (3.37 t DM ha^{-1}) and contents of N (3.35 %) and P (0.23 %) in the biomass significantly higher than those reached with the ryzobium alone or with the application of N. The fungal structures of the plants were also favored with the combination of the ryzobium and the AMF. Under the conditions of this experiment, the synergic effect of both microorganisms was proved, as well as the advantages of their co-inoculation to improve yield and nutritive value of kusdzú at least during its establishment.

Key words: *Rhizobium, Glomus cubense, forage legumes, nitrogen biological fixation, nutrition*.

The effective management of the rhizospheric microorganisms may be an ecological alternative and economically feasible to increase productivity, persistence and nutritive value of forage legumes and reduce the use of chemical fertilizers (Nascimento *et al*. 2008 and Corbera and Nápoles 2011).

It has been proved that the symbiotic associations established between these plants, the arbuscular mycorrhizial fungi (AMF) specialized in capturing phosphorous and other nutrients from the soil, together with bacteria from the Rhizobium genera, specialized in capturing the atmospheric N_2 and other microorganisms from the rhizosphere, not only contribute to improve the nutritional stage of forage legumes, but also to preserve the fertility of the soils (Castillo *et al*. 2008 and Toro *et al*. 2008).

From these premises, the objective of this study was to assess the effect of the co-inoculation of two strains of rhizobium and one of AMF on the nutritional stage and yield of kusdzú (*Pueraria phaseoloides* vc. CIAT-9900) during its establishment.

The experiment was conducted in the Microstation of Pastures and Forages of the Genetic Cattle Enterprise "Niña Bonita", in Artemisa province, Cuba. The soil was red lixiviated ferralitic (Anon 1999), with pH close to neutrality, mean content of OM and assimilable P and low interchangeable K content (Paneque and Calaña 2001). Its main chemical characteristics are presented in table 1.

Eight treatments were assessed, distributing the rhizobium strains, named conventionally K1 and K2, the application of 50 kg of N ha^{-1} and a control without, without rhizobium or nitrogen fertilizer, rhizobium alone or combined with *Glomus cubense* (Rodríguez *et al*. 2011), in a random block design with four replicates. The test was conducted in plots constituting the experimental unit. They had a total surface of 21 m^2 and a calculation area of 14 m^2. The kusdzú was sown at drill, in June 2009, in furrows separated at 70 cm, with 5 kg of total seeds ha^{-1}.

The rhizobium strains were isolated from the kusdzú plants without inoculation, which grew spontaneously in a grassland of *Panicum maximum* cv. Likoni, cultivated under similar conditions to those of the experimental area. The strains were purified and characterized (Pérez 2008, unpublished data).

Due to its high efficiency, proved in previous studies of AMF-pastures species strains, conducted under the same edaphic conditions of this experiment (González *et al*. 2008), the strain HMA *G. cubense*,

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

pH H_2O	MO (%)	P_2O_5 (mg 100 g^{-1})	Ca	Mg	Na	K	CCB
			(cmolc kg^{-1})				
6.7	3.25	2.6	9.7	2.2	0.15	0.21	12.3

named Glomus hoi-like before its classification by Rodríguez *et al*. (2011) was selected. For the application of microorganisms, the respective solid inoculants were used, containing, in the first case, 10 x 10^{10} cfu g^{-1} of one or other rhizobium strain and, in the second, 25 spores g^{-1} of the strain AMF *G. cubense*. Both bio-preparations were produced in the National Institute of Agricultural Sciences (INCA) and were applied by the method of covering the seed when sowing, in amounts equivalent to 50 and 100 g of rhizobium inoculum and AMF, respectively, per each seed kilogram. The N was applied as urea, at 30 d after the kusdzú germination, in a small furrow of 10 cm depth, made with hoe at 10 cm of each plant row, covered later with the soil.

The first cut of kusdzú was conducted after three months of the sowing. The green mass of the calculation area of each plot was weighed. Samples of 200 g were collected to determine the percentage of DM and the contents of N, P, K of the biomass (AOAC 1995). The DM yield was estimated from that of the green mass (GM) and the DM percentage. Previously, samples of the rhizosphere soil and roots of 10 plants were collected per plot to determine the number of nodules and their color (FAO 1985), the percentages of mycorrhizial colonization (Giovanetti and Mosse 1980) and the visual density or intensity of the colonization (Trouvelot *et al*. 1986), as well as the density of AMF spores in the rhizosphere (Gerderman and Nicholson 1963, modified by Herrera *et al*. 1995).

The data were processed with the analysis of variance. The multiple comparison test of Duncan (1955) was used when significant differences between treatments were found. The information of the colonization percentages were transformed previously through the function arcsine $\sqrt{x/100}$. For the number of spores 50 g^{-1} and that of nodules per plant, the function log (x+0.375) was used.

In absence of the *G. cubense* inoculation, the rhizobium strains produced similar amounts of nodules per plant, although with K2 nodulation was more effective. However, both of them produced a significantly higher nodulation when inoculating with the strain of AMF (table 2).

