

Biomass production of *Cenchrus purpureus* (Schumach.) Morrone, associated with *Canavalia ensiformis* (L.), co-inoculated with mycorrhizae and rhizobium and combined with mineral nitrogen

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Abstract

Objective: To evaluate the benefits of inoculation of *Canavalia ensiformis* (L.) with arbuscular mycorrhizal fungi and rhizobia in biomass production of *Cenchrus purpureus* (Schumach.) Morrone cv. Cuba CT-169, fertilized with medium doses of mineral nitrogen.

Materials and Methods: The research was carried out in a low-fertility soil of the Technical Scientific Basic Unit Soils, Barajagua, Cienfuegos, Cuba. A randomized block design was applied, with six treatments and three replicas: T1) *C. ensiformis* + *C. purpureus* CT-169, T2) *C. ensiformis* + *C. purpureus* CT-169 50 % N, T3) *C. ensiformis* + AMF + *C. purpureus* CT-169 50 % N, T4) *C. ensiformis* + AMF + rhizobia + *C. purpureus* CT-169 +50 % N, T5) *C. purpureus* CT-169 100 % N and T6) *C. purpureus* CT-169. *C. ensiformis* was intercropped 20 days after the CT-169 leveling cut. At 60 days, it received a first cut and was deposited between the furrows for decomposition. The regrowth of *C. ensiformis* was allowed to grow for 65 days, to coincide with the 145 days of CT-169. At this time, the final cut was made to both species. Total biomass production was calculated in the treatments combining CT-169 and canavalia, for which both species were weighed independently and the percentage corresponding to the contribution of canavalia was determined. In the rest of the treatments, only the biomass of CT-169 was calculated. At the time of cutting, the height of CT-169 was measured, as well as the length and width of the fourth leaf. Subsequently, leaf area and total biomass production were calculated.

Results: The morpho-physiological indicators of treatments T5 and T6 differed from the others, with extreme values (high and low), respectively. Between treatments T3 and T4, there were no differences in stem diameter, length and width of the fourth leaf and leaf area. Regarding biomass production, treatment T5 reached the highest production with significant differences compared with the others, followed by treatment T3. In the treatments where *C. purpureus* CT-169 was associated with *C. ensiformis*, the legume constituted between 14,7 and 18,1 % of the total biomass production, respectively.

Conclusions: With the application of 100 % mineral nitrogen to *C. purpureus* CT-169, better yield was achieved in terms of the morphophysiological indicators as well as biomass production. In addition, the inclusion of *C. ensiformis* improved the forage quality, due to the protein contribution of the legume.

Keywords: fertilizers, legumes, yield

Introduction

The adoption of agroecological practices favors the sustainable intensification of agriculture, as it addresses problems related to negative environmental impact and low productivity and efficiency in specialized agricultural systems (Bover-Felices and Suárez-Hernández, 2020). Grassland agroecosystems, which cover more than a quarter of the earth's land surface, are crucial for milk and meat production (Martínez-Sáez *et al.*, 2018). Agroecological strategies include the use of vegetative covers, which protect the soil against erosion, control weeds, increase organic matter and improve soil properties (Alonso-Ayuso *et al.*, 2020). Legumes,

such as Fabaceae, are particularly valuable, as in addition to these benefits, they enrich the soil with nitrogen through their symbiosis with atmospheric nitrogen-fixing bacteria that act as green manures (People *et al.*, 2019).

Canavalia ensiformis (L.) is a plant that stands out for its resistance, adaptability to diverse soil conditions, capacity for atmospheric nitrogen fixation and positive response to mycorrhizal inoculation, which makes it an ideal green manure for diverse crops in Cuba (Rivera *et al.*, 2020). Its biomass decomposes rapidly, releasing nitrogen and other nutrients that improve the nutrition of associated crops and increase the efficiency of applied fertilizers (Simó *et al.*, 2020).

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Arbuscular mycorrhizal fungi (AMF) are critical for crop nutrition and agroecosystem resilience, enhance nutrient and water uptake by plants and protect them from pathogens (Ortas, 2019; Rillig *et al.*, 2019; Schaefer *et al.*, 2021). In legumes, the tripartite symbiosis between plants, rhizobia and AMF further enhances these benefits (Calderon and Dangi, 2024). Inoculation of *C. ensiformis* with efficient strains of these mycorrhizae improves the nutrition of associated crops, reduces the need for fertilizers and facilitates effective mycorrhization, without requiring direct inoculations on other crops (Rivera *et al.*, 2020; 2023).

