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CONTRIBUTION OF ARBUSCULAR MYCORRHIZAL INOCULATION TO THE REDUCTION OF PHOSPHORUS FERTILIZATION IN *Brachiaria decumbens*

Contribución de la inoculación micorrízica arbuscular a la reducción de la fertilización fosfórica en *Brachiaria decumbens*

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ABSTRACT. An experiment was conducted on a Red Ferralitic Lixiviated soil in order to assess the contribution of arbuscular mycorrhizal inoculation to reduce phosphorus fertilization in *Brachiaria decumbens* cv. Basilisk. Doses of 0, 30, 60 and 90 kg P_2O_5 ha^{-1} $year^{-1}$, alone and combined with the inoculation of arbuscular mycorrhizal fungal strain *Glomus cubense*, previously selected for its high efficiency, were distributed in a randomized block design with 4x2 factorial arrangement and four replications. The experiment was conducted under irrigated conditions and lasted two years. *G. cubense* significantly increased mycorrhizal colonization percentages, visual density and spore number in the rhizosphere, although the values of those variables decreased with the highest doses of P_2O_5. In non inoculated treatments, the highest yields and P extractions in the aerial biomass were obtained with doses of 30-90 kg of P_2O_5 ha^{-1}, with no significant differences between them, but only with the inoculation of *G. cubense*, during the first year of the establishment of grass both indicators reached values similar to those obtained with phosphoric fertilizer applications without inoculation. The influence of *G. cubense* was observed until the rainy season of the second year, although fungal variable values, yields and P exports with the aerial biomass indicated a decrease in the effectiveness of inoculation, in relation to the first year.

Key words: Glomus, nutrition, fertilization, forage grasses

RESUMEN. Se realizó un experimento en un suelo Ferralítico Rojo Lixiviado, con el objetivo de evaluar la contribución de la inoculación micorrízica arbuscular a la reducción de la fertilización fosfórica en *Brachiaria decumbens* cv. Basilisk. Se estudiaron dosis de 0, 30, 60 y 90 kg de P_2O_5 ha^{-1} $año^{-1}$, solas y combinadas con la inoculación de la cepa de hongo micorrízico arbuscular *Glomus cubense*, seleccionada previamente por su alta eficiencia, en un diseño de bloques al azar con arreglo factorial 4x2 y cuatro réplicas. El experimento se condujo bajo condiciones de riego y tuvo una duración de dos años. La inoculación de *G. cubense* incrementó significativamente los porcentajes de colonización micorrízica, la densidad visual y el número de esporas en la rizosfera, aunque los valores de estas variables disminuyeron con la dosis más alta de P_2O_5. En los tratamientos no inoculados, los mayores rendimientos y extracciones de P en biomasa aérea del pasto se obtuvieron con las dosis de 30 a 90 kg de P_2O_5 ha^{-1}, sin diferencias significativas entre ellas; sin embargo, solo con la inoculación de *G. cubense*, durante el primer año del establecimiento del pasto ambos indicadores alcanzaron valores similares a los obtenidos con las aplicaciones de fertilizante fosfórico sin inocular. La influencia de *G. cubense* se observó hasta el período lluvioso del segundo año, aunque los valores de las variables fúngicas, los rendimientos y las exportaciones de P en la biomasa de la parte aérea indicaron una disminución de la efectividad de la inoculación, en relación con el primero.

Palabras clave: Glomus, nutrición, fertilización, gramíneas forrajeras

INTRODUCTION

Improved pasture fertilization is a tool to increase forage supply per unit area and time and, consequently, animal production. By this way, not only soil nutrients are restored through the biomass consumed by cattle,

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but also the nutritional value and pasture persistence are enhanced (1).

So far, studies on grass fertilization have mainly focused on increasing biomass production per unit area and evaluation of the economic impact of fertilizer use. At present, regarding the economic aspect, given the increasing prices of fertilizers, environmental preservation becomes very important; thus, there is a greater need to design fertilization strategies to guarantee an adequate nutrition of grasses which, in turn, ensures the protection of natural resources (2, 3).