The treatments inoculated with *G. cubense* showed significantly higher percentages of mycorrhizial colonization, visual density and density of spores in the rhizosphere than those not-inoculated. This corroborated the effectiveness of this strain for reaching, at least under the conditions of this experiment, higher root occupation levels than those of the native AMF (González *et al*. 2008). However, these fungal variables reached their highest values when the inoculation of *G. cubense* was combined with any rhizobium strain.

Although the two rhizobium strains increased yield and N content in the air biomass of kusdzú compared with the non-inoculated control, they reached significantly higher values with K2 than those with K1, and were similar to those achieved with 50 kg of N ha^{-1} (table 3).

The DM yields and the N and P concentrations in the air biomass, obtained with the co-inoculation of one or other rhizobium ecotype and that of the AMF strain were significantly higher than those achieved with the inoculation of each microorganism separately, and even with the nitrogen fertilization. Karti (2009) also observed that the forage species *Pueraria phaseoloides* and *Centrosema pubescens* produced the highest biomass yields with the combined use of biological fertilizers based on AMF and bacteria of the rhizobium genus, independently with each of them.

The results of this study suggest an important kinetics effect between the rhizobium ecotypes and

Table 2. Effect of the treatments on the nodulation and structures of kusdzú

Treatments			Nodulation		Mycorrhizia structures		
Rhizobium strain	AMF	N (kg ha^{-1})	Nodules/plant	Effective nodules, %	Colonization, %	Visual density, %	Spores/ 50 g
No	No	0	(14) 1.52^c	39	(33,7) 0.62^c	1.19^c	(183) 2.64^c
K1	No	0	(26) 1.79^b	62	(35,3) 0.64^c	1.23^c	(192) 2.66^c
K2	No	0	(30) 1.85b	96	(32,4) 0.61^c	1.25^c	(181) 2.63^c
No	No	50	(16) 1.58^c	41	(36,1) 0.64^c	1.20^c	(185) 2.64^c
No	Sí	0	(13) 1.49^c	38	(48,3) 0.77^b	2.25^b	(297) 2.85^b
K1	Sí	0	(42) 2.00^a	93	(57,3) 0.86^a	2.81^a	(382) 2.96^a
K2	Sí	0	(46) 2.04^a	89	(59,4) 0.88^a	2.88^a	(395) 2.97^a
No	Sí	50	(17) 1.61^c	43	(38,6) 0.67^c	1.24^c	(190) 2.65^c
SE			0.02**		0.02**	2.0**	0.04**

[abc]Averages with different letters in each row differ significantly at $P < 0.05$ (Duncan 1955).
( ) Original data. **$P < 0.01$

Table 3. Effect of the treatments on the DM yield and the nutrient content on the air biomass of kusdzú

Treatments			MS ($t\ ha^{-1}$)	Nutrient conten, %		
Rhizobium strain	HMA	N ($kg\ ha^{-1}$)		N	P	K
No	No	0.00	1.57^{e}	1.93^{e}	0.17^{b}	1.27
K1	No	0.00	2.28^{c}	2.28^{c}	0.18^{b}	1.32
K2	No	0.00	2.73^{b}	2.53^{b}	0.16^{b}	1.25
No	No	50.00	2.70^{b}	2.56^{b}	0.18^{b}	1.30
No	Sí	0.00	2.05^{d}	2.16^{d}	0.20^{ab}	1.29
K1	Sí	0.00	3.27^{a}	2.79^{a}	0.22^{a}	1.27
K2	Sí	0.00	3.35^{a}	2.81^{a}	0.23^{a}	1.33
No	Sí	50.00	2.61^{b}	2.51^{b}	0.18^{b}	1.26
SE±			0.08*	0.03*	0.01*	0.05

[abcde]Averages with different letters in each row differ significantly at $P < 0.05$ (Duncan 1955). $^*P < 0.05$

the AMF strain *G. cubense*, manifested with increases in the number of nodules per plant and the nodulation effect, increase of the fungic structures of the plants and increase of the N and P content in the air biomass and kusdzú yield. Rabie *et al*. (2005) state that the benefits of the tripartite symbiosis bacteria-AMF-legume, related with the nodulation process of the nitro-fixer bacteria and the establishment of the arbuscular mycorrhizia, could occur simultaneously and kinetically. Likewise, while fungi mobilizes P and other nutrients from the soil, bacteria supply N, not only to the plant but also to the AMF.

The AMF and nitro-fixer bacteria could act kinetically, stimulating the kusdzú growth through a better adquisition of nutrients (Saravia *et al*. 2010). Besides, it is stated that the root colonization by the AMF could affect, directly or indirectly, the bacteria communities associated with the rhizosphere. The direct interactions include the energy supply through compound rich in carbon, transported from the hostage plant to the mycorrhizia through the fungi hair (Bonfante and Anca 2009). The indirect interactions refer to the mycorrhizia effects on the hostage plant, the exudation of growth promoter substances and the improvement of the soil structure. All these factors increase the activity of the nitro-fixer bacteria (Antoun and Prévost 2005).

Under the conditions of this experiment, the kinetic effect of the isolated rhizobium strains and the AMF strain was proved, as well as the advantages of co-inoculating both microorganisms to improve the kusdzú nutritional stage and yield, at least during its establishment.

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