Scientific Paper

Effect of green manure from mycorrhized *Canavalia ensiformis* (L.) on the successor crop *Cenchrus purpureus* (Schumach.) Morrone Cuba CT-169

Lázaro Jesús Ojeda-Quintana[1] https://orcid.org/0000-0001-8629-5695, Ramón Rivera-Espinosa[2] http://orcid.org/0000-0001-6621-7446, Pedro José González-Cañizares[2] https://orcid.org/0000-0003-3206-0609, Juan José de la Rosa-Capote[1] https://orcid.org/0000-0001-6860-7296, Osvaldo Arteaga Rodríguez[3] https://orcid.org/0000-0001-8749-6026 y Consuelo Hernández-Rodríguez[3] https://orcid.org/0000-0002-8281-3376

[1]*Centro Universitario Municipal (CUM) Cumanayagua, Universidad de Cienfuegos Carlos R. Rodríguez, Calle Los Filtros No. 18, Cumanayagua, Cienfuegos, Cuba.* [2]*Instituto Nacional de Ciencias Agrícolas, San José de las Lajas, CP 32700, Mayabeque, Cuba.* [3]*Unidad Científico Tecnológica de Base (UCTB) Suelos Cienfuegos, Carretera a Manicaragua, km 13½, Barajagua, Cumanayagua, Cienfuegos, Cuba. *E-mail:* joberoverde@azurina.cult.cu

Abstract

Objective: To evaluate the effect of green manure from mycorrhized *Canavalia ensiformis* (L.) on the biomass yield and quality of the successor crop *Cenchrus purpureus* (Schumach.) Morrone Cuba CT-169.

Materials and Methods: A study was conducted on a soil of low natural fertility of the Base Soil Scientific Technological Unit, Barajagua, Cienfuegos, Cuba. A randomized block design was used, with three replicas and four treatments: inoculation of jack bean with the arbuscular mycorhizal fungi species *Funneliformis mosseae*, *Glomus cubense* and *Rhizoglomus irregulare* and a treatment without inoculation (control). After 69 days, jack bean was incorporated to the soil and 70 days later *C. purpureus* cv Cuba CT-169 was planted. Morphological indicators, biomass yield and crude protein content of C. purpureus were evaluated, in addition to mycorrhizal colonization of the roots and their visual density.

Results: There were no statistical differences in height, length of the fourth leaf, leaf area and stem diameter. Regarding the width of the fourth leaf, the best response was obtained with the green manure of *C. ensiformis*, mycorrhized with the strain *R. irrregulare*, without differing from the other inoculated variants, although it differed from the control ($p \leq 0,05$). The biomass production showed significant differences in the variants inoculated with *G. cubense* and *F. mosseae* with regards to the control. *R. irregulare* did not differ from the two above-mentioned strains or from the control. In the crude protein content no statistical differences were found among the inoculated treatments, but they differed from the control.

Conclusions: The results suggest a favorable combination of green manure from mycorrhized *C. ensiformis* and *C. purpureus* cv Cuba CT-169. The inoculation of jack bean with the mycorrhizal strains increased the biomass and crude protein yield of *C. purpureus* cv Cuba CT-169.

Keywords: inoculation, biomass, yield

Introduction

The demand of foodstuffs from animal origin could be satisfied, to a large extent, through the production systems of tropical countries, because they have the best conditions to increase significantly food production, from their capacity to generate biomass (Chará *et al.,* 2015).

Grazing grasses constitute the main feeding basis of cattle. In general, cattle husbandry faces limitations in the availability, quality and productivity of pasturelands; in addition, long dry or very rainy periods occur, which affect the forage offer to supply the nutritional requirements of the animals in terms of maintenance, growth and production, which makes it necessary to complement feeding in order to keep acceptable levels of beef and milk production (Bueno-Guzmán *et al*., 2015).

Green manures are an agronomic practice that consists in the incorporation of a non-decomposed plant mass of cultivated plants, with the purpose of improving soil nutrient availability and properties. Other benefits are associated with the increase of the activity and diversity of soil microorganisms (Martín-Alonso and Rivera-Espinosa, 2015).

