Scientific Paper

Biofertilization with mycorrhizal fungi on *Urochloa* grasses in a low-fertility acid soil

Juan Francisco Ramírez-Pedroso[1] *https://orcid.org/0000-0003-3384-3904*, Mildrey Paz-Medel[2] *https://orcid.org/0000-0002-3334-995x*, Pedro José González- Cañizares[3] *https://orcid.org/0000-0003-3206-0609*, Ramón Rivera-Espinosa[3] *https://orcid.org/0000-0001-6621-7446* and Alberto Hernández-Jiménez[3] *https://orcid.org/0000-0002-6138-0620*

[1]*Centro de Investigaciones en Plantas Proteicas y Productos Bionaturales, Calle 5ta Av. y 246 Complejo Barlovento, Playa. La Habana, Cuba.* [2]*Estación Experimental de Pastos y Forrajes, Carretera Central, Crucero Digna, Cascajal. Villa Clara, Cuba.* [3]*Instituto Nacional de Ciencias Agrícolas, Gaveta Postal No. 1. San José de las Lajas, Mayabeque, Cuba. E-mail: jpedroso@bionaturasm.cu, mildrey@pastos,ucl.minag.cu, pgonzalez@inca.edu.cu*

Abstract

Objective: To evaluate the response to biofertilization with arbuscular mycorrhizal fungi of four grass species of the genus *Urochloa*, cultivated on a low-fertility acid soil in the Manacas Savanna region, Cuba.

Materials and Methods: Twelve different treatments were evaluated, generated from the combination of four grass species and three mycorrhizal inoculants. The used species were *Urochloa brizantha* cv. Marandú, *Urochloa decumbens* cv. CIAT 606, *Urochloa híbrido* cv. 36087 Mulato II and *Urochloa híbrido* cv. BR02/1752 Yacaré. The mycorrhizal inoculants were formulated with the arbuscular mycorrhizal fungi strains *Funneliformis mosseae*, *Glomus cubense* and *Rhizoglomus irregulare*, respectively. In addition, a control group, without inoculation, was included. The experiment was carried out according to a randomized block design, with a 4 x 4 factorial arrangement and four replications. The total duration was three years. The evaluated variables were: frequency and intensity of mycorrhizal colonization, number of spores in the rhizosphere, macronutrient concentrations (NPK) in aerial biomass, participation of inoculants in nutrition and dry mass yield.

Results: There was no interaction between inoculation with arbuscular mycorrhizal fungi strains and grass species for the measured variables. The inoculation factor treatments showed differences among themselves. The highest values of frequency and intensity of mycorrhizal colonization, number of spores in the rhizosphere, nutrient concentrations in the biomass and participation of the inoculants in the nutrition and yield of the grasses were reached with *F. mosseae*, whose effect was maintained up to two years after the inoculants were applied.

Conclusions: Biofertilization with *F. mosseae* is effective in improving the nutritional status and yields of grasses of the genus *Urochlola*, grown on a low-fertility acid soil.

Keywords: nutritional status, yield, edaphic factors

Introduction

Grasses of the genus *Urochloa* (syn. *Brachiaria*) are widely cultivated, due to their high palatability and capacity to produce biomass with adequate nutritive value under adverse edaphic and climatic conditions for other species (Baptistella *et al*., 2021). In Cuba, although they are widespread throughout the country, they have an important presence in the animal husbandry areas located in the geographical region of Manacas Savanna. This area partially covers Matanzas, Villa Clara and Cienfuegos provinces. Its soils are of light texture, low natural fertility and high acidity, conditions that limit the productivity of pastures and their lifespan, leading to the appearance of invasive plants of low nutritional value for animal feeding (Pereira *et al*., 2018).

The use of high doses of fertilizers is essential to increase forage supply per unit area and time and improve the nutritive value of biomass (Silva-Parra *et al*., 2021; Cabral *et al*., 2023). However, high fertilizer prices and the need to promote environmentally friendly animal husbandry lead to the search for biological alternatives. These are based on the management of interactions between soil microorganisms and plants, with the purpose of increasing the productivity of agricultural crops and reducing dependence on external inputs.

