*Multiplication of AMF infective propagules in a mulberry (*Morus alba *L.) plantation*

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ABSTRACT

A field essay was conducted at the Experimental Station of Pastures and Forages "Indio Hatuey", on a Ferralitic Red soil of good surface and internal drainage in order to evaluate the effect of mineral fertilization and intercropping of *Canavalia ensiformis* (jack-bean), inoculated with AMF, on the multiplication of infective propagules in a mulberry (*Morus alba* L.) plantation. The mulberry planting frame was 1,0 by 0,50 m (20 000 plants/ha). The plantation area was 384 m^2, with 40 plants in the plots. The forage was harvested with a 90-day interval at a height of 30 cm. The evaluated treatments were: control (without fertilization and without intercropping of jack-bean, WFWJBAMF); AMF incorporation through the intercropping of jack-bean, without mineral fertilization (WFJBAMF); mineral fertilization (F) and AMF incorporation through intercropping of jack -bean, with mineral fertilization (FJBAMF). The jack-bean was planted inoculated with AMF at a distance of 0,5 m from mulberry, in the first 15 days of growth of this plant since cutting, at the beginning of each season. The design was randomized blocks with factorial arrangement and three repetitions. Low content of native infective propagules was evidenced in the rhizosphere of the mulberry plantation (190-295 spores/50 g). The effect of FJBAMF was highly significant on the multiplication of infective propagules during the rainy season and during the first 45 days of the dry season (370 and 469 spores/50 g, respectively), while it contributed to increase the organic matter and K contents of the soil. It is concluded that the combination of mineral fertilization with AMF incorporation through the intercropping of jack-bean constitutes an effective procedure to multiply the AMF infective propagules up to moderate levels.

Key words: *Canavalia ensiformis*, inoculation, *Morus alba*, propagules

INTRODUCTION

The loss of food self-sufficiency in the planet, among other reasons, is due to the adoption of technological models based on the irrational use of inputs which are not in agreement with our countries' demands. Such models have caused ecological damage in the rural areas of the tropical and subtropical zones, with the consequent depletion of natural resources, erosion and natural loss of soil fertility, as well as alarming reduction of biomass and biological diversity (Martín 2009). In the face of this situation, the development of more suitable alternatives based on a reasonable use of endogenous resources, managed from the knowledge of each of the elements that comprise them, with a holistic approach that strengthens agroecosystem sustainability, can not be postponed (Ferrera and Alarcón, 2001).

Arbuscular mycorrhizal fungi (AMF) constitute one of the elements of the agricultural environment; they are part of a huge range of soil microorganisms used in biofertilizer production (Calderón and González, 2007). Among them the following can be found: *rhizobium* and *bradyrhizobium* (bacteria or symbiotic N_2-fixing), free or associated rhizobacteria (N_2-fixing and/or plant growth stimulators), *azotobacter*, *azospirillum* and P-solubilizing bacteria.

The arbuscular mycorrhizal effectiveness can be interpreted in different ways. It is more related to the yield of a certain crop, that is, the endophyte effectiveness on plant growth; to the nutrient transference per unit of exchanged carbohydrates during the symbiosis; and to the propagule number in the natural ecosystem. Its high impact is guaranteed with management practices which stimulate the multiplication of infective propagules and their permanence (González *et al*., 2008).

In such sense, green manures as *in situ* nutrient and organic matter sources guarantee the necessary nutrient supply for an effective mycorrhization and allow to diminish or substitute the use and transportation of the classical sources of organic manure (earthworm humus, farmyard manure), thus reducing dependence on the "outside" of the system (Rivera and Fernández, 2003).

Hence the objective of this study was to evaluate the effect of mineral fertilization and inoculation of arbuscular mycorrhizal fungi, through the intercropping of a temporary crop, on the multiplication of infective propagules in an established mulberry (*Morus alba*) plantation.

