Scientific Paper

Management of the nutrition and defoliation of Morus alba *(L.) var. tigreada for leaf production*

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ABSTRACT: In an established plantation of mulberry [*Morus alba* (L.) var. tigreada], without irrigation and subject to cutting intervals, the effect of intercropping jack bean [*Canavalia ensiformis* (L.)] inoculated with arbuscular mycorrhizal fungi (AMF), complemented with mineral fertilizers, in both seasons was determined. A randomized block design with factorial arrangement and four replications was used. The treatments were: cutting intervals of 30, 60 and 90 days; and nutrition management: without mineral fertilizers or intercropped AMF-inoculated jack bean; mineral fertilization with 150 and 75 kg ha^{-1} of N and K_2O per season, respectively; and intercropped jack bean inoculated with *Glomus cubense*. The season influenced the production, which was three times higher in the rainy season than in the dry season. The leaf N concentration in mulberry was higher with the mineral fertilization and decreased with the cutting interval; the values were below the optimum one reported for the species. It is concluded that the 90-day cutting interval showed the best results and in this treatment it was feasible to intercrop jack bean inoculated with AMF in the rainy season.

Keywords: vesicular arbuscular mycorrhizae, yield, survival

INTRODUCTION

Mulberry [*Morus alba* (L.)] is a promising forage species which grows well on different soil types, mainly on the high-fertility ones. It stands out due to its high yield of leaves, which are used for feeding the silkworm (*Bombyx mori*) and other monogastric species (Martín, 2004; Prieto, 2015).

To obtain a high yield in leaves, which have high bromatological and biochemical quality, high amounts of fertilizers are generally used, implying high production costs. In order to reduce such inputs, it is important to evaluate agroecological practices of nutrition management, such as green manures intercropped between the mulberry plants and the biofertilizers based on mycorrhizal inoculants –AMF– (Martín, 2009).

The cutting interval also constitutes a determining factor in the leaf production and is influenced in turn by fertilization and season (Martín *et al*., 2013). The results that appear in literature about this aspect have been generated in monocrop plantations, where cutting intervals of 45, 60, 75, 90 and 120 days were studied (Martín, 2004; García *et al*., 2011).

In the established mulberry plantations, the associated mycorrhiza-inoculated crop should be intercropped for its use as green manure. This is satisfactorily integrated with the other cultural practices.

There is some information about plantations of mulberry intercropped with cash crops (Srinivas, 2005), but no results were found of its association with green manures, or of their potential as a way to inoculate the mulberry plantations with AMF. For such purpose, a study was conducted in an established plantation of mulberry (*M. alba* var. tigreada), without irrigation and subject to cutting intervals, in order to determine the effect of intercropped AMF-inoculated jack bean [*Canavalia ensiformis* (L.)], complemented with mineral fertilizers.

MATERIALS AND METHODS

Geographic location. The trial was conducted in areas of the Research Station Indio Hatuey, located between 22º 48' and 7" North latitude and 81º and 2" West longitude, at 19,01 masl; in the Perico municipality, Matanzas province, Cuba.

Edaphoclimatic characteristics. The soil corresponds to the genetic type lixiviated Ferralitic Red, according to the criteria expressed by Hernández *et al*. (2015); and to the Nitisol Ferralitic Rhodic, Lixic, Euthric, according to FAO (2014). The topography is flat, with slope of 0,5 to 1,0 %, and the depth to the limestone is 1,50 m.

The pH and the concentrations of Ca^{2+} and exchangeable Mg^{2+} (table 1) were typical of these soils, which also showed low values of exchangeable K^{+}. The concentrations of available P and OM can be considered moderate for the group of Ferralitic soils (Hernández *et al*., 2014).

The evaluative period was two years and it was characterized by an annual mean of 1 487 mm of rainfall, from them 19 % occurred in the dry season –which is framed between November 15 and April 15–. The mean annual temperature was 23,6 ºC and varied between 25,90 ºC and 21,20 ºC in the rainy and dry seasons, respectively. These values were similar to those of the last 20 years, which correspond to 1 393,3 mm of mean annual rainfall; 1 120,25 mm and 25,95 ºC in the rainy season, and 273,05 mm and 21,65 ºC in the dry season (Meteorological Station Indio Hatuey, CITMA, Matanzas).

Description of the experiment

The study was conducted per season. The planting frame of mulberry was 0,50 x 1,00 m, with a density of 20 000 plants ha^{-1}. The experimental plots measured 24 m^2 and had 48 plants; for the calculation area 12 plants, homogeneous in age, located towards the center of the plots, were used. The experimental design was randomized blocks with factorial arrangement and four replications. The following factors were studied:

- Cutting interval (A). The cutting intervals of 30, 60 and 90 days were evaluated.
- Nutrition management (B): a) without mineral fertilizers or intercropped AMF-inoculated jack bean (without MF and CeAMF); b) mineral fertilization (MF) 150 and 75 kg ha^{-1} of N and K_2O per season, respectively; c) intercropped jack bean inoculated with *Glomus cubense* at the moment of being planted in each season.

The cutting intervals in each season started and ended in unison. The cutting dates for each interval were: 30 (every first day of each month of the year), 60 days (the first day of the month every two months since July) and 90 days (the first day of the month every three months since August).

The fertilizers were manually applied, on the soil surface and on the stem basis; the carriers used were urea (46 %) and potassium chloride (60 %). P was not applied, because the low requirement of the mulberry crop and the initial concentration of P in the soil (equivalent to 99,62 kg ha^{-1}) were taken into conideration.

The jack bean was manually planted, in each season. The planting density was 25 000 plants ha^{-1} (with a frame of 0,4 x 1,0 m), at a distance of 0,50 m with regards to the mulberry rows (60 plants in each plot).

The jack bean was inoculated with a *G. cubense* strain. The seeds were covered, through the method established by Rivera *et al.* (2006), with 0,15 g of inoculant per seed in each season. The mycorrhizal inoculant consisted in spores and other propagules, and it was prepared by the technology of EcoMic® in the department of biofertilizers and plant nutrition of the National Institute of Agricultural Sciences (INCA for its initials in Spanish) –Mayabeque, Cuba–, with a titer of 25 spores per each gram of inoculant as minimum. After cutting the jack bean as green manure, the aerial biomass was fractioned into equal parts and placed as mulch around the mulberry, in a proportion of 1,25 plants of jack bean per each mulberry plant.

Measurements

Dry matter yield (t ha^{-1}) of the leaves in each season. It was determined through the addition of the dry matter yield in each cutting and expressed as cumulative dry matter in each season (seasonal yield).

N concentration in the leaves of mulberry (g kg^{-1} DM). In each season the N concentration was determined as percentage of the dry matter of

Table 1. Chemical indicators of the soil, at the beginning of the experiment

	pH H_2O	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}
		(%)	(mg kg^{-1})		(cmol$^{(+)}$ kg^{-1})		
	6,51	2,94	21,75	0,15	0,07	11,26	3,94
$\pm Z_{1.}$ SEx	0,13	0,27	2,49	0,02	0,01	0,80	0,24

$\pm Z_{1.}$ SEx: confidence limit for α = 0,05 the leaves, from the humid digestion with H_2SO_4 + Se, by the colorimetric determination method (Paneque *et al*., 2010).

Survival of the mulberry plantation (%). At the end of the experiment the number of live plants in the calculation area of each plot was counted, and expressed as percentage with regards to the initial quantity.

Statistical analysis. Variance analysis and mean comparison through Duncan's (1955) multiple comparison test were carried out. Paired-sample analysis (Steel and Torrie, 1992) was also made and later, t-test. The statistical package used was Infostat 2008 (Di Rienzo *et al*., 2008).