There are several commercial inoculants based on AMF strains, available for agriculture, whose effectiveness has been evaluated in several studies (Elliott *et al.*, 2021; Frew, 2021). In Cuba, research has proven the effectiveness of *C. ensiformis* inoculated with generalist strains of these mycorrhizae on different crops and soils (Rivera *et al.*, 2020). However, more research is required on the use of *C. ensiformis*, inoculated with AMF and rhizobia in plantations of *Cenchrus purpureus* (Schumach.) Morrone in Grayish-brown dystric soils, common in the animal husbandry areas of the country.

The objective of this study was to evaluate the benefits of inoculation of *C. ensiformis* with AMF and rhizobia on biomass production of *C. purpureus* cv. Cuba CT-169, fertilized with medium doses of mineral nitrogen.

Materials and Methods

Location of the study area. The research was carried out in the Scientific-Technical Basic Unit of Soils, Barajagua, belonging to the Ministry of Agriculture, located at coordinates 22° 09' north latitude and 80° 12' west longitude, at 60 m a.s.l., in the town of Barajagua, Cumanayagua municipality, Cienfuegos province, central-southern region of Cuba.

General characteristics of the soil. The soil is classified as grayish brownish-grayish dystric (Hernández-Jiménez *et al.*, 2015). The values of some components of its fertility at the time of forming the experimental plots were: pH (KCl) 5,15; organic matter 1,84 %; assimilable phosphorus and potassium 3,01 and 5,72 mg kg⁻¹ of soil, respectively.

Experimental design and treatment. The experiment was conducted in a randomized block design with six treatments and three replicas as described below:

- T1) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169

- T2) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169 - 50 % N
- T3) AMF *C. ensiformis* - *Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N
- T4) *C. purpureus* cv. Cuba CT-169 - 50 % N
- T5) *C. purpureus* cv. Cuba CT-169 -100 % N
- T6) *C. purpureus* cv. Cuba CT-169

Experimental procedure. The experiment was conducted in an area of *C. purpureus* cv. Cuba CT-169, established for four years. Plots of 8,4 m² were formed, with four furrows 3,0 m long by 0,7 m wide and 0,5 m apart. The two central furrows with discarded edges were used as the evaluation unit, for an area of 2,1 m².

Before the formation of the plots, a homogenizing cut was made to *C. purpureus* throughout the experimental area, at a height of 15 cm above the ground. After 20 d, in the corresponding plots, a furrow of *C. ensiformis* was interspersed 35 cm away from each furrow of *C. purpureus*, not only in the lanes (between furrows of *C. purpureus*) but also 35 cm from the outer edges, for a total of five furrows of *C. ensiformis* in each plot. *C. ensiformis* was planted at 0,25 m plant spacing for a total of 60 plants per plot.

The inoculated mycorrhizal strain was *Funneliformis mosseae* (T.H. Nicolson and Gerd.; *C. Walker* and A. Schüßler) INCAM-2, from the AMF strain collection of the National Institute of Agricultural Sciences (INCA, for its initials in Spanish). The inoculant was formulated in solid state on a clayey substrate, containing 30 spores per gram of inoculant, as well as non-quantified amounts of rootlet and hyphal fragments.

Inoculation with AMF was carried out by the coating method, for which *C. ensiformis* seeds were immersed in a fluid paste, prepared by mixing an amount of inoculant equivalent to 10 % of the weight of the seeds and water, in the proportion of 50 mL of water per 100 g of inoculant (Simó *et al.*, 2020).

The rhizobium strain used was Can 2 from the Department of Plant Physiology and Biochemistry of INCA, at a concentration of 1 × 10⁸ CFU mL⁻¹. The recommended dose for the rhizobium strain was 250 mL per 50 kg of seed in imbibition. First the rhizobium application was carried out, followed by inoculation with the mycorrhizal strain.

At 60 d, at the flowering stage and with the first pods forming, *C. ensiformis* received a first cut at 15 cm above the ground. All the foliage was deposited

between the furrows for decomposition. At that time, CT-169 was 80 d old. The regrowth of *C. ensiformis* was allowed to grow for 65 days, coinciding with the 145 days of CT-169, at which time the final cut was performed on both species.

Measurements. At the time of cutting, the height of CT-169 was measured with a graduated ruler from the ground to the point of growth, as well as the length and width of the fourth leaf, completely open from the apex down, according to the methodology of Herrera and Ramos (2006). Subsequently, the leaf area resulting from the multiplication of the leaf length by width was calculated.