MATERIALS AND METHODS

The study was conducted in areas of the EEPF "Indio Hatuey", Perico municipality, Matanzas province, Cuba, which is located at 22°48'7 latitude North and 81°2' longitude West, at 19,01 masl.

The soil of the site is Ferralitic Red (Hernández, *et al*., 2003), with good surface and internal drainage and with an average native population of arbuscular mycorrhizal fungi of 299 spores/50 g of soil. Table 1 shows that the chemical composition of the soil was characterized by low availability of minerals, with low concentrations of K_2O and moderate contents of organic matter. The pH oscillated between slightly acid and neutral, according to Martín Alonso (2011).

The essay was conducted during two years, from the establishment of the mulberry plantation (12 months after planting). The planting frame was 1,0 by 0,50 m, equivalent to 20 000 plants/ha. The plantation area was 384 m^2 and in the experimental plots there were 40 plants. The forage was harvested with an interval of 90 days and it was cut at a height of 30cm. Mineral fertilization was applied at the beginning of each season, at a rate of 150 and 75 kg of N and K/ha/ season, respectively. In the treatments with jack-bean (*Canavalia ensiformis*) a row was associated, inoculated with AMF, at a distance of 0,5 m from mulberry. The jack-bean planting coincided with the first 15 days of growth of mulberry after cutting, at the beginning of each season. The distance between the jack-bean plants was 0,4 m.

The commercial product used to inoculate the mycorrhizal fungi was EcoMic®. The AMF strain (Glomus hoi-like) was obtained from a certified mycorrhizal inoculum (Fernández, Gómez, Martínez and Noval, 2001), which is produced in the department of biofertilizers and plant nutrition of the National Institute of Agricultural Science (Mayabeque, Cuba). This inoculum had 20 spores per gram of inoculant. The inoculation was conducted by the method of seed coating. After 60 days of being planted, the jack-bean plants were cut and used to mulch the mulberry row, which contributed to their slow and natural decomposition in order to achieve higher multiplication of infective propagules (Rivera and Fernández, 2003; Peña *et al*., n.d.).

Mineral fertilization and intercropping of jack-bean inoculated with AMF were the study factors. The management treatments were the following:

Mineral fertilization	Intercropping of inoculated jack-bean with AMF
Without fertilization	Without intercropping (WFWJBAMF): control With intercropping + AMF (WFJBAMF)
With fertilization	Without intercropping (F) With intercropping + AMF (FJBAMF)

The number of spores was evaluated per 50 g of soil. The samples were taken 45 days after beginning the essay in the control plots and in the treatment with mineral fertilization, to characterize the native population of AMF.

Likewise, samples were taken in all the management treatments 45 days after the dry season intervention, and 180 days after the intervention of each season.

At the beginning of the dry season of each year, the potassium and phosphorous content in the topsoil was determined through the method proposed by Oniani (1964); the organic matter content in this layer was evaluated by the Walkley-Black method.

The design was randomized blocks, with factorial arrangement and three repetitions. The result processing was based on the descriptive analysis of the soil chemical composition, and ANOVA was used through the General Linear model to analyze the development of the infective propagules of AMF. To

Table 1. Initial characteristics of the topsoil in the experimental area.

	Potassium	Calcium	Magnesium	Phosphorus	Organic matter	pH
	(cmol/kg)			(ppm)	(%)	(H_2O)
Lower value	0,1	9,4	2,4	7	1,5	6,2
Higher value	0,25	13,3	4,8	48	3,53	7,0

compare the means Duncan's test (1955) was used, with the help of the statistical package InfoStat, free version.

RESULTS AND DISCUSSION

The results in the control treatment and in the fertilization alone (table 2) allowed to consider the content of native infective propagules in the rhizosphere of the mulberry plantation between moderate and low, according to the classification proposed by Ruiz (2001) and Rivera and Fernández (2003), who consider that the presence of such propagules in a density lower than 200 spores/50 g of soil is considered low; moderate, when it is between 300 and 500 spores/50 g of soil; and high when it exceeds 600 spores/50 g of soil.

Regarding this, it is known that AMF are obligatory symbionts and their distribution in cultivated soils is strongly influenced by vegetation. The spore number of AMF increases significantly with the increase of the number of the plant species present (Chen, Tang, Fung and Shimizu, 2004), which does not happen in the mulberry as monocrop. Peña *et al.* (2006), analyzing appearance frequency of native spores of AMF per cover type, found that agroforestry systems, followed by the natural forest, showed the highest averages of root colonization (over 30 %). On the contrary, the plot covers, the monocrop and the paddocks showed the lowest percentages. In another study these authors proved that the native arbuscular mycorrhizal symbiosis was the most effective in ecosystems with highly heterogeneous covers; hence the importance of promoting biodiverse agricultural production systems (Peña, Cardona, Arguelles and Arcos, 2007) that minimize the frailty of the monocrop plantations, as in the case of mulberry for forage.

Regarding the multiplication of the infective propagule content in the soil, it was observed that after two years of management with the different treatments, the effect of the FJBAMF combination was significant in the rainy season, with regards to the control (table 2); while the treatments of mineral fertilization alone (F) and intercropped jack-bean (WFJBAMF) did not differ from it.

The inoculation with AMF in the dry season in the WFJBAMF and FJBAMF treatments was manifested in a significant proliferation of the infective propagules 45 days after the intervention, which was not achieved with mineral fertilization alone.

The superiority of the FJBAMF treatment, six months after intervention, is in correspondence with the criteria expressed by Martín (2009), who proved in crop rotation systems the high effect of permanence of the symbiosis process on the crop after inoculation. In the case of perennial crops it is known that the persistence of symbiotic structures is directly linked with root renewal: the mycorrhizal symbiosis generates operation cycles, which are renewed from time to time with root change (Rivera and Fernández, 2003). In the case of the mulberry plantation, the vegetative growth cycle re-starts every three months, which guarantees a constant renewal of the root system.

It is known that the development of the structures of mycorrhizal fungi depends, to a large extent, on the availability of the necessary nutrients to complement the plant requirements. In this study, such availability was guaranteed with the N and K mineral fertilization, combined with the jack-bean incorporated to the soil (figs 1, 2 and 3). In the treatments with fertilization the available potassium in the soil was doubled (fig. 1); while in the treatment of intercropping of jack-bean without fertilization it underwent a slight decrease in the concentration of this element (0,03 meq/100 g), comparing the two years.

Regarding the P content in the soil, the treatments with fertilization underwent slight decreases with respect to the first year; however, the WFJBAMF treatment favored its stability (fig. 2), as well as the organic matter increase (fig. 3). This last aspect had a similar trend in all the treatments, with percentage increases between 0,29 and 0,7 units, which is related

Table 2. Effect of the management treatments on the multiplication of infective propagules (number of spores per 50 g of soil).

Treatment	November (180 days after intervention in rainy season)	December (45 days after intervention in dry season)	May (180 days after intervention in dry season)
WFWJBAMF	295^{b}	210^{b}	190^{b}
WFJBAMF	350^{ab}	510^{a}	278^{a}
F	335^{ab}	202^{b}	182^{b}
FJBAMF	370^{a}	469^{a}	233^{ab}
SE ± log (x)	0,30*	0,55*	0,24*

Values with different letters in the column differ statistically at P<0,05 (Duncan, 1955) *P<0,05

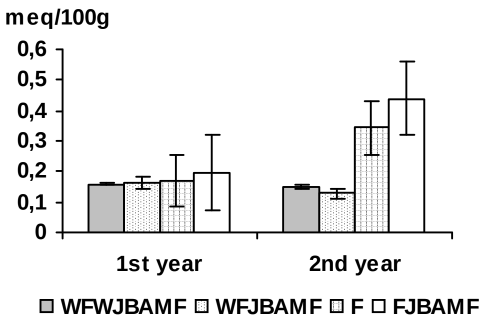

Fig. 1. Effect of management treatment on K content in the soil.