In general, pasture species are mycotrophic. They establish in their roots a symbiosis with arbuscular mycorrhizal fungi (AMF), which is mutually beneficial. Mycorrhizae receive carbonated sources from the plant; while through the fungal structures the soil exploration capacity is increased, leading to increases in nutrient and water absorption, higher plant growth and development (Jung *et al.,* 2012), improvement in soil aggregates (Lehmann *et al.,* 2017) and higher agrosystem resilience (van der Heijen *et al.,* 2015).

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In Cuba, a progressive advancement has been achieved in the study about the use and management of AMF in a varied range of agricultural crops, which have contributed high yields through the inoculation of efficient strains (Rivera *et al.,* 2015). With them, between 30 and 50 % of mineral or organic fertilizers has been substituted, depending on the crop, soil and nutrient availability (Ruiz *et al.,* 2011).

AMF management is foreseen as an economically and ecologically viable alternative to decrease the costs of pasture fertilization and the risks of environmental contamination (González *et al.,* 2015).

This work was conducted in order to evaluate the effect of green manure from mycorrhized *Canavalia ensiformis* (L.) on the biomass yield and quality of the successor crop *Cenchrus purpureus* (Schumach.) Morrone Cuba CT-169.

Materials and Methods

Location of the study area. The study was conducted in the Base Soil Scientific Technological Unit, belonging to the Ministry of Agriculture, located at the coordinates 22º 09′ North latitude and 80º 12′ West longitude, at 60 m.a.s.l., in the Barajagua town, Cumanayagua municipality, Cienfuegos province, central-southern region of Cuba.

General soil characteristics and synoptic variables in the period. The soil is classified as grayish brown (Hernández-Jiménez *et al.*, 2015). After the incorporation period of *C. ensiformis* (jack bean), and before sowing *C. purpureus* Cuba CT-169 (Pennisetum), the values of some components of soil fertility were: pH (KCl) 5,16; organic matter 1,77 %; phosphorus and assimilable potassium 5,41 and 16,72 mg kg^{-1} of soil, respectively.

The performance of the synoptic variables (rainfall and temperature) during the period in which the work was developed is shown in figure 1.

Design and experimental treatment. For the research a randomized block design with three replicas, and the following treatments, were applied:

- T1-Green manure of *C. ensiformis* mycorrhized with the strain *Funneliformis mosseae*, incorporated to the soil before sowing *C purpureus* Cuba CT-169.
- T2-Green manure of *C. ensiformis* mycorrhized with the strain *Glomus cubense,* incorporated to the soil before sowing *C. purpureus* Cuba CT-169.
- T3-Green manure of *C. ensiformis* mycorrhized with the strain *Rhizoglomus irrregulare*, incorporated to the soil before sowing *C. purpureus* Cuba CT-169.
- T4-Green manure of *C. ensiformis* without mycorrhization, incorporated to the soil before sowing *C. purpureus* Cuba CT-169 (control).

Experimental procedure

The inoculated mycorrhiza species were: *Funneliformis mosseae*/INCAM-2 (Nicol. and Gerd. Walker and Schüßler (Schüßler and Walker, 2011), *Glomus cubense/* INCAM-4 Y. Rodr. and Dalpé (Rodríguez *et al.*, 2011) and *Rhizoglomus irrregulare/* INCAM-11 N.C. Schenck & G.S. Sm. Sieverd., G.A. Silva and Oehl (Sieverding *et al.*, 2014). All of them belong to the collection of the National Institute of Agricultural Sciences (INCA) of Cuba. The inoculants showed concentration of 30 spores/g, for each of the strains.