The management of mycorrhizal symbiosis can be considered among these strategies, since the benefits of arbuscular mycorrhizal fungi (AMF) in grassland agroecosystems are closely related to a higher absorption of mineral elements, water and other substances, through a network of interconnected hyphae that increases the volume of soil explored by roots and makes easier plant access to less available nutrients (4, 5). Therefore, the fertilizer doses to be applied to pastures could be reduced based on a better profitability and efficient use of soil nutrients and even fertilizers.

P is one of the most important nutrients whose absorption is favored by AMF action, since 95 to 99 % of its amounts present in the soil are not available for plants (6). In the specific case of cattle agroecosystems in tropical regions, where the low P profitability by many forage grass species is inseparably linked to the complex dynamics of this element in soils (7, 8), the management of mycorrhizal associations may constitute a valid choice for improving the efficiency of phosphorus fertilization.

Considering such aspects, this work was aimed at evaluating the contribution of arbuscular mycorrhizal fungal (AMF) inoculation to reduce phosphorus fertilization in *Brachiaria decumbens* cv. Basilisk.

MATERIALS AND METHODS

The experiment was conducted under field conditions, at the Micro-Station of Pasture and Forage from “Niña Bonita” Cattle Genetic Enterprise, located in the municipality of Bauta, Artemisa province, on a Lixiviated Red Ferralitic soil (9), whose main chemical characteristics (Table I) showed a slightly acid pH, an average organic matter content as well as low amounts of available P_2O_5 and exchangeable K (10).

Table I. Soil chemical characteristics (depth: 0-20 cm)

pH H_2O	MO (%)	P_2O_5 (mg 100 g^{-1})	Exchangeable cations (cmolc kg^{-1})) Ca	Mg	Na	K
6,4	3,23	2,1	9,51	2,52	0,17	0,26

The methods established at the laboratory of soils and plant tissue from the Department of Biofertilizers and Plant Nutrition of the National Institute of Agricultural Sciences (INCA) were used for soil analysis (11).

Four doses of phosphoric fertilizer (0, 30, 60 and 90 kg P_2O_5 ha yr^{-1}) were evaluated under two conditions of mycorrhizal inoculation (non-inoculated and inoculated with AMF *Glomus cubense* strain) in a randomized block design with 4x2 factorial arrangement and four replications. Treatments were distributed in plots, making up the experimental unit with a total area of 28 m^2 and a calculation area of 21 m^2.

The soil was broken down by plowing, harrowing and the crossing of both at intervals of 20 days approximately. The grass was seeded by drilling rows spaced 70 cm, on March 15, 2005, using a dose of 6 kg total seeds ha^{-1} (1 kg pure seeds ha^{-1} with germinating ability) at 1,5 cm deep. Phosphoric fertilizer rates were applied as recommended per treatment at seeding time and 12 months later. All treatments had a fixed basal dressing of 210 kg N ha^{-1} yr^{-1}, splitting doses of 35 kg ha^{-1} at seeding time and after each cut, and 100 kg K_2O ha^{-1} yr^{-1} in a single dose also applied at seeding time and 12 months later. Triple superphosphate, urea and potassium chloride were used as carriers.

Crop requirements, the amount of nutrients in the soil, the number of cuts per year and expected yield were taken into account for selecting rates and frequencies of phosphoric fertilization as well as fixed basal dressing of N and K_2O. AMF INCAM-4 strain from *Glomus cubense* species (12), named as *Glomus hoi-like* before its classification, was used due to the high rate of efficiency shown in trials performed under similar conditions to those of this experiment (13). A certified solid inoculant containing 25 spores g^{-1} substrate was applied, which was obtained at the Department of Biofertilizers and Plant Nutrition of INCA. The grass was inoculated at seeding time by the seed coating method (14).