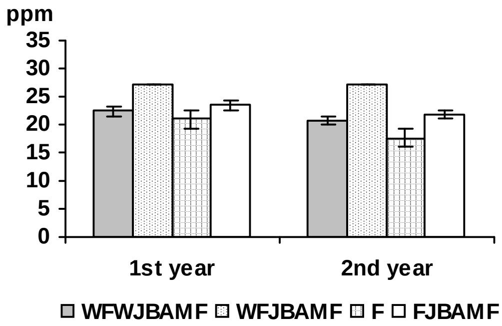

Fig. 2. Effect of management treatment on P content in the soil.

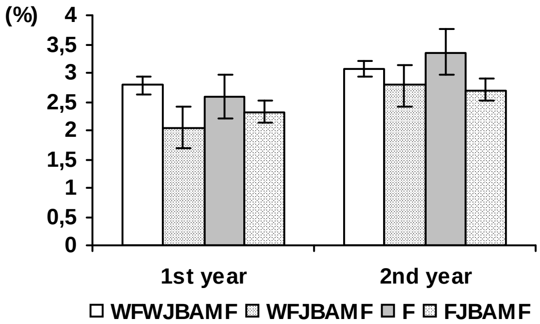

Fig. 3. Effect of management treatment on Organic matter content in the soil.

to the productive cycle of mulberry crop for cut and carry.

These results coincide with results informed by Ochoa (2007), who pointed out that jack-bean plants are capable of increasing in a year the content of ammoniacal nitrogen in the soil in 7,36 ppm; maintaining the potassium concentration balanced, with little losses (0,05 meq/100 g); and propitiate slight increases of organic matter in the soil.

Some authors state that the highest effects of the inoculation with efficient AMF strains are achieved with a moderate availability of nutrients in the soil. If the availability is low or non-existent, the symbiosis does not operate properly and the inoculation effectiveness is low (Mohammad, Mitra and Khan, 2004; Gamper, Hartwig y Leuchtmann, 2005). It is considered that the association between the host and the AMF consumes between 5 and 10 % of the photosynthesis products, which will be compensated if the plant is under suboptimal conditions of nutrient supply (Siqueira and Franco, 1988). For the symbiosis to be efficient the nutrient availability in the system should be lower than the one commonly used for non-mycorrhizated plants. The attainment of plants with optimal growth in the presence of lower quantities of nutrients is due to the increase in the efficiency of the absorption process by the mycorrhizated plants and, therefore, the increase of the nutrient utilization coefficient (Rivera and Fernández, 2003). On the contrary, the high availability of nutrients causes a decrease in the presence of mycorrhizal structures inside the roots, which indicates that the reduction in the mycorrhizal effectiveness is consequence of bad functioning or symbiosis inhibition. That is why in this research a dose of nitrogen and potassium fertilization was used below the optimum value suggested by Martín (2004), which contributed to the highest values of infective propagules when combined with a short-cycle legume, intercropped in a spaced way and inoculated with AMF.

The low native population of AMF in the control plots (table 2), with values between 295 and 190 spores/50 g of soil, confirmed the need of artificially introducing these microorganisms through inoculation and thus favor symbiosis, because in low-fertility soils a higher amount of fungal structures is necessary to guarantee the proper functioning of the symbiosis (Azcón, Ambrosano and Charest, 2003).

From the results obtained it is concluded that the presence of native infective propagules within the established plantation of mulberry was low, with values which oscillated between 190 and 299 spores/ 50 g; therefore, the inoculation of AMF through jack-bean complemented with mineral fertilization constituted a way to raise the presence of propagules up to moderate levels and to increase some indicators of soil quality.

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Received: January 25, 2012
Accepted: October 19, 2012