Biomass production was determined according to the following equation:

$$\text{Biomass (t ha}^{-1}\text{)} = \frac{\text{green mass, kg plot}^{-1} \times \text{dry matter, g kg}^{-1}}{\text{calculation area, m}^2 \times 100}$$

Total biomass production included the treatments that combined CT-169 and *C. ensiformis*, for which both species were weighed independently and the percentage that corresponded to the contribution of *C. ensiformis* was determined. The others considered only the biomass of the CT-169 treatments. In each plot, 200 g of leaf samples of the species present were taken to determine the total dry matter (Paneque *et al.*, 2010).

Statistical analysis. Statistical processing of the data was done by variance analysis. When significant differences were found among treatments, means were compared by Duncan (1955). Previously, the corresponding tests for normality (Shapiro-Wilks) and homogeneity of variance (Levene) were performed. The IBM Statistical Package for Social Sciences (SPSS) for Microsoft Windows®, version 15.0 (2012) was used for data analysis.

Results and Discussion

The performance of some morpho-physiological indicators of *C. purpureus* Cuba CT-169 at the time of the establishment cut is shown in table 1. Treatments T5) *C. purpureus* cv. Cuba CT-169 - 100 % N and T6) *C. purpureus* cv. Cuba CT-169 differed from the others, with the highest and lowest values of the morpho-physiological variables, respectively. The favorable effect of 100 % mineral nitrogen fertilization, without addition of green manure or biofertilizers, was observed with regards to the absolute control and the other variants. Between treatments, T3) *C. ensiformis* - *Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N and T4) *C. purpureus* cv.

Cuba CT-169-50 % N there were no differences in the indicators stem diameter, length and width of the fourth leaf and leaf area (table 1).

A favorable effect of the green manure T1) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169 was found in relation to the absolute control, T6) *C. purpureus* cv. Cuba CT-169 and T3) *C. ensiformis*-*Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N with respect to T2) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169 - 50 % N. This seems to indicate the benefit of joint inoculation of mycorrhizae and rhizobia combined with 50 % nitrogen doses. In the variants where *C. ensiformis* was intercropped, independently from being inoculated or not with the biofertilizers, the height of CT-169 was lower compared with CT-169 with mineral nitrogen fertilization. This could indicate a discrete effect of competition.

Table 1. Morpho-physiological indicators of *C. purpureus* in the establishment cut.

Treatment	Diameter, cm	Length four leaf, cm	Width fourth leaf, cm	Height, m	Leaf area, cm ²
T1	1,71 ^d	80,3 ^c	2,6 ^c	2,1 ^c	211,6 ^c
T2	1,78 ^c	80,0 ^d	2,7 ^c	2,3 ^c	221,5 ^c
T3	1,88 ^b	82,2 ^b	2,8 ^b	2,3 ^c	230,3 ^{bc}
T4	1,84 ^b	82,7 ^b	3,0 ^b	2,4 ^b	245,3 ^b
T5	1,94 ^a	87,0 ^a	3,5 ^a	3,0 ^a	301,6 ^a
T6	1,64 ^c	78,8 ^c	2,4 ^d	1,9 ^d	189,1 ^d
SE ±		2,65 ^{***}	0,36 ^{***}	0,38 ^{***}	37,34 ^{***}

a, b, c, d and e: Different letters in the same column indicate significant differences for $p \leq 0,001$

T1) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169, T2) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169 - 50 % N, T3) *C. ensiformis* - *Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N, T4) *C. purpureus* cv. Cuba CT-169 - 50 %, T5) *C. purpureus* cv. Cuba CT-169 -100 % N, T6) *C. purpureus* cv. Cuba CT-169

This performance seems to be related to the increased utilization of soil nutrients from the formation of quantities of mycorrhizal structures that can facilitate plant access to such resources from the soil (Igiehon and Babalola, 2017) and, in addition, to the inoculation of *C. ensiformis* with rhizobium and the contribution to the soil after its decomposition, once deposited.

Ojeda-Quintana *et al.* (2019) studied under field conditions, similar to those of this work, the effect of green manure of *C. ensiformis*, inoculated with AMF and used as a preceding crop and incorporated into the soil, on the yield and biomass quality of *C. purpureus* Cuba CT-169 (successor crop) in its establishment stage. No differences were found in height, fourth leaf length, leaf area and stem diameter of CT-169, but differences were found in fourth leaf width, as well as in biomass production.