The effective management of mycorrhizal associations constitutes an avenue for the above-mentioned intentions, due to the ability of arbuscular mycorrhizal fungi (AMF) to increase the volume of soil explored by roots and, consequently, improve plant access to soil nutrients and water, in addition to other benefits such as the induction of response mechanisms to pathogen attack (Chandrasekaran, 2020).

The genus *Urochloa*, inoculation with efficient AMF strains has been shown to improve yields and the nutritive value of biomass, contribute to plant recovery after cutting or grazing, and reduce the doses of mineral or organic fertilizers normally required

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by these crops (Karti *et al*., 2021; Chang *et al*., 2023). However, factors related to soil characteristics and the resident AMF community, the ability of the strains to adapt to the ecological niche where they are introduced, the species or cultivars and their management, can influence the effectiveness of inoculation (Tshibangu-Kazadi *et al*., 2020; Silva-Parra *et al*., 2021). Hence the importance of evaluating the effect of biofertilization with these microorganisms in different agroecosystems.

Based on these premises, the objective of this study is to evaluate the response to inoculation with arbuscular mycorrhizal fungi strains of four grass species of the genus *Urochloa*, cultivated in a low-fertility acid soil in the Manacas Savanna region, Cuba.

Materials and Methods

Location. The experiment was conducted at Cascajal Pasture and Forage Station, located at 22° 39′ 44” north latitude and 80° 29’ 36” west longitude, in the geographic region Manacas Savanna, Villa Clara province, Cuba.

Soil characteristics. The study was executed on a ferruginous nodular gley soil (Hernández-Jiménez *et al*., 2015), whose main chemical characteristics are shown in table 1.

The soil had high acidity, characterized by a strongly acid pH, high values of exchangeable acidity ($H^+ + Al^{3+}$) and very low percentage of base saturation (V), in addition to low organic matter content and very low levels of assimilable phosphorus and exchangeable cations (Paneque and Calaña, 2001). For the chemical characterization of the soil in the experimental area, five samples were taken by the zigzag method, at a depth of 0-20 cm. The samples were analyzed according to the Manual of Analytical Techniques for the analysis of soil, foliar, organic fertilizers and chemical fertilizers of the National Institute of Agricultural Sciences (INCA, for its initials in Spanish) (Paneque *et al*., 2010).

The performance of rainfall during the period in which the experiment was conducted is shown in figure 1.

Experimental design and treatments. In a randomized block design, with a 4 x 4 factorial arrangement and four replications, 12 treatments were evaluated, resulting from the combination of four grass species of the genus *Urochloa* (*U. brizantha* cv. Marandú, *U. decumbens* cv. CIAT 606, *U. hybrid* cv. 36087 Mulato II and *U. hybrid* cv. BR02/1752 Yacaré) and inoculation with three AMF strains Funnelnela (*U. hybrid* cv. BR02/1752 Yacaré). hybrid cv. BR02/1752 Yacaré) and inoculation with three strains of AMF *Funneliformis mosseae*, strain INCAM-2 (Nicol. & Gerd.) Walker & Schüßler,

Table 1. Soil chemical characteristics (depth 0-20 cm).

pH H_2O	OM, %	P, mg 100 g^{-1}	Ca^{2+}	Mg^{2+}	Na^+	K^+	BEC	$H^+ + Al^{3+}$	Al^{3+}	V, %
			(cmol kg^{-1})							
4,8	2,52	5,9	3,32	1,12	0,05	0,1	4,59	4,33	0,06	51
(0,2)	(0,17)	(0,3)	(0,3)	(0,1)	(0,01)	(0,02)	(0,31)	(0,33)	(0,01)	

OM: organic matter, BEC: base exchange capacity, $H+ + Al^{3+}$: exchangeable acidity, V: base saturation.
Values in parentheses indicate confidence intervals (α = 0,05).

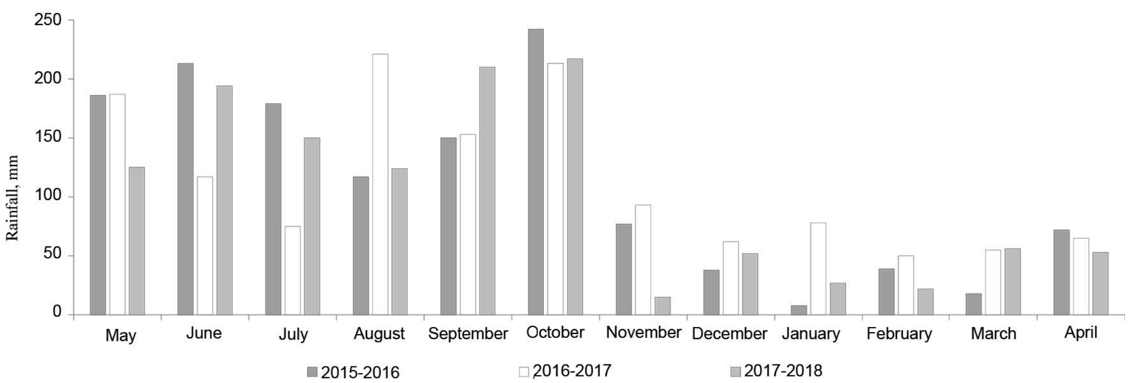


Figure 1. Rainfall distribution during the period in which the experiment was conducted.

Glomus cubense strain INCAM-4, (Y. Rodr. & Dalpé), *Rhizoglomus irregulare* strain INCAM-11 (N. C. Schenck & G. S. Sm. Sieverd, G. A. Silva & Oehl), plus a non-inoculated control. The plots constituted the experimental unit, and had a total surface area of 21 m2 and a calculation area of 14 m^2.

Experimental procedure. The soil was prepared by rototilling (plowing), harrowing, crossing (plowing) and harrowing, at intervals of approximately 25 days between each one. Sowing was carried out in furrows spaced 50 cm apart, at a rate of 1 kg of pure germinable seed ha^{-1} and at a depth of 1,5 cm. The experiment was conducted under rainfed conditions and the pastures were fertilized with 35 kg ha^{-1} of N 30 days after sowing, and after each cut made during the rainy season of each year with applications of (30 and 120 kg of P_2O_5 and K_2O ha^{-1} $year^{1}$, respectively) at the beginning of each rainy season. Urea, triple superphosphate and potassium chloride were used as carriers. The experiment lasted three years.

Inoculation was carried out at the time of sowing by the seed coating method, for which the seeds were immersed in a fluid paste, prepared by mixing a quantity of solid inoculant, equivalent to 10 % of their weight and water, in a 1:0,6 m/v ratio. Once the seeds were coated and the inoculant solidified, the seeds were sown. The inoculants were produced in the biofertilizer and plant nutrition department of INCA. They had a concentration of 35 spores of each AMF species to be evaluated per gram of inoculant, as well as abundant fragments of rootlets of the host plant used for multiplication.

The first cutting was made 120 days after sowing, and thereafter at intervals of approximately 60 and 90 days during the rainy and light rainy periods, respectively. In each cutting, the fresh mass of the aerial part of the plants occupying the calculation area of the plots was weighed. Samples of 200 g were taken and placed in an air circulation oven at 70 ºC for 72 hours to determine the percentage of dry mass (DM), estimate the DM yield and the concentrations of N, P and K in the biomass (Paneque *et al*., 2011).

Measurements. For the evaluation of mycorrhizal variables in alternate cuts, five root samples were taken per plot, at a depth of 0-20 cm with the use of a metal cylinder 5 cm in diameter and 20 cm long. The samples were pooled to form a composite sample from which 1 g of rootlets was extracted for staining and clarification (Rodríguez-Yon *et al*., 2015). The frequency of mycorrhizal colonization was determined using the intercept method (Giovanetti and Mosse, 1980), the visual density or intensity of colonization (Trouvelot *et al*., 1986) and the number of spores in the rhizosphere from wet sieving and decanting of these structures and their observation under the microscope (Herrera *et al*., 1995).

The participation of inoculants in pasture nutrition was determined (Rivera *et al*., 2003) using the following formula:

Participation, % = N, P and K concentrations, g kg^{-1} DM in the aerial biomass of the inoculated treatment-N, P and K concentrations, g kg^{-1} DM in the aerial biomass of the non-inoculated treatment) / N, P and K concentrations, g kg^{-1} DM in the aerial biomass of the inoculated treatment x 100.

Statistical analysis. For statistical processing, after checking the normality and homogeneity of variance of the data, the analysis of variance was applied, according to the experimental design and Duncan's multiple range test ($p < 0,05$). For soil chemical characterization, as well as to evaluate mycorrhizal structures and the participation of inoculants in grass nutrition, the confidence interval at $\alpha = 0,05$ was used (Payton *et al*., 2000). In all cases, the statistical program SPSS 25 (IBM SPSS Statistics, 2017) was used.

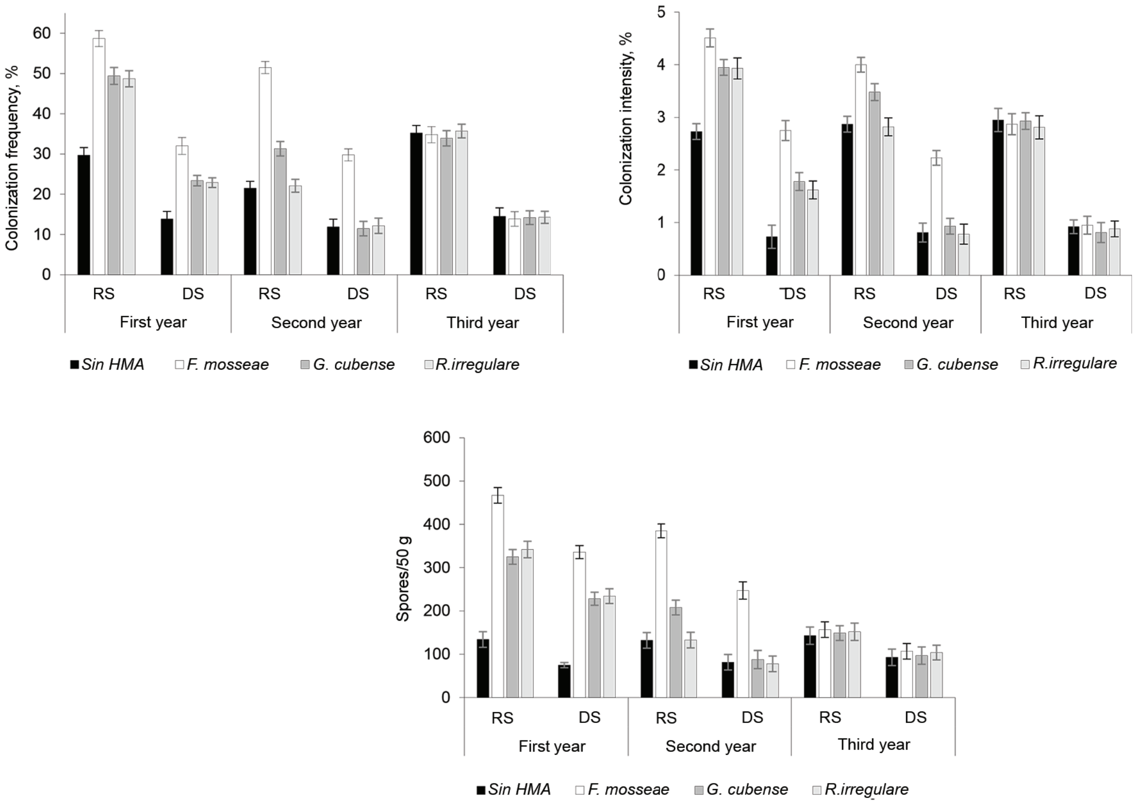
Results and Discussion

There was no interaction between inoculation between AMF strains and grass species on mycorrhizal structures, but the inoculation factor treatments showed differences among themselves. As shown in figure 2, all strains increased the frequency and intensity of colonization and the number of spores in the rhizosphere with regards to the non-inoculated control, which reflected the level of radical occupancy of resident AMF. However, the highest values of these variables were reached with *F. mosseae*, whose effect was maintained until the dry season of the second year.

The inoculants with *G. cubense* and *R. irregulare* not only produced lower frequency and intensity of colonization and number of spores than *F. mosseae*, but their effects had less permanence, since they were only observed until the rainy and dry seasons of the second and first year, respectively. In the third year, no effect of inoculation on mycorrhizal variables was observed.

No significant differences were found among grass species for fungal variables, which also showed the highest values in the rainy season of each year (figure 3).

These results corroborate those found by González-Cañizares (2014), who, when evaluating the effect of inoculation with AMF strains on *Brachiaria*



RS: rainy season, DS: dry season.

Vertical bars show the confidence interval of the means. Overlapping confidence intervals do not differ significantly (α = 0,05).

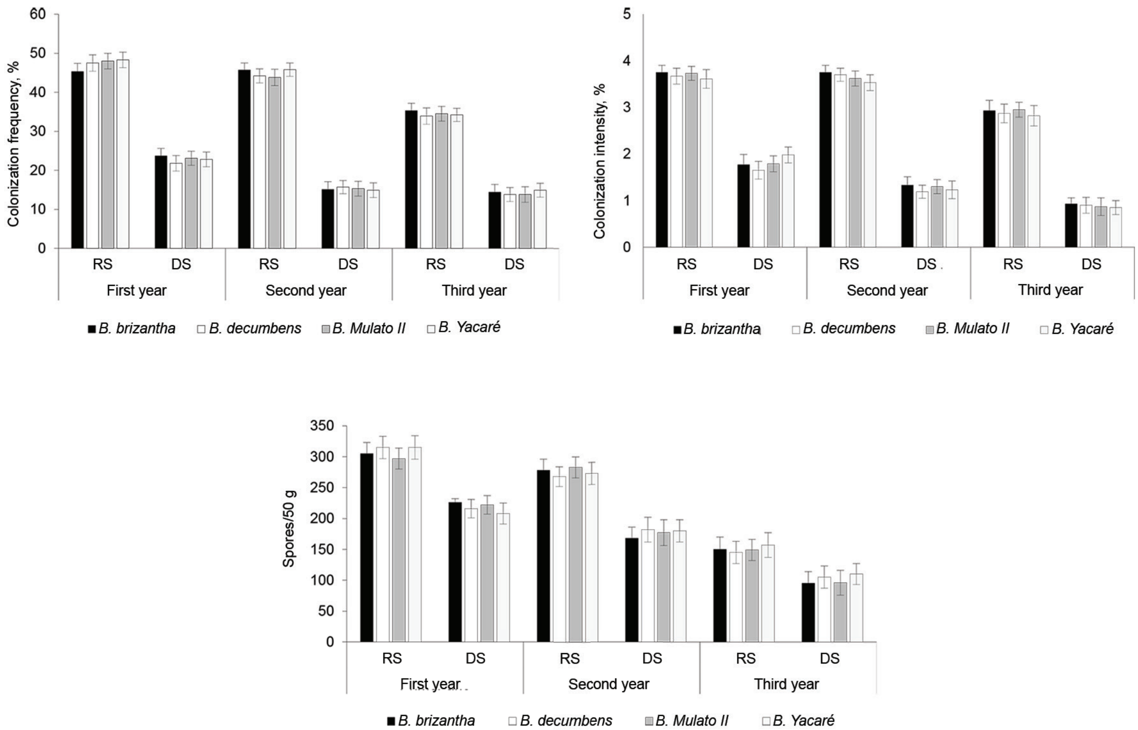
Figure 2. Effect of inoculation with AMF strains on the frequency and intensity of colonization and the number of spores in the rhizosphere.

grasses grown on two soil types, concluded that for each edaphic condition there was at least one strain highly effective in increasing the values of mycorrhizal variables in all grass species. This indicates, according to the above-cited author, high compatibility between the efficient AMF strain and the soil type and low specificity between the efficient AMF strain and the grass species. These regularities have also been found in other crops with different growth patterns and nutritional requirements in an important group of soils (Rivera *et al*., 2020; Simó *et al*., 2020).

There is consistent evidence that soil properties, specifically pH, markedly influence the effectiveness of inoculation with AMF strains (Tshibangu-Kazadi *et al*., 2020). In fact, inoculation with *F. mosseae* has been more effective in edaphic environments characterized by high acidity and low fertility (Rivera-Espinosa *et al*., 2015), conditions similar to those of the soil where this study was conducted.

The higher values of frequency and intensity of colonization and the number of spores in the rhizosphere, recorded in rainy periods compared with dry periods, can be related to the seasonal variation in biomass production of grasses. Its greater growth in the season of higher rainfall, temperature and luminosity, favors more demand for nutrients and, in fact, greater formation of mycorrhizal structures to access soil resources (Cera *et al*., 2021).

There was no interaction between inoculation with AMF strains and grass species for the concentrations (annual averages) of macronutrients in biomass, but the inoculation factor did show significant differences between treatments (table 2). As with the fungal variables, all strains increased N, P and K concentrations relative to the non-inoculated control. However, the highest values were achieved with *F. mosseae*, whose effect, unlike the inoculants formulated with *G. cubense* and *R. irregulare*, lasted until the second year. The concentrations of these nutrients showed no differences among the grass species.



RS: rainy season, DS: dry season.
Vertical bars show the confidence interval of the means. Overlapping confidence intervals do not differ significantly (α = 0,05).
Figure 3. Effect of grass species on the frequency and intensity of colonization and number of AMF spores in the rhizosphere.

Table 2. Effect of inoculation of AMF strains and grass species on concentrations (annual averages) of N, P and K in aboveground biomass, g kg^{-1}.

Treatment	First year			Second year			Third year		
	N	P	K	N	P	K	N	P	K
Inoculation effect									
Without AMF	12,2^{c}	1,6^{c}	11,1^{b}	11,9^{b}	1,7^{b}	11,9^{b}	11,8	1,7	11,7
F. mosseae	15,5^{a}	2,2^{a}	14,5^{a}	14,3^{a}	2,2^{a}	12,5^{a}	12,3	1,5	12,3
G. cubense	13,3^{b}	1,9^{b}	13,2^{b}	12,1^{b}	1,8^{b}	10,1^{b}	12,1	1,6	11,9
R. irregulare	13,8^{b}	1,8^{b}	13,5^{b}	12,0^{b}	1,7^{b}	10,5^{b}	12,2	1,5	12,5
SE ±	0,311	0,102	0,421	0,315	0,103	0,415	0,500	0,101	0,432
P - value	0,00	0,00	0,00	0,00	0,00	0,00	0,322	0,489	0,377
Pasture species effect									
U. brizantha	13,7	1,9	12,7	12,6	1,9	11,6	12,1	1,6	12,5
U. decumbens	12,8	2,1	12,9	12,4	2,0	10,9	11,8	1,5	12,3
U. Mulato II	13,9	1,8	13,0	12,7	1,7	11,5	12,3	1,7	11,7
U. Yacaré	13,7	1,7	12,8	12,7	1,8	10,7	11,8	1,5	11,5
SE ±	0,411	0,104	0,371	0,387	0,110	0,502	0,425	0,112	0,507
P - value	0,436	0,252	0,327	0,391	0,222	0,411	0,415	0,332	0,451

Non-common letters in the same column differ significantly at p < 0,05, according to Duncan's test.

The participation of mycorrhizal inoculants in pasture nutrition was evaluated by the percentage increase of N, P and K concentrations in aerial biomass with regards to the non-inoculated treatment (figure 3). Only the results of the first two years are shown, since as table 2 shows, in the third year there was no effect of inoculation on the concentrations of these nutrients in the biomass.

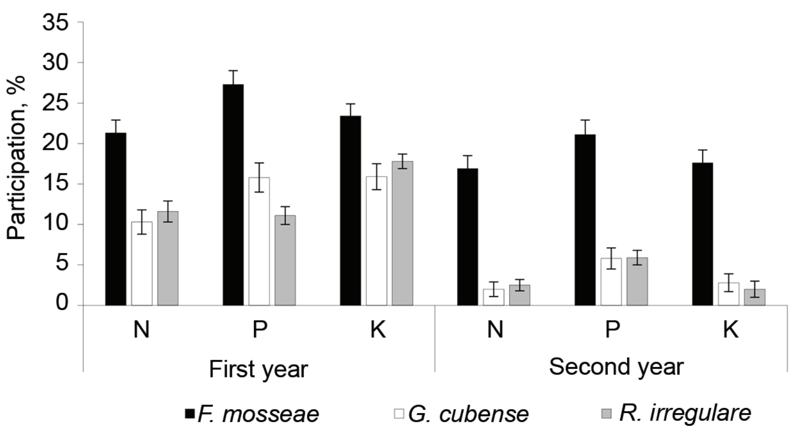
As in the concentrations of nutrients in the aerial biomass, the greatest participation corresponded to the inoculant formulated with *F. mosseae*, whose effect was maintained until the second year, although with lower values than in the first year, in the three elements. *G. cubense* and *R. irregulare*, besides having lower participation than *F. mosseae* during the first year, had a very low effect in the second year, because their contribution to the increase of nutrients in biomass barely exceeded 5 %, only in phosphorus.

In forage crops inoculated with AMF, it has been found that higher levels of mycorrhizal colonization are generally associated with increased nutrient uptake (Tran *et al*., 2019; Cavagnaro *et al*., 2021). This could explain the higher concentrations of N, P and K in the aerial biomass of grasses inoculated with *F. mosseae*, as well as their higher participation in the nutrition of the three elements during the two years in which response to inoculation was found. As shown in figure 2, during this period, the levels of root occupancy reached with the inoculant containing this strain were significantly higher than with the others.

Figure 4 shows that, during the first and second year, mycorrhizal inoculants contributed more to phosphorus nutrition than to nitrogen and potassium. However, according to the achieved values, it was more noticeable with *F. mosseae*. This behavior could be related to the role of mycorrhizae in increasing phosphate uptake from the soil, even of poorly assimilable forms (Teutscherova *et al*., 2019; Chandrasekaran, 2020) and to the increased utilization coefficient of the phosphate fertilizer that was applied in the background, especially in the soil where this experiment was conducted. There, the assimilable phosphorus contents were very low per se and were apparently not sufficient to meet the needs for this nutrient in the inoculated pastures.

There was no interaction between inoculation with AMF strains and grass species for dry mass yield. However, the inoculation factor and species showed differences between them (table 3).

In the first year, in both rainy and non-rainy periods, inoculation with AMF strains increased yield compared with the non-inoculated treatment. However, the highest values were achieved with *F. mosseae*. In the second, with the inoculant formulated with *F. mosseae*, the highest yield was also obtained during both seasons. *G. cubense* again



Participation, % = Concentration of N, P and K, g kg^{-1} in the aerial biomass of the biofertilized treatment - concentration of N, P and K, g kg^{-1} of the aerial biomass of the non-inoculated treatment / Concentration of N, P and K, g kg^{-1} of the aerial biomass of the inoculated treatment x 100.

Vertical bars show the confidence interval. Means with overlapping confidence intervals are not significantly different (α= 0,05).

Figure 4. Participation of mycorrhizal inoculants in pasture nutrition.

Table 3. Effect of inoculation with AMF strains and grass species on DM yield during the three years the experiment was conducted (t ha^{-1}).

Treatment	First year			Second year			Third year		
	DM RS	DM DS	TDM	DM RS	DM DS	TDM	DM RS	DM DS	TDM
Inoculation effect									
Without AMF	8,3^{c}	3,2^{c}	11,5^{c}	9,3^{c}	3,2^{b}	12,5^{c}	8,6	3,5	12,1
F. mosseae	10,5^{a}	3,9^{a}	14,4^{a}	11,0^{a}	4,1^{a}	15,1^{a}	8,5	3,8	12,3
G. cubense	9,6^{b}	3,3^{b}	12,9^{b}	10,0^{b}	3,2^{b}	13,2^{b}	8,7	3,7	12,4
R. irregulare	9,4^{b}	3,4^{b}	12,8^{b}	9,6^{c}	3,3^{b}	12,8	8,7	3,6	12,2
SE ±	0,210	0,102	0,231	0,223	0,112	0,231	0,180	0,109	0,198
P - value	0,000	0,000	0,000	0,000	0,000	0,000	0,113	0,143	0,115
Pasture species effect									
U. brizantha	9,1^{b}	3,4^{b}	12,4^{b}	9,9^{b}	3,1^{b}	13,0^{b}	8,4^{b}	3,4^{b}	11,8 ^{b}
U. decumbens	8,9^{b}	3,2^{b}	12,1^{b}	9,9^{b}	3,0^{b}	13,0^{b}	8,5^{b}	3,4^{b}	11,9 ^{b}
U. híbrido Mulato II	9,1^{b}	3,3^{b}	12,4^{b}	9,9^{b}	3,2^{b}	13,1^{b}	8,3^{b}	3,3^{b}	11,6 ^{b}
U. híbrido. Yacaré	10,6^{a}	4,1^{a}	14,7^{a}	11,0 ^{a}	4,5^{a}	15,5^{a}	9,5^{a}	4,5^{a}	14,0 ^{a}
SE ±	0,231	0,122	0,267	0,224	0,108	0,251	0,207	0,111	0,243
P - value	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000