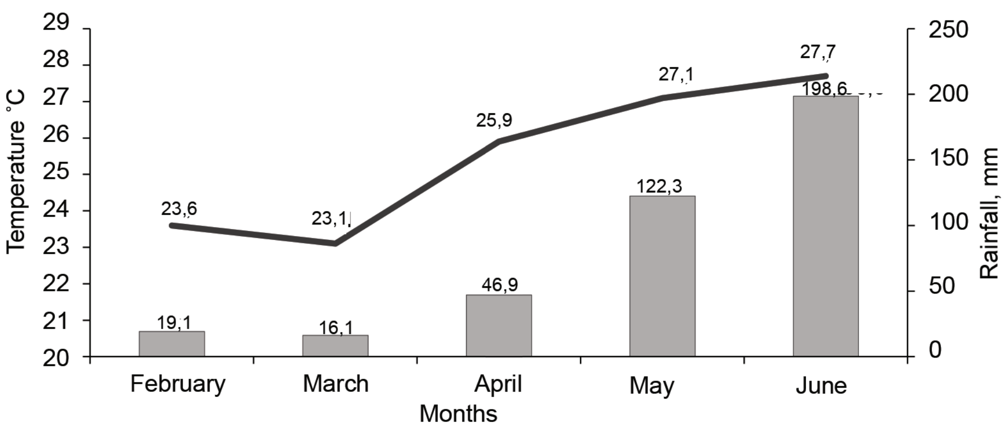


Figure 1. Rainfall (mm) and mean monthly temperature (°C) during the experimental period.

The inoculation with the AMF species was carried out by covering the seeds and 10 kg ha^{-1} of inoculant were applied (Martín *et al.,* 2012).

Two plots of 8,40 m^2 were prepared, with separation of 0,5 m between them. As evaluation unit the two central rows were taken, discarding the edges, for an area of 2,10 m^2/plot. The precedent jack bean was sown on September 28, 2016, with sowing frame of 0,25 m between plants and 0,70 m between rows, which originated 32 evaluable plants, incorporated to the soil 69 days later in all the treatments.

The plantation of *C. purpureus* Cuba CT-169 was carried out on February 16, 2017, in the same plots of the predecessor crop. The cuttings (25 cm long) were placed on the bottom of the row, with an overlapping of 0,10 m on the tips. Once the plant material was located, it was covered with the earth from between the rows. Until the plantation of *C. purpureus* Cuba CT-169, a period of 70 days passed.

After 133 days, the establishment cut of *C. purpureus* Cuba CT-169 was performed (June 29, 2017), moment at which height was measured with a graduated ruler from the soil to the growth point, as well as the length and width of the fourth leaf, completely opened from the apex down, according to the methodology proposed by Herrera (2006). Afterwards, the leaf area was calculated.

In each plot, the green mass was weighed, corresponding to the two central rows, and 200 g of plant material were taken to calculate total dry matter (TDM) and nitrogen percentage through humid digestion with H_2SO_4 + Se and colorimetrics with Nessler reagent (Paneque *et al.,* 2010). Both crops were not fertilized or irrigated. Neither pests nor diseases appeared during the phenological cycle.

The biomass yield and crude protein were calculated according to the following formulas:

$$\text{Biomass} = \frac{\text{Green mass (kg plot}^{-1}\text{) x Dry matter (g kg}^{-1}\text{)}}{\text{Calculation area (m}^2\text{) x 100}}\text{, t ha}^{-1}$$

Crude protein = Leaf nitrogen (%) x 6,25, %

In the establishment cut, the roots in each plot were sampled. For the collection a metallic cylinder of 5 cm diameter and 20 cm of height was used, which was introduced in the soil and the sample was taken.

The roots were clarified by the staining technique suggested by Rodríguez *et al.* (2015). The mycorrhizal colonization (percentage of MC) according to the methodology proposed by Giovannetti and Mosse (1980), and the visual density of colonization (% VD) were quantified. The latter expresses more clearly the intensity of mycorrhizal colonization in the root. The categories to value the percentage of visual density (VD) were the following: 0-absence of AMF; 1-1 %; 2-2,5 %; 3-15,5 %; 4-35,5 % and 5-47,5 % (Herrera-Peraza *et al.*, 2004).

For determining the differences among treatments, Duncan's multiple rage comparison test was used for $p \leq 0,05$. All the data were processed with the statistical package *Stadistical Package for Social Sciences* (SPSS) for Microsoft Windows®, version 15.0.