Figure 1 shows the biomass production obtained in the establishment cut of CT-169 and *C. ensiformis* (total biomass and only of CT-169). All treatments differed statistically from each other. Treatment T5) *C. purpureus* cv. Cuba CT-169 -100 % N achieved the highest biomass yield. However, most interesting was the response of treatment T3) *C. ensiformis* - *Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N, which outperformed the others, except for 100 % nitrogen fertilization, indicating a favorable response of *C. ensiformis* inoculation

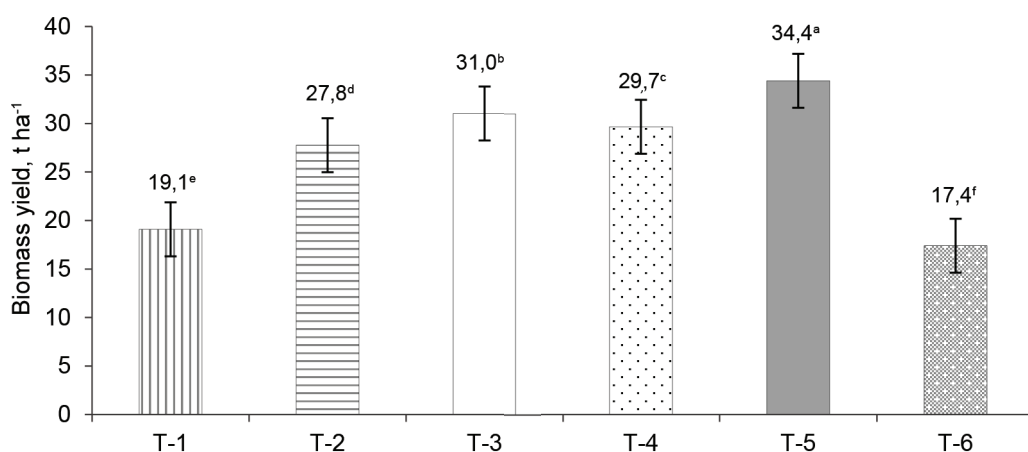
with the biofertilizers in the presence of 50 % nitrogen fertilization. The results indicate that the use of inoculated *C. ensiformis*, although with a favorable effect, failed to replace 50 % of the fertilization. They suggest that subsequent experiments should work with higher percentages of fertilizers, although always less than 100 % of fertilization or combined with organic fertilizers to achieve higher biomass yields and equivalent to or higher than those obtained in the treatment with 100 % fertilization (Rivera *et al.*, 2023).

The combination of *C. ensiformis* without inoculation (T-1) with *C. purpureus* was remarkable, which outperformed the absolute control (CT-169), showing the favorable effect of green manure on the growth of CT-169 and the increase in its biomass.

It is known that AMF increase the absorption and translocation of nutrients due to the morphological and physiological modifications they produce in the roots of the host plant, which increase the surface area in contact with the soil (Carrillo-Saucedo *et al.*, 2022).

Martín *et al.* (2015) evaluated the response of *C. ensiformis* to rhizobium and AMF co-inoculation in ferruginous nodular gley and red ferralitic soils. The results in those works showed that *C. ensiformis* responded positively to rhizobium/AMF co-inoculation in both types of soils, with improved biomass yield of *C. ensiformis*.

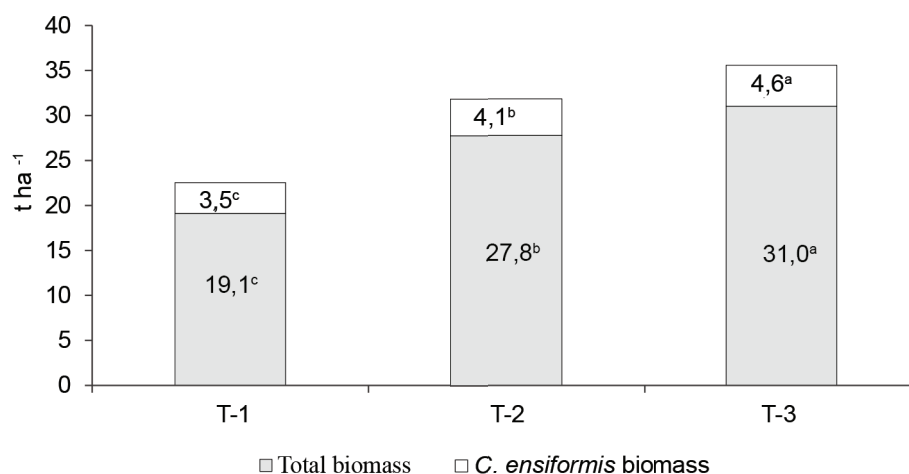
Figure 2 shows the structure of total biomass production in the treatments that intercropped *C.*



a, b, c, d and e: Different letters in the same column indicate significant differences for $p \leq 0,001$

