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Arbuscular mycorrhizae in legumes of the livestock production enterprise El Tablón, Cuba)

L. Ojeda[1], E. Furrazola[2] and Consuelo Hernández[3]

[1]*Centro Universitario Municipal (CUM) Cumanayagua, Universidad de Cienfuegos Carlos R. Rodríguez, Calle Los Filtros No. 18, Cumanayagua, Cienfuegos*
[2]*Instituto de Ecología y Sistemática, Ministerio de Ciencia, Tecnología y Medio Ambiente, La Habana, Cuba*
[3]*Estación Experimental de Suelