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la respuesta agroproductiva de Morus alba

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SCIENTIFIC PAPER

Effect of the spatial arrangement and the intercropping with mycorrhiza-inoculated Canavalia ensiformis on the agroproductive response of Morus alba

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ABSTRACT: A study was conducted at the Research Station Indio Hatuey in order to determine the effect of the spatial arrangement of the plantation and the intercropping of AMF-inoculated jack bean (CeAMF), without irrigation and without mineral fertilization, on the agroproductive response of mulberry. The treatments consisted in single row (0,50 x 1,00 m), with 20 000 plants ha⁻¹ with and without CeAMF; and double row (0,50 x 0,50 x 1,00 m), with 26 666 plants ha⁻¹, with and without CeAMF. The edible dry mass yield and the concentration of N, P and K were evaluated for two years. The concentration of N in the edible biomass did not vary, and its values (2,09 and 2,4 %) were below the optimum range for the mulberry crop. Significant effects were found in the concentration of P, of the CeAMF treatment as well as of the highest planting density and the double row, and the values were 0,16 and 0,19 %. The highest planting density and the double row influenced the increase of the concentration of K, unlike CeAMF. The highest values (2,22 and 2,37 %) were ranked, just like in P, within the optimum range reported for the species. The edible biomass yield did not differ among treatments and varied from 8,38 to 10,51 t ha⁻¹. It is concluded that the spatial arrangement with higher planting density, double row and CeAMF guaranteed a better agroproductive response of mulberry in terms of P and K concentration in the edible biomass; although, from the point of view of forage yield, no treatment proved to be feasible under the conditions of insufficient nutrient availability in the soil and without irrigation.

Keywords: forages, phosphorus, potassium

INTRODUCTION

The studies about spatial arrangements of *Morus alba* L. plantations suggest a large range of choices, determined by the soil and climate conditions and by the purpose of the forage production.

In this sense, planting densities in single rows of 20 000 and 25 000 plants ha⁻¹ (Ting Zing *et al.*, 1998; Martín *et al.*, 2002) and up to 27 777 plants ha⁻¹ (Boschini *et al.*, 1999) have been recommended. Xuanc and Duc (2003) and Rojas *et al.* (2005) studied higher densities (between 40 000 and 100 000 plants ha⁻¹). Under Cuban conditions, Martín *et al.* (2014) proved the effectiveness of the spatial arrangement of the plantation with 50 000 plants ha⁻¹ and a planting frame of 1 m x 0,20 m.

Several works from Latin America, China and India recommend spatial arrangements in double row with densities between 7 751 plants ha⁻¹ (Lea and Lee, 2001) and 26 666 plants ha⁻¹ (Uribe, 2002); in all these cases the agronomic management was made with irrigation or with a high rainfall regime.

The studies in mulberry plantations intercropped with temporary crops, used as green manure or vehicle to incorporate arbuscular mycorrhizal fungi (AMF) are insufficient; although there are some experiences of intercropping herbaceous legumes, with planting frames in double rows, in which the forage yield increased up to 16 % compared with the one reached when single rows were used (Shankar *et al.*, 2000; Hadimani *et al.*, 2004).

With regards to AMF, there are many results that approach the benefits of mycorrhizal inoculants in different crops and soil types, because an effective symbiosis is established, which increases the nutrient uptake, guarantees high yields and decreases the fertilizer requirements (González, 2014). In that sense, mulberry, as mycotrophic crop (Ram Rao *et al.*, 2007), does not constitute an exception; although the works that study the importance of inoculation in this crop are scarce.

The purpose of this study consisted in determining the agroproductive response of mulberry to the

changes of spatial arrangement of the plantation and the intercropping of AMF-inoculated jack bean (*Canavalia ensiformis*), cultivated with neither irrigation nor application of fertilizers.

MATERIALS AND METHODS

Geographical location. The study was conducted in areas of the Research Station Indio Hatuey, located between 22° 48' and 7" North latitude, and 81° and 2' West longitude, at 19,9 m.as.l., in the Perico municipality, Matanzas province, Cuba.

Edaphoclimatic characteristics. The soil has flat topography, with slope from 0,5 to 1,0 %, classified by Hernández *et al.* (2015) as lixiviated Ferralitic Red, hydrated ferruginous nodular humic, of fast desiccation, clayey on limestone. The average depth is 1,50 m.

According to the analysis of chemical fertility at the beginning of each experiment (table 1) and its characterization according to the charts of agrochemical interpretations (Cancio, 1982; Paneque and Calaña, 2001), the soil shows a slightly acid pH; the values of K and exchangeable Na are low, which is typical of ferralitic soils; and the exchangeable Mg content is high. The Ca values are considered moderate, compared with those of other Cuban soils settled on sedimentary rock; however, these contents can be considered sufficient for the crops.

The organic matter content is ranked between low and moderate, with regards to most Cuban soils; but it is relatively high for the Ferralitic grouping and indicates that this soil is little degraded.

In the experimental stage an annual mean rainfall of 1 500 mm occurred (table 2), with 80, 3 % of the rain in the rainy season. The mean of the four years was close to the mean of the last 20 years, which was 1 120,25 mm in the rainy season and 273,05 mm in the dry season (Estación Meteorológica Indio

Hatuey, CITMA). Nevertheless, important variations of the annual rainfall occurred: a year with high rainfall (2008), characterized by an active hurricane season; and a year with low rainfall (2007), with affectations in both seasons. In years 2007 and 2008 it rained more than in later years.

The mean temperature of the air per year and per season was more homogeneous, and varied as average of the four years between 25,91 °C in the rainy season and 21,23 °C in the dry season.

In general, the rainfall regime varied more from year to year and between seasons than the mean temperature of the air.

Design and treatments. A completely randomized design and four replications were used, and the treatments consisted in:

1. Spatial arrangement in single row (0,50 x 1,00 m), with 20 000 plants ha⁻¹, without CeAMF
2. Spatial arrangement in single row (0,50 x 1,00 m), with 20 000 plants ha⁻¹, with CeAMF
3. Spatial arrangement in double row (0,50 x 0,50 x 1 m), with 26 666 plants ha⁻¹, without CeAMF
4. Spatial arrangement in double row (0,50 x 0,50 x 1 m), with 26 666 plants ha⁻¹, with CeAMF

Experimental procedure. Two plantations were used. Plantation 1 had the plots with planting frame of 0,50 x 1,00 m, with 20 000 plants ha⁻¹, without CeAMF (1) and with CeAMF (2); these plots had 24 m² and 48 plants, from which the central twelve were considered for the calculation area.

Plantation 2 included the plots with planting frame of 0,50 x 0,50 m x 1 m, with 26 666 plants ha⁻¹, without CeAMF (3) and with CeAMF (4). These plots had 13,5 m² and 36 plants, and from them the central twelve were taken into consideration for the calculation area.

Both plantations were previously established with the variety tigreada; six months before starting

Table 1. Some indicators of the chemical characteristics of the soil at the beginning.

Plantation	Na	K	Ca	Mg	P	OM (%)	pH
	cmol.kg ⁻¹				mg.kg ⁻¹		(H ₂ O)
1							
x	0,07	0,15	11,26	3,94	21,75	2,94	6,51
± Z ₁ SE _x	0,01	0,02	0,80	0,24	2,49	0,27	0,13
2							
x	0,14	0,09	11,10	3,90	7,90	4,17	6,14
± Z ₁ SE _x	0,02	0,01	0,20	0,57	5,04	1,13	0,20

± Z₁ SE_x: confidence limit for α= 0,05

Table 2. Performance of the climate variables rainfall and mean temperature of the air during the research period.

Season	2007		2008		2009		2010	
	Ra.	Tm.	Ra.	Tm.	Ra.	Tm.	Ra.	Tm.
Dry	336,60	21,72	367,20	21,52	156,30	21,17	322,70	20,52
Rainy	1 264,10	25,54	1492,70	25,72	932,80	26,07	1 129,20	26,32
Total/average	1 600,70	23,63	1 859,90	23,62	1 089,10	23,62	1 451,90	23,42

Data obtained from the Meteorological Station Indio Hatuey (CITMA, Matanzas, Cuba).

Ra: rainfall, Tm: mean temperature.

the evaluations a homogenization cut was performed and a period of stabilization of the treatments was established, which constitutes a conventional practice in the experiments about mulberry biomass production (Rojas *et al.*, 2005). The rows were oriented from East to West.

The jack bean was manually planted 15 days after the beginning of mulberry growth in each season (November 15 and May 15). A planting frame of 0,4 x 1,0 m was used with a density of 25 000 plants ha⁻¹, intercropped between the mulberry rows.

Jack bean was inoculated with *Glomus cubense*, efficient AMF strain for this soil type (Martín *et al.*, 2010; González, 2014); the seeds were covered through the method established by Rivera *et al.* (2006), with 0,37 g of inoculant per seed, equivalent to 18,5 kg ha⁻¹ in each season. Two seeds were planted and thinning was applied, leaving one plant.

The mycorrhizal inoculant consisted in spores and propagules, and was prepared through the technology of EcoMic® (Fernández *et al.*, 2000) in the department of Biofertilizers and Plant Nutrition of the National Institute of Agricultural Sciences, with a titer as minimum of 20 spores g⁻¹ of inoculant.

Sixty days after 75 % of the jack bean plants emerged, they were cut close to the soil, fragmented in equal parts and mulched around the mulberry plants in a proportion of 1,25 jack bean plants per each mulberry plant.

The cutting interval of mulberry was 90 days and the height, 0,30 m. The cuttings were framed in the rainy season between August and November, and in the dry season, between February and May. The cutting dates coincided with the first day of each month since August.

The manual weeding was maintained throughout the experimental period; and the criterion of not applying irrigation was assumed, in correspondence with the reality of most farming exploitations.

The determinations were the following:

- Dry matter yield of the edible biomass (t.ha⁻¹ per year). It was determined from the sum

of the dry matter yield of the leaves and fresh stems.

- Concentration of N, P and K in the edible biomass. In the last cutting of each season the concentrations of N, P and K were determined, as percentage of the dry mass of the leaves and fresh stems, according to the analytical methods described by Paneque *et al.* (2010), which include the humid digestion with H₂SO₄ + Se and the color development.

Statistical analysis. The data distribution normality in all the variables was verified through the modified Shapiro-Wilk test; and the variance homogeneity, by Levene's test. The comparison was made through the analysis of confidence intervals ($\pm Z_1$ Esx), for a significance of 0,05. As statistical program Infostat 2008 was used (Di Rienzo *et al.*, 2008).

RESULTS AND DISCUSSION

The nitrogen concentration did not vary in any of the treatments, and the values were below the optimum range for the mulberry crop (3,0 – 4,0 %) acknowledged by Sannappa *et al.* (2000) and González and Cáceres (2011).

The intercropping of AMF-inoculated jack bean (CeAMF) did not favor the increase of the N concentration in the edible biomass (fig. 1). This differs from the results obtained by Hadimani *et al.* (2004), who observed an increase of N in the leaves of mulberry intercropped with soybean, with irrigation. It is inferred that the low N concentration resulted from the dilution of this element in the plant and it should have been related to an insufficient N availability in the soil, because no chemical fertilizer was applied and the crop was developed without irrigation.

Regarding the P concentration (fig. 2), a significant effect was found of the intercropping of AMF-inoculated jack bean as well as of the spatial arrangement. In the plots with intercropped and inoculated jack bean there were higher P concentrations, just like in the plots with double row.

The association of mulberry with AMF-inoculated jack bean should have facilitated the

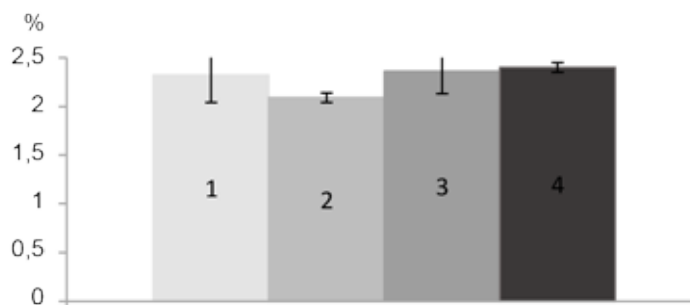


Figure 1. Concentration of N in the edible biomass of mulberry.

1. 0,50 x 1,00 m without CeAMF; 2. 0,50 x 1,00 m with CeAMF; 3. 0,50 x 0,50 x 1,00 m without CeAMF; 4. 0,50 x 0,50 x 1,00 m with CeAMF.

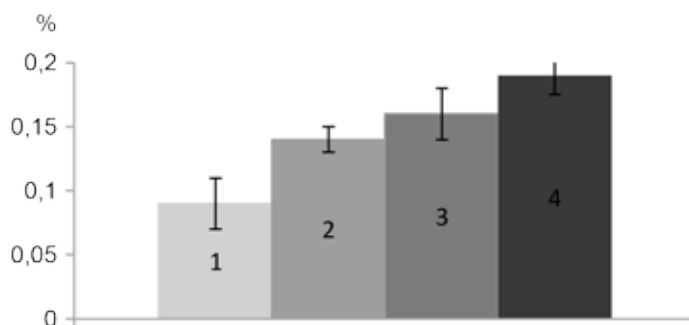


Figure 2. Concentration of P in the edible biomass of mulberry. 1. 0,50 x 1,00 m without CeAMF; 2. 0,50 x 1,00 m with CeAMF; 3. 0,50 x 0,50 x 1,00 m without CeAMF; 4. 0,50 x 0,50 x 1,00 m with CeAMF.

absorption of phosphorus and guaranteed higher concentrations in the leaves and fresh stems; this coincides with the report by Baqual *et al.* (2005). It is known that the extraradical hyphae of AMF are extended several centimeters in the soil and help plants to absorb the mineral nutrients that are found in less available forms (Marschner and Dell, 1994); in addition, they are more efficient than the roots to absorb nutrients, because due to their extremely long and fine structure they can explore soil volumes that cannot be reached by the root structures of plants (Read and Pérez-Moreno, 2003).

The absorption and transference of P to the plant through the AMF hyphae is a fast and efficient process, because of the presence of high-affinity H_2PO_4 transporters (Requena *et al.*, 2003; Yao *et al.*, 2008). The absorbed H_2PO_4 is rapidly transformed into polyphosphate in the extraradical mycelium (Ezawa *et al.*, 2004), and the presence of several H_2PO_4 transporters has been reported in the cells colonized

by arbuscules which are responsible for the entrance of P in the host cell (Harrison *et al.*, 2002).

The jack bean intercropped, inoculated and mulched as green manure on the mulberry row should have improved the physical condition of the soil, by acting on the compaction, water holding, aeration and other physical characteristics, which, along with the narrower planting frame (in double row), favored the growth and development of the root system of the plants. All this has implications in the absorption of nutrients, because to satisfy the plant demand, the minerals must reach the root surface, mainly through diffusion or transport with the soil solution, and the root growth decreases the length of the trajectories of the minerals.

The highest values of P in the edible biomass were ranked in the range recommended for the species by Martín (2004).

The spatial arrangement remarkably influenced the increase of the concentration of K (fig. 3), unlike the intercropping of inoculated jack bean. This showed the

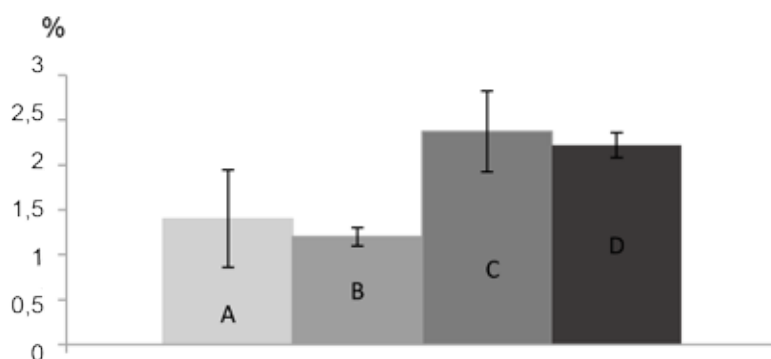


Figure 3. Concentration of K in the edible biomass of mulberry. 1. 0,50 x 1,00 m without CeAMF; 2. 0,50 x 1,00 m with CeAMF; 3. 0,50 x 0,50 x 1,00 m without CeAMF; 4. 0,50 x 0,50 x 1,00 m with CeAMF.

insufficient availability of this element in the soil, which limited its efficiency of extraction by AMF; but it was compensated with the increase of density and the planting frame in double rows, which should have been translated into a higher production and distribution of the roots.

The higher concentrations of K in the mulberry leaves, between 25 g kg⁻¹ and 30 g kg⁻¹, according to Martín (2004) and Chen *et al.* (2009), are related to fertilization and to the soils that have a high content of available potassium (Shankar and Rangaswamy, 1999).

In this regard, Chen *et al.* (2009) proved that the fertilization with K significantly increased the concentration of K in the leaves. Shankar and Rangaswamy (1999), with different combinations of N and K from 300 and 400 kg of N ha⁻¹; 120, 160 and 200 kg of K₂O ha⁻¹ and with a basal dose of P (120 kg ha⁻¹ of P₂O₅), obtained an increase of the concentration

of K in the mulberry biomass up to 23,9 g kg⁻¹ in the treatment with the highest dose of N and K₂O.

K, just like P, moves in the soil through diffusion as the main mechanism. The driving force of diffusion is a gradient of concentration between the adjacent soil and the root surface. With time depletion profiles are developed, and the availability of the exchangeable or barely exchangeable elements mainly depends on the balance between the absorption by the roots, the replenishment through fertilization or release of the elements in the soil (Paulo and Torres de Toledo, 2006); which proves the need to complement the intercropping of AMF-inoculated jack bean with fertilization.

The edible biomass yield did not differ among the treatments (fig. 4), and proved the need to apply fertilizer under similar conditions to the ones in this study.

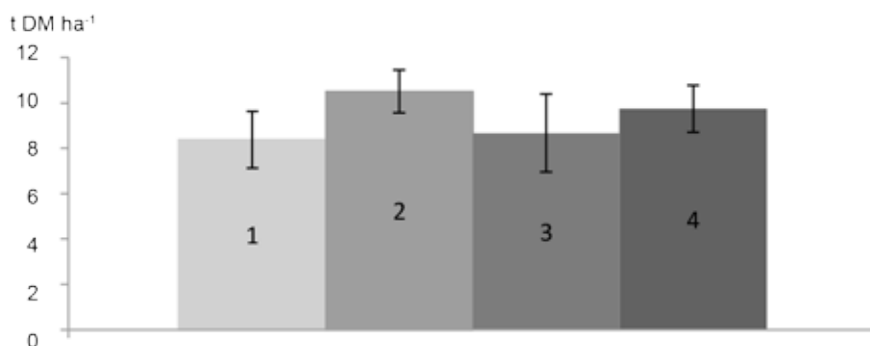


Figure 4. Edible biomass yield of mulberry. 1. 0,50 x 1,00 m without CeAMF; 2. 0,50 x 1,00 m with CeAMF; 3. 0,50 x 0,50 x 1,00 m without CeAMF; 4. 0,50 x 0,50 x 1,00 m with CeAMF.

It is known that mulberry allows the use of planting densities and frames which include spatial arrangements from 5 000 to 100 000 plants ha⁻¹. Some authors refer that with higher planting densities more edible biomass is produced per surface unit (Cifuentes and Kee Wook, 1998), reason for which in China the traditional planting density is 10 000 plants ha⁻¹, but for the crop with an intensive cutting management it is 25 000 plants ha⁻¹ (Ting Zing *et al.*, 1998).

CONCLUSIONS

The spatial arrangement of the mulberry plantation with higher planting density and frame in double row, and the intercropping of AMF-inoculated jack bean, guarantee a better agroproductive response of mulberry in terms of P and K concentration in the edible biomass. However, from the point of view of forage yield, these alternatives of agronomic management under conditions of insufficient nutrient availability in the soil and without irrigation did not prove to be feasible.

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