RESULTS AND DISCUSSION

The biomass yield, its chemical composition and the survival of the plantations constitute an expression of the productivity and stability of mulberry management systems as forage tree (Martín *et al*., 2007); hence the importance of its integral analysis.

Table 2 shows the effect of the cutting interval and nutrition management on the leaf biomass yield; interaction was found between both factors in all the seasons and years of evaluation. The best treatments in any of the seasons were associated with the 90-day cutting interval, and the highest values were obtained in the rainy season with intercropped AMF-inoculated jack bean, and in the dry season, with the mineral fertilization treatment.

The treatments which did not receive MF or intercropped AMF-inoculated jack bean showed the lowest yields.

Independently from the fact that the best yields were different between seasons, this factor influenced the leaf yield, so that the highest value was always reached in the rainy season.

The best results coincided with the ones reported by Martín *et al.* (2000), who found that the leaf yield of mulberry in monocrop was higher as the cutting interval increased from 45 to 90 days.

From the response of the crop to the 60-day cutting interval, it is inferred that the intercropped jack bean must have limited the entrance of light to the mulberry regrowths, as a consequence of its exuberant growth form and the broad surface of its leaves, with negative effects for the mulberry growth, which is a heliophilous species. Such phenomenon did not occur with the 90-day cutting interval, because the branches always remained over the height of the jack bean, because the latter was planted aftert 15 days of mulberry regrowth in May and November, and the cutting and mulching of the green manure was performed 15 days before the first cutting of mulberry in August and February.

About this inter-species behavior, Willey *et al*. (cited by Pentón, 2015) stated that the increase of the yield of mulberry associated with legumes depends on the species differing in their needs of resources for growth. The effect of competition is minimized when the cutting moment of mulberry and the planting and harvest of the intercropped species are adjusted in benefit of the main crop, which was satisfactory in this study in the 30- and 90-day intervals, and supports the statement in this regard by Srinivas (2005).

Table 2. Effect of the cutting interval and nutrition management on the seasonal yield (t ha^{-1}) of dry biomass.

Nutrition management (B)	Cutting interval (A), days											
	Rainy season 2007			Dry season 2008			Rainy season 2008			Dry season 2009		
	30	60	90	30	60	90	30	60	90	30	60	90
Without MF and CeAMF	$1{,}07^{e}$	$1{,}99^{cd}$	$4{,}99^{b}$	$0{,}49^{e}$	$1{,}00^{cd}$	$1{,}52^{b}$	$1{,}06^{f}$	$1{,}38^{ef}$	$3{,}92^{c}$	$0{,}15^{e}$	$0{,}48^{d}$	$1{,}04^{c}$
Mineral fertilization	$1{,}42^{de}$	$5{,}72^{b}$	$5{,}89^{b}$	$0{,}73^{de}$	$1{,}72^{b}$	$2{,}59^{a}$	$0{,}99^{f}$	$4{,}40^{b}$	$4{,}47^{b}$	$0{,}27^{e}$	$1{,}46^{b}$	$1{,}77^{a}$
CeAMF	$2{,}35^{c}$	$2{,}27^{cd}$	$7{,}91^{a}$	$0{,}91^{cd}$	$1{,}09^{c}$	$1{,}60^{b}$	$1{,}58^{e}$	$2{,}08^{d}$	$5{,}08^{a}$	$0{,}49^{d}$	$0{,}96^{c}$	$1{,}37^{b}$
SE (A x B) ±		0,29***			0,08***			0,16***			0,05***	
Season mean		3,74			1,29			2,77			0,89	
				7,55***						9,96***		

Different letters indicate significant differences $p \leq 0{,}05$ according to Duncan (1955). SE (A x B) ± indicates the standard error of the interaction between the factors: A (factor cutting interval), B (factor nutrition management). ***$p < 0{,}001$.

These results also coincide with the report by Delgado and Rodríguez (2012) in mulberry associated with alfalfa, in which nitrogen fertilization was applied; and also with the successful experiences of intercropping temporary cash crops in mulberry plantations, under different conditions of soil and spatial arrangements of the plantation, with cutting intervals of mulberry higher than 60 and up to 90 days (Srinivas, 2005).

The mulberry leaf yield in the 30-day cutting interval (table 2) decreased, compared with that of the most productive treatments: mineral fertilization with 60-day interval and intercropped AMF-inoculated jack bean with the 90-day interval; and this was related to a low vegetative growth rate during the first regrowth weeks. Such phenomenon is due, mainly, to the fact that non-structural carbohydrates which are retained in the basis of the stem and in the roots are used for regrowth, and only after the plant can produce new leaves capable of performing the photosynthesis the recovery and biomass production stage is started (Stür *et al.*, cited by Pentón, 2015).

The results in favor of the treatment of intercropped AMF-inoculated jack bean in the 90-day cutting interval indicate the scope and magnitude of the intercropped crop placed as mulch on the mulberry row as green manure, in the rainy season.

The beneficial effects of intercropped jack bean can be related to the high concentration of N in its biomass, its high productivity and the effective symbiosis that is established with the rhizobium, which guarantees a nutrient contribution and recycling in the soil which is translated into a higher sufficiency of nutrients for the associated cash crops (Martín, 2009).

Regarding the AMFs, Reddy *et al.* (2002) and Setua *et al.* (2005) reported their influence on the physiological and productive response of the inoculated mulberry plants.

The seasonality of the crop in terms of leaf yield reaffirms its characteristics of deciduous species, with variations of its phenological status between seasons. According to Pentón and Martín (2006), in the rainy season the plant experiences vigorous growth during the months from May to November, with abundance of leaves, of intense green color and succulent appearance; while in the dry season the vegetative growth rate is reduced, which is translated into a decrease of leaf yield, and is related to the lower rainfall accumulations and the slight decrease of the air mean temperature (Pentón, 2015).

The concentration of N in the leaves did not show interaction between the studied factors (table 3). In the first year significant effects were found, of the factor cutting interval as well as the factor nutrition management in each season. The N concentration in the 90-day interval was lower compared with those of the 30- and 90-day intervals. Such behavior is explained by a dilution phenomenon of the chemical element as the yield increases with plant age, and is in correspondence with the report by Martín (2004) and García *et al.* (2011) regarding the fact that during the first weeks after cutting, the plants emit leaves and branches with a high N concentration, but the dry matter content is low, due to the restricted quantity of carbohydrates that remain as reserve in the stem basis and in the roots after cutting and which are used in the emission of regrowths. In addition, in this period the root renewal and growth are slow, and this limits the access to water and nutrients such as K^+, which is a cation that participates in the active formation of many photosynthesis and respiration process catalyzer enzymes and in the transport of soluble sugars; the low K concentration in the leaves, mainly in the early growth stages, is manifested in a limited content of soluble carbohydrates in the plant tissue and in a higher N concentration.

In the second year there was no important variation among the treatments; the low productivity of the mulberry plantation influenced this, with higher incidence in the dry season, along with a possible depletion of the N, P and K contents in the soil.

The highest value of N in the leaves was obtained with the mineral fertilization and, in general, in all the treatments it was below the optimum range for the mulberry crop (30 and 40 g N kg^{-1} DM), reported by González *et al.* (2011).

The treatment of intercropped AMF-inoculated jack bean did not favor the increase of the N concentration; this is explained in the rainy season, by an effect of dissolution of the N element due to the increase of the biomass yield of mulberry. In the dry season, the influence of AMF-inoculated jack bean was lower because of the climate conditions inherent to this season. These results differ from the ones obtained by Srinivas (2005), who reported an increase in the N concentration in the mulberry leaves when intercropping soybean under irrigation conditions.

Table 3. Effect of the cutting interval and nutrition management on the N concentration in the mulberry leaves (g kg^{-1} DM).

Nutrition management (B)	Cutting interval (A), days															
	Rainy season 2007				Dry season 2008				Rainy season 2008				Dry season 2009			
	30	60	90	Factor B	30	60	90	Factor B	30	60	90	Factor B	30	60	90	Factor B
Without CeAMF and fertilizer	28,2	25,9	18,0	24,0^{b}	31,0	28,3	19,7	26,3^{b}	28,2	28,5	25,8	27,5	30,9	31,1	28,3	30,1
Mineral fertilization	33,0	30,2	26,2	29,8^{a}	36,1	33,0	28,6	32,6^{a}	29,0	29,0	26,5	28,2	31,8	31,7	29,0	30,8
CeAMF	23,4	21,4	15,5	20,1^{c}	25,7	23,5	17,0	22,0^{c}	25,6	26,9	23,5	25,3	28,1	29,4	25,7	27,7
Means of factor A	28,2^{a}	25,8^{a}	19,9^{b}		30,9^{a}	28,3^{a}	21,8^{b}		27,6	28,1	25,3		30,3	30,8	27,7	
SE ±	(A) 1,2**; (B) 1,2**				(A) 1,4**; (B) 1,4**				(A) 1,5ns; (B) 1,5ns				(A) 1,7ns; (B) 1,7ns			
Season means	24,7				27,0				27,0				29,6			
t				13,47**								44,06**				
Year means				25,86								28,28				
t	2,51*															

Different letters indicate significant differences $p \leq 0,05$ according to Duncan (1955). SE ± indicates the standard error of the factors: A (factor cutting interval), B (factor nutrition management). *$p < 0,05$ **$p < 0,01$ ns: not significant

There was a significant effect of the season on the N concentration of the leaves, and in all the treatments it was higher in the dry season; this is explained by the reduction of the vegetative growth rate in this season, which is related to the scarce rainfall, lower extraction of water and nutrients from the soil, lower synthesis of carbohydrates, decrease of the cell multiplication and differentiation indexes, and higher accumulation of N in the tissues.

Although there was no interaction between the factors, the influence of the cutting interval was significant. The positive effect of the 90-day interval must be emphasized, with values of up to 93 % survival in the treatment with intercropped AMF-inoculated jack bean; this indicates that wider cutting intervals tend to guarantee a higher useful life of the plantation.

In the 60-day interval there was a survival of 71,28 % and the report by Benavides (2000) was corroborated, regarding the fact that 60 days is a necessary limit time for the recovery of mulberry plants after cutting. On the other hand, Delgado and Rodríguez (2012) proposed to prolong the recommended interval for the mulberry monocrop in 10 or 15 days for the associations, due to the competition represented by the intercropped crop.

Table 4. Influence of the cutting interval and nutrient management on the plantation survival.

	Factor	Survival % (√x)
Cutting interval (days)	30	68,52 (8,21^{b})
	60	78,24 (8,82^{b})
	90	93,52 (9,67^{a})
SE ±		0,21***
Nutrition management	WF or CeAMF	80,09 (8,92)
	Mineral fertilization	78,24 (8,76)
	CeAMF	81,94 (9,02)
SE ±		0,21 ns

Different letters indicate significant differences according to Duncan (1955).

In the 30-day interval the survival of mulberry was lower than 70 %. The advantage represented by this cutting interval is related to the highest concentrations of N and P in the leaves and their high palatability, digestibility and acceptability; nevertheless, it was proven that during the first 30 days of regrowth the mulberry plants are not capable of recovering their reserves.

It is concluded that the 90-day cutting interval in mulberry was the best, and in this one it was feasible to intercrop the AMF-inoculated jack bean in the rainy season.

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Received: July 19, 2015

Accepted: May 4, 2016