The first cut was done 120 days after seeding whereas the others at intervals of 45 and 90 days during the rainy and poorly rainy seasons, respectively, making up a total of six cuts per year, which were made at 10 cm high from soil surface and weighing the green mass (MV) of the aerial part of plants from the calculation area of plots; then, samples of 200 g were taken to determine the percentage of dry mass (MS) and P concentrations of the aerial biomass (11). MS yield and P extraction of the aerial biomass were calculated; in the first case, based on MV yield and MS percentage whereas in the second one, based on MS yield and P concentrations in the aerial biomass, respectively.

In alternate cuts, 10 sub-samples of roots and soil from the rhizosphere were taken at 0-20 cm deep, by using a metal cylinder of 5 cm diameter and 20 cm high. Sampling points were equidistantly distributed and separated 10 cm from the rows (4).

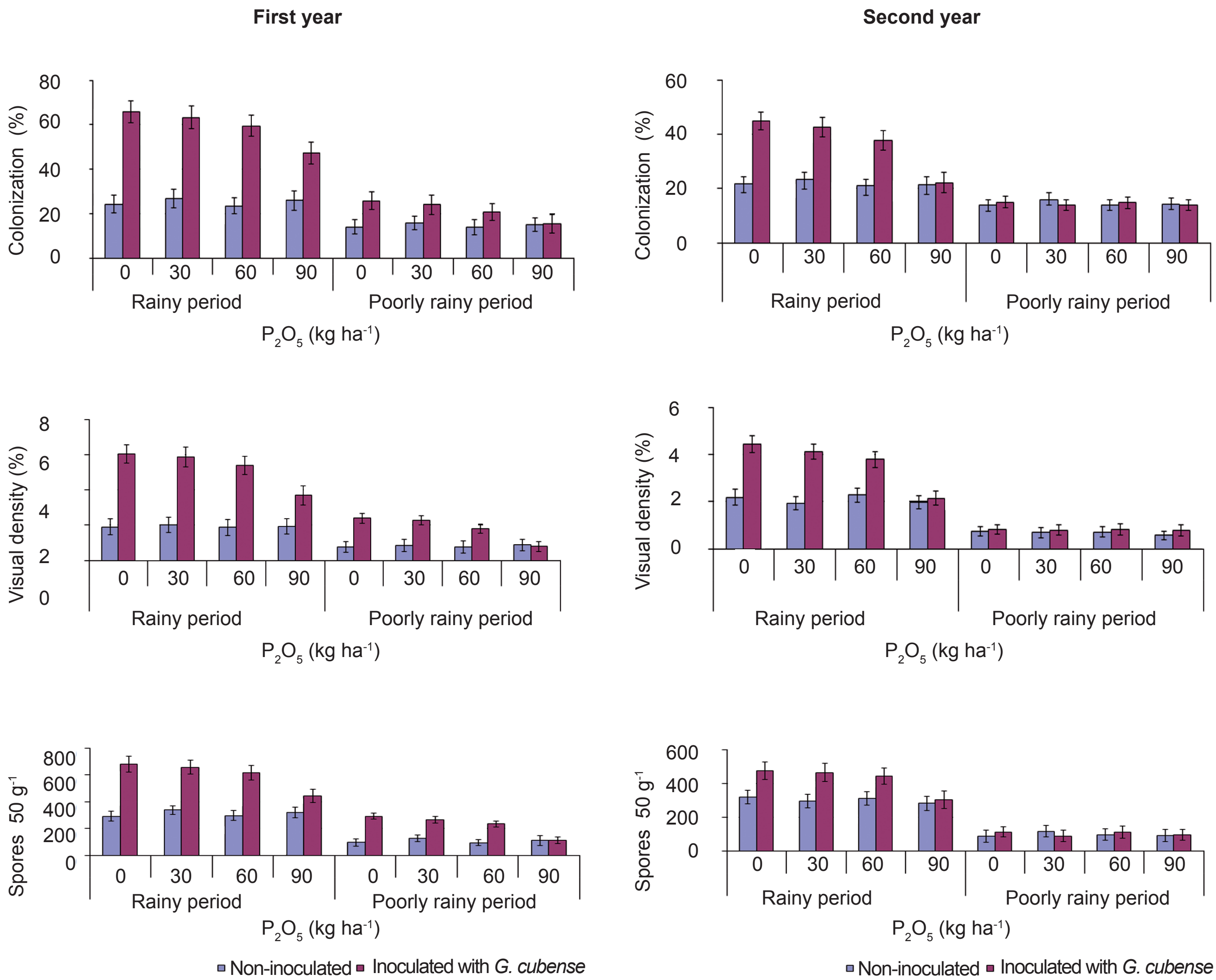
These were homogenized to form a composite sample per plot, from which 1 g rootlets was extracted for its staining and clarification (15). Mycorrhizal colonization (16), visual density or colonization intensity (17) and spore number in the rhizosphere (18) were evaluated.

Data were processed through a bifactorial variance analysis, according to the experimental design employed and Duncan's test at $p < 0.05$ (19) or the confidence interval at $\alpha = 0.05$ (20), to establish significant differences between the means of treatments. The SPSS 11.5 statistical program for Windows was used.

RESULTS AND DISCUSSION

The figure shows the effect of inoculation with *G. cubense*, alone or combined with applications of increasing phosphoric fertilizer doses on pasture mycorrhizal variables.

The mean values of each variable during the rainy and poorly rainy seasons of every year are presented. *G. cubense* increased colonization percentage, visual and spore densities in the rhizosphere, compared to non-inoculated treatments, which showed the level of roots taken in resident or native AMF. Phosphorus fertilization did not influence these variables, except the treatment where *G. cubense* was combined with the highest doses of P_2O_5, which showed significantly lower colonization levels, visual density and amounts of spores than the other treatments inoculated.



Las barras verticales muestran el intervalo de confianza (α = 0,05)

Effects of phosphoric fertilization and *G. cubense* inoculation on the performance of mycorrhizal variables in pasture

The effect of inoculation with *G. cubense* remained until the rainy season of the second year, except in the treatment with the highest dose of P_2O_5; however, absolute values of fungal variables were lower than during the same period of the previous year. Percentages of colonization, visual and spore densities in the rhizosphere were significantly higher during the rainy season than in the dry period of each year.

A significant interaction was recorded between phosphorus fertilization and inoculation or not with *G. cubense* for grass MS yield (Table II). In non-inoculated treatments, the highest yields were achieved with doses of 30 to 90 kg P_2O_5 ha^{-1} yr^{-1}, without significant differences between them; nevertheless, just by inoculating with *G. cubense*, during the first year values were similar to those achieved with phosphorus fertilizer without inoculation, so that applications of this nutrient were unnecessary.

Over the second year, the influence of *G. cubense* on grass yield was lower than in the first one, because it only lasted until the rainy season, despite values were significantly higher than those recorded in the control without P_2O_5 or inoculation, and its effect for reducing grass response to phosphorus fertilizer was not maintained, so that it was necessary to apply this nutrient to attain the highest yields.

Neither P_2O_5 applications nor *G. cubense* inoculation influenced P concentrations in the aerial part biomass (Table III); however, a significant interaction was observed between both factors for the extraction of such element by the grass aerial biomass (Table IV).

As P extraction was related to grass MS yield, both variables showed very similar performance; that is, the highest P extractions were achieved by applying 30 to 90 kg P_2O_5 ha^{-1} yr^{-1} to non-inoculated treatments, without significant differences with the extraction during the first year just with *G. cubense* inoculation. Over the second year, strain effect was only kept until the rainy season, but it did not reach the levels of P extraction obtained when applying phosphorus fertilizer.

Several aspects should be highlighted from the comprehensive analysis of these results. Firstly, under these experimental conditions, the possibility of improving phosphoric nutrition and pasture productivity by introducing an efficient AMF strain was proved, coinciding with other results (21, 22) about the contribution of inoculating by efficient AMF strains to increase yields and reduce the requirements of phosphorus fertilizer in forage species.

Table II. Effects of phosphoric fertilization and *G. cubense* inoculation on MS yield (t ha^{-1}) in pasture

Treatments		First year			Segundo año		
P_2O_5 (kg ha^{-1})	Inoculation	Rainy period	Poorly rainy period	Total	Rainy period	Poorly rainy period	Total
0	NI	11,82 b	5,23 b	17,05 b	9,01 c	4,24 b	13,25 c
30	NI	15,24 a	6,10 a	21,34 a	12,10 a	5,12 a	17,22 a
60	NI	15,11 a	5,70 a	20,81 a	11,84 a	4,93 a	16,77 a
90	NI	15,24 a	6,07 a	21,32 a	12,25 a	4,82 a	17,07 a
0	I	14,89 a	5,97 a	20,86 a	10,40 b	4,31 b	14,71 b
30	I	15,06 a	6,08 a	21,13 a	11,92 a	4,98 a	16,90 a
60	I	14,94 a	5,98 a	20,92 a	12,11 a	4,93 a	17,04 a
90	I	15,28 a	5,95 a	21,24 a	11,80 a	5,00 a	16,80 a
ES		0,40**	0,21**	0,43**	0,37**	0,19**	0,41**

NI: non-inoculated I: inoculated with *G. cubense*
Means with non-common letters in the same column differ significantly to p<0,05, according to Duncan's test

Table III. Effect of treatments on P (% MS) concentrations in pasture biomass

Treatments		First year		Segundo año	
P_2O_5 (kg ha^{-1})	Inoculation	Rainy period	Poorly rainy period	Rainy period	Poorly rainy period
0	NI	0,18	0,22	0,20	0,23
30	NI	0,19	0,24	0,20	0,22
60	NI	0,20	0,23	0,22	0,24
90	NI	0,19	0,22	0,21	0,22
0	I	0,20	0,23	0,20	0,23
30	I	0,19	0,22	0,21	0,24
60	I	0,18	0,24	0,21	0,22
90	I	0,20	0,22	0,20	0,23
ES		0,01	0,01	0,01	0,01

NI: non-inoculated I: inoculated with *G. cubense*

Table IV. P (kg ha^{-1}) extraction with aerial biomass

Treatments		First year			Second year		
P_2O_5 (kg ha^{-1})	Inoculation	Rainy period	Poorly rainy period	Total	Rainy period	Poorly rainy period	Total
0	NI	21,28 b	10,98 b	32,26 b	18,02 c	9,75 b	27,77 c
30	NI	28,96 a	14,03 a	42,99 a	24,20 a	11,26 a	35,46 a
60	NI	30,22 a	13,57 a	43,79 a	26,05 a	11,34 a	37,39 a
90	NI	28,96 a	13,96 a	42,92 a	25,73 a	10,60 a	36,33 a
0	I	29,78 a	13,13 a	42,91 a	20,80 b	9,91 b	30,71 b
30	I	30,12 a	13,38 a	43,50 a	25,03 a	10,96 a	35,99 a
60	I	28,39 a	13,75 a	42,14 a	25,43 a	10,85 a	36,28 a
90	I	30,56 a	13,09 a	43,65 a	26,18 a	11,50 a	37,68 a
ES		1,3**	0,6**	1,4**	1,2**	0,5**	1,3**

NI: non-inoculated I: inoculated with *G. cubense*
Means with non-common letters in the same column differ significantly to p<0,05, according to Duncan's test

The effectiveness of *G. cubense* to attain a suitable mycorrhizal functioning and, consequently, an effective agronomic response in pastures was confirmed, which are previously tested aspects (12) in *Brachiaria* forage grasses and other plants grown in agroecosystems with a similar type of soil to that used for the experiment (23, 24).

Regarding the effect of AMF inoculation, although it is known that pasture response tends to vary depending on many factors, as the ecophysiology of these microorganisms in grassland agroecosystems is quite complex (25), several authors agree in stating that the introduction of selected strains adapted to environmental conditions and with a high level of functional and ecological compatibility for the soil-plant system, may be a desirable and even necessary management choice, in those cases where resident strains are not effective enough to attain a desirable agronomic response in these crops (26). The contribution of *G. cubense* to phosphoric nutrition of grass can be attributed to a higher profitability coefficient of the nutrient in a greater amount of soil explored by roots (27); it is inferred from its effect on mycorrhizal variables, since its high values for the rainy and the poorly rainy seasons of the first year appear to have decisively contributed to reduce the need of phosphorus fertilizer during that period. Similarly, this hypothesis is supported by the decrease observed in these variables during the second year, due to the reduced effectiveness of the strain introduced and the need of applying phosphorus fertilizer to achieve higher yields during that time.

In addition to the physical effect of *G. cubense* in root system extension, other mechanisms related to AMF ability to access even less available P sources and makes easier its uptake by plants, could also explain its influence on grass phosphoric nutrition (28, 29, 30).

Moreover, when *G. cubense* inoculation was combined with the highest dose of P_2O_5, there was a spatial and temporary reduction of fungal variables, which appears to be a physiological consequence of the diminishing role of mycorrhizae on its uptake, because the amount of phosphoric fertilizer applied was obviously much higher than that required by the grass. In this regard, some authors state that nutrient availability manages mycorrhizal structure growth and the distribution of arbuscles and extra-radical hyphae is reduced when pastures have been adequately fertilized, since the delivery of soil resources to the host plant through AMF loses importance (31, 32).

The fact that the application of a low dose of phosphorus (30 kg P_2O_5 ha^{-1} yr^{-1}) has been enough to get the highest yield in non-inoculated grass draws the attention, despite the low initial content of available P_2O_5 in the soil (9). Besides, the fact that neither P fertilization nor *G. cubense* inoculation have increased P amount in biomass also seems to confirm its low external requirement of grass. In this sense, it was proved that *B. decumbens* roots can access to less available P forms in the soil (33, 34).

The fact that when inoculating with an efficient AMF strain, phosphorus fertilization is unnecessary to improve yield, at least during the first year of its establishment, indicates that in addition to the morphological characteristics of roots and biochemical mechanisms that may be involved in the low response of this species to P applications, *G. cubense* played a decisive role in improving its phosphoric nutrition and indeed in reducing the external requirements of this element.

Not less interesting were the seasonal variations of mycorrhizal variables, since in all treatments for two years there were significantly higher colonization levels, visual and spore densities during the rainy season than in the poorly rainy season, which coincided with the distribution of grass biomass production in one and the other seasons.

Therefore, some authors point out that the rapid growth of pastures during the period of higher levels of rainfall, temperature and light demands a greater amount of nutrients and water; consequently, greater amounts of mycorrhizal structures to access to such resources (35, 36). Moreover, as the cost of mycorrhizal colonization is usually high (it is said that up to 20 % C can be delivered to microbial symbiont) during the greatest growing period, plants would be better able to allocate resources for making and keeping these structures (37).

CONCLUSIONS

Regarding these results, it can be concluded that inoculating an efficient AMF strain increases mycorrhizal structures and reduces the need to apply phosphoric fertilizer to pasture *B. decumbens*, cv. Basilisk, at least during the first year of its establishment. Thus, AMF management is seen as an economical and ecological choice to reduce fertilization costs in pastures as well as the risks of environmental pollution associated to its extensive and systematic implementation of these crops.

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