Results and Discussion

The performance of some morphological indicators of *C. purpureus* Cuba CT-169 in its establishment stage is shown in table 1. No statistical differences were appreciated in height, length of the fourth leaf, leaf area and stem diameter. Regarding the width of the fourth leaf, the best response was obtained with the green manure from *C. ensiformis*, mycorrhized with the strain *R. irrregulare*, without differing from the other inoculated variants, but it differed from the control ($p \leq 0,05$).

The variables height, length and width of the fourth leaf completely open from the apex, as well as the length of the fourth internode from the soil level and green matter yield, were key indicators to explain, with higher accuracy degree, the performance of the clone *C. purpureus* (Herrera *et al.,* 2013).

Biomass production showed significant differences in the variants inoculated with *G. cubense* and *F. mosseae* with regards to the control. Nevertheless, the green manure of *C. ensiformis,* mycorrhized with the strain *R. irrregulare*, did not differed from the two above-mentioned strains or from the control either. This performance indicates that the period of synchrony between incorporation of inoculated jack bean and planting of the successor crop caused increase of the biomass yield compared with the control, and the first two AMF strains.

The yield was obtained without irrigation and the rainfall by the establishment cut were below the historical mean of the locality (47,0; 60,2; 64,5; 176,0 and 243,4 mm in February, March, April, May and June, respectively), according to reports of the Delegación Provincial de Recursos Hidráulicos, Cienfuegos (2018). This could have influenced the growth and development of *C. purpureus* CT-169.

Table 1. Morphological indicators at the moment of the establishment cut of CT-169.

Treatment	Height, cm	Fourth leaf width, cm	Fourth leaf length, cm	Leaf area, cm^2	Stem diameter, cm
T1	2,3	3,0^{ab}	84,0	251,1	1,6
T2	2,1	3,1^{ab}	81,3	248,9	1,7
T3	2,1	3,6^{a}	80,6	288,3	1,6
T4 (control)	2,0	2,8^{c}	80,8	228,2	1,5
SE ±	0,319	0,315*	0,940	0,863	0,722

T1-Green manure from *C. ensiformis* mycorrhized with the strain *Funneliformis mosseae*, T2-Green manure from *C. ensiformis* mycorrhized with the strain *Glomus cubense*, T3-Green manure from *C. ensiformis* mycorrhized with the strain *Rhizoglomus irrregulare* and T4-Green manure from*C. ensiformis* without mycorrhization (Control)
a, b, c: Different letters in the same column indicate significant differences for $p \leq 0,05$, $*p < 0,05$

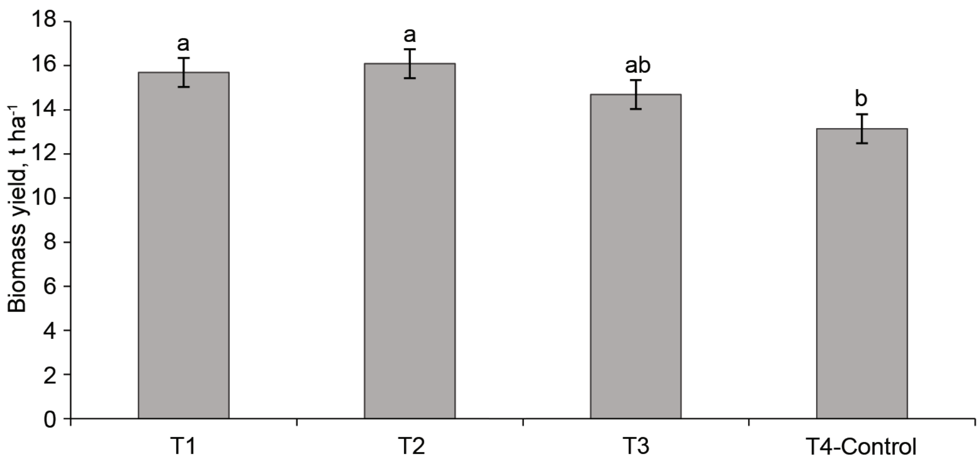
The use of mycorrhized jack bean as green manure (strains *F. mosseae* and *G. cubense*) was also evaluated by (García-Rubido *et al.*, 2017) on a lixiviated reddish yellow ferralitic soil with pH (KCl) of 5,5 and 1,38 % of organic matter.