Different letters in the same column differ significantly according to Duncan ($p < 0,05$).
DM RS: dry mass yield during the rainy period, DM DS: dry mass yield during the light rainy period, TDM: total dry mass yield (rainy + light rainy period).

increased yield with respect to the non-inoculated treatment, but its effect remained only until the rainy period. The influence of the inoculum with *R. irregulare* on this variable did not last beyond the first year. In the third year, no effect of inoculation on yield was observed. Among the grass species, the highest yields during the three years were achieved with *B.* Yacaré.

A comprehensive analysis of the results obtained in this experiment concluded that the mycorrhizal inoculant formulated with *F. mosseae* was more effective in improving the performance of *B. brizantha*, *B. decumbens*, *B.* Mulato II and *B.* Yacaré grasses, grown in a ferruginous nodular gley soil with low fertility and high acidity. Yacaré, grown in a ferruginous nodular gley soil of low fertility and high acidity. This ratified, under the conditions in which the trial was conducted, the low specificity between the inoculated AMF strains and the grass species, as observed earlier when evaluating mycorrhizal structures. This effect remained until the low rainfall period of the second year.

Although generally strict fungus-host plant specificity is not manifested, not all AMF species colonize with the same intensity and efficiency the different plant species, thus proving the existence of different degrees of compatibility in the symbiosis, as a result of environmental influences on the genotypic expression of both symbionts (Morales-Londoño *et al*., 2019; Rosales-Jenqui *et al*., 2021).

Works carried out in Cuba, related to AMF inoculation in different agricultural crops and soil types, have shown a strong relationship between edaphic factors, mainly pH, and the effectiveness of the inoculated strain in increasing yields. Inoculants formulated with *F. mosseae* stand out as the most efficient for plants grown in low fertility soils (Martín-Alonso *et al*., 2017).

The effect of the mycorrhizal inoculant containing the *F. mosseae* strain on increasing pasture yield was related to the improvement of the nutritional status of the latter, which was evident by the higher concentrations of N, P, K in the aerial biomass reached with the inoculation, as well as by its greater participation in the nutrition of the pastures compared with *G. cubense* and *R. irregulare*. This fact is related to the increased utilization of soil nutrients and fertilizers applied to pastures from the formation of sufficient amounts of mycorrhizal structures that facilitated plant access to these resources (Huang *et al*., 2020; Bouskout *et al*., 2022).

No less interesting was the better performance of Yacaré grass with regards to the other evaluated species. Although in some regions of Cuba this cultivar has shown higher yields and persistence levels than other *Urochloa* species (Pentón-Fernández *et al*., 2018), the fact that on acid and low fertility soils it has achieved higher productivity, indicates that in addition to inoculation with AMF, the inclusion of Yacaré grass in the varietal structure of forage species can also be an option to increase biomass production under these edaphic conditions.

Conclusions

Biofertilization with mycorrhizal inoculant formulated with *F. mosseae* is an effective way to improve the nutritional status and yields of *Urochlola* grasses grown on acid soils with low fertility, and the effect of the inoculant remains during the first two years after its application.

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Conflict of interests

The authors declare that there is no conflict of interests among them.

Authors' contributions

- Juan Francisco Ramírez-Pedroso. Led the research, participated in the design and execution of the experiment from which this paper was derived. He collaborated in the statistical processing and interpretation of the experimental results and in the manuscript writing.
- Mildrey Paz-Medel. Contributed to the execution of the experiment, data recording and sampling, data analysis and interpretation.
- Pedro José González-Cañizares. Directed the research task from which the paper was conceived, participated in the design of the experiment and interpretation of the results, and in the paper writing.
- Ramón Rivera-Espinosa. Participated in the execution of the experiment that led to the conception of the paper and took part in the interpretation of results.
- Alberto Hernández-Jiménez. Participated in the execution of the experiment from which the paper was developed. He contributed to the design and interpretation of the results of the experiment.

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