T1) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169, T2) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169 - 50 % N, T3) *C. ensiformis* - *Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N, T4) *C. purpureus* cv. Cuba CT-169 - 50 %, T5) *C. purpureus* cv. Cuba CT-169 -100 % N, T6) *C. purpureus* cv. Cuba CT-169

Figure 1. Biomass production per treatment.



a, b and c: Different letters in the same column indicate significant differences for $p \leq 0,001^{***}$

T1) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169, T2) *C. ensiformis* + *C. purpureus* cv. Cuba CT-169 - 50 % N, T-3) *C. ensiformis* - *Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N, T4) *C. purpureus* cv. Cuba CT-169 - 50 %, T5) *C. purpureus* cv. Cuba CT-169 - 100 % N, T6) *C. purpureus* cv. Cuba CT-169

Figure 2. Structure of biomass production in the treatments associated with *C. ensiformis*.

ensiformis. The contribution of the legume intercropped with CT-169 constituted between 14,7; 14,7 and 18,1 % to the total biomass accounting obtained, indicating that the production of CT-169 was lower, an aspect that can be ascribed to the effect of competition between the two species. However, total biomass production was favored by the inclusion of *C. ensiformis* inoculated with the biofertilizers, since it was not only higher ($p \leq 0,05$) than that obtained with the treatment that received 50 % of the fertilization, but the forage improved its protein quality by including 18,0 % of *C. ensiformis*, which shows a nitrogen concentration close to 3 %, higher than those of CT-169, which is commonly found in the order of 1,5 % (Simo *et al.*, 2020).

Experimental information has established that *C. ensiformis* shows higher growth, biomass production, nutrient content and nodule number in the presence of co-inoculation of efficient AMF and rhizobial strains (Tamayo *et al.*, 2015). This could be observed in the response of treatment T3) *C. ensiformis* - *Funneliformis mosseae*/ INCAM-2 - Azofert-Can 2 + *C. purpureus* cv. Cuba CT-169 - 50 % N in relation to biomass production. This idea also coincides with Tamayo-Aguilar *et al.* (2021), regarding the increase in biomass production of *C. ensiformis* with inoculation with rhizobia and AMF strains and through their symbiotic interactions that provide benefits in yield and crop quality of associated economic crops.

Consideration should be given to Bakshi *et al.* (2020), who point out that to achieve more efficient crop nutrition, the integration of fertilizers and organic sources is a current necessity. Under these circumstances, integrated nutrient management has become the best technology, by enriching agricultural crops with essential nutrients, increasing production and sustaining productivity and soil fertility from the integrated use of organic manures, chemical fertilizers and biofertilizers, which also encompasses crop rotation, introduction of legumes and efficient water management.

Conclusions

The application of 100 % mineral nitrogen to *C. purpureus* CT-169 resulted in the best performance in morpho-physiological indicators such as biomass production. In addition, the inclusion of *C. ensiformis* improved forage quality, due to the protein contribution of the legume.

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Cienfuegos for processing and analyzing the foliar and soil samples.

Conflict of interests

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

Authors' contributions

- Lázaro Jesús Ojeda-Quintana. Conceptualized, formulated and designed the research. Conducted the field research, evaluated and collected the data in the experiment tests, interpreted the results of the statistical analysis, and drafted the manuscript. Maintained a sequence of photographic images of all activities for the duration of the experiment.
- Ramón Antonio Rivera-Espinosa. Directed the project from which the research is derived. Participated in the design of the research, as well as in the interpretation of the achieved results. Critically reviewed the draft and recommended modifications, deletions and additions that enriched the manuscript.
- Enrique Casanovas-Cosío. Contributed to the conception of the experiment, performed the statistical analyses and proposed tables and figures for the paper. Intervened in the revision of the draft and recommended modifications.
- Juan José de la Rosa-Capote. Responsible for planning the field activities, as well as providing the materials and resources necessary for the execution of the research, as well as the conservation of the data and notes recorded during the course of the research. Suggested ideas during the whole process of execution of the experiment and in the preparation of the paper.
- Consuelo Hernández-Rodríguez. Participated in field activities and evaluations, as well as in the revision of the original manuscript.
- Yanoris Bernnal-Carrazana. Intervened in the field activities and evaluations, as well as in the revision of the original manuscript.

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