There was increase of biomass yield of the successor crop in the variants in which jack bean was inoculated with the AMF. Although no statistical differences were obtained among the different strains (figure 2).

Rainfall, according to Nava *et al.* (2013), influence forage production and the fundamental problem of tropical animal husbandry is animal feeding in the dry season. In this study, in spite of the low rainfall, in all the treatments yield exceeded the mean for this cultivar, estimated in higher yields than 12 t ha^{-1}, without irrigation.

In all the treatments with inoculation very low values of mycorrhizal colonization and visual density were obtained, without statistical differences from T4-Green manure from *C. ensiformis* without mycorrhization (table 2).

Colonization with mycorrhizae was lower than 5 % (very low). However, the species *F. mosseae* and *G. cubense* increased the biomass yield with regards to the control (table 1). Martín *et al.* (2018) considered that the soil type, nutrient availability and pH, are closely related to the response in the jack bean crop to the inoculation with AMF. The mycorrhization of *C. purpureus* CT-169 (table 2) could have been related to the performance of propagules in the soil complex and the period of synchrony between incorporation of green manure and sowing of the successor crop.



T1-Green manure from *C. ensiformis* mycorrhized with the strain *Funneliformis mosseae*, T2-Green manure from *C. ensiformis* mycorrhized with the strain *Glomus cubense*, T3-Green manure from *C. ensiformis* mycorrhized with the strain *Rhizoglomus irrregulare* and T4-Green manure from *C. ensiformis* without mycorrhization (Control)
a, b: Different letters in the same column indicate significant differences for $p \leq 0,05$,* $p < 0,05$

Figure 2. Biomass yield of *C. purpureus* Cuba CT-169.

Table 2. Biological indicators of mycorrhization.

Treatment	Colonization, %	Visual density, %
T1	1	0,01
T2	1	0,01
T3	2	0,02
T4 (control)	5	0,05

T1-Green manure from *C. ensiformis* mycorrhized with the strain *Funneliformis mosseae*, T2-Green manure from *C. ensiformis* mycorrhized with the strain *Glomus cubense*, T3-Green manure from *C. ensiformis* mycorrhized with the strain *Rhizoglomus irrregulare* and T4-Green manure from *C. ensiformis* without mycorrhization (Control)

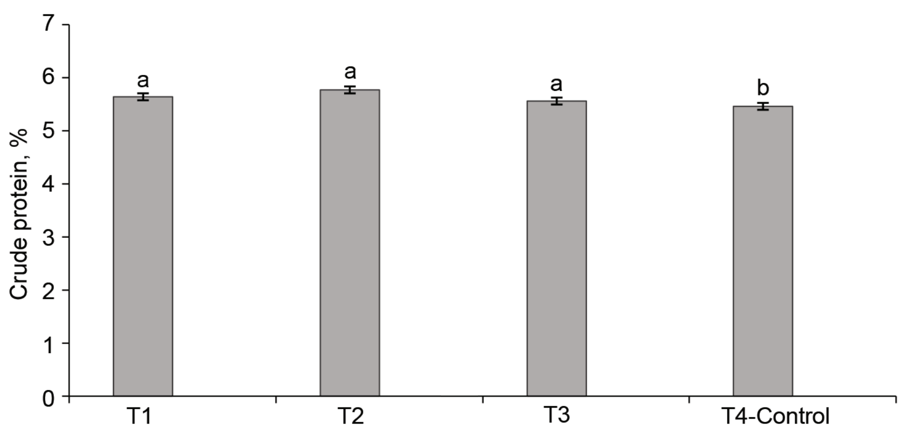
Ojeda-Quintana *et al.* (2018a) reported that the fungal colonization of rootlets in *C. ensiformis* before the incorporation to the soil, reached percentages of 59,39 % in *F. mosseae*, followed by *G. cubense (*55,35 %) and *Rhizoglomus intraradices (*50,94 %), which shows a favorable response to the inoculation with AMF under the soil conditions where the cited authors conducted the research.

In this study, the fungal colonization of the rootlets decreased in time, to very low percentages (table 2). This could have been due to the days passed between incorporation of jack bean to the soil and sowing of *C. purpureus* CT-169, period which perhaps did not favor the permanence of mycorrhizal propagules inoculated to the antecessor crop, and decreased the colonization of the successor crop.

In works conducted in Cuba, when using jack bean inoculated with efficient AMF strains, positive effect of permanence was obtained, with periods not longer than 30 days between jack bean cutting and incorporation and sowing of the successor crop, without having to inoculate the latter so that it would reach effective mycorrhization (Simó-González *et al.*, 2016; João *et al.*, 2017).

Oliveira *et al.* (2014) reported higher effectiveness of the introduced strains compared with the resident mycorrhizae. These authors indicated that the management of arbuscular mycorrhizal symbiosis via inoculation could be assumed as an effective agronomic practice to improve the productivity of forage species.

Figure 3 shows the crude protein content. There were no statistical differences among treatments 1, 2 and 3; while the control (T4) differed from the



T1-Green manure from *C. ensiformis* mycorrhized with the strain *Funneliformis mosseae*, T2-Green manure from *C. ensiformis* mycorrhized with the strain *Glomus cubense*, T3-Green manure from *C. ensiformis* mycorrhized with the strain *Rhizoglomus irrregulare* and T4-Green manure from *C. ensiformis* without mycorrhization (Control).

a, b: Different letters in the same column indicate significant differences for $p < 0,05$, * $p < 0,05$

Figure 3. Crude protein content of *C. purpureus* Cuba CT-169.

others. Pasture quality is extremely important for the evaluation of any forage species that is used in a technology aimed at animal production. In addition, it complements the agronomic and morphophysiological indicators, for which it offers answers to the influence of environmental and management factors (Fortes *et al.,* 2012).

The pasture species of the *Cenchrus* genus are promising for animal husbandry. Suárez (2016) stated that the content of crude protein of *C. purpureus* is, in general, between 3 and 20 % and even more, in younger plants. The values obtained in this study are included in this range.

Ojeda *et al.* (2018b), under similar soil conditions, inoculated the species *F. mosseae, G. cubense* and *R. irregulare*, directly at the moment of planting *C. purpureus* cv. Taiwán morado. These authors achieved a significant response to the inoculation with these mycorrhizal species. The crude protein percentages reported in the establishment cut were 5,25; 4,31 and 5,87 % respectively; while in the control treatment they were 5,12 %.

The inclusion of green manures inoculated with AMF in fertilization systems for pastures and forage crops is a way to improve yield and nutritional value. Zhang *et al.* (2016) observed that the benefits of these microorganisms in pasture agroecosystems are closely related to the increase of the root absorption surface and, consequently, to the improvement in the efficiency of nutrient utilization by the plants.

The inoculation of jack bean with the mycorrhizal strains increased biomass yield and crude protein of *C. purpureus* cv Cuba CT-169.

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Authors' contribution

- Lázaro J. Ojeda-Quintana. Conceptualized, formulated and designed the research. Also led the field research, evaluated and compiled the data in the experimental tests, interpreted the results of the statistical analysis and wrote the manuscript draft. Rectified the issues indicated by the referees.
- Ramón Rivera-Espinosa. Collaborated in the interpretation of the results of the statistical analysis. Did the critical revision of the draft and recommended modifications, supressions and additions in the manuscript.
- Pedro J. González-Cañizares. Did the critical revision of the draft an recommended modifications, supressions and additions in the document.
- Juan J de la Rosa-Capote. Responsible for providing the necessary materials and resources for conducting the research, besides the conservation of the data and notes taken throughout the research.
- Osvaldo Arteaga Rodríguez. Responsible for the acquisition of necessary funds for conducting the project that led to this publication.
- Consuelo Hernández-Rodríguez. Responsible for the management, coordination, planning and execution of the research activities

Conflicts of interests

The authors declare that there are no conflicts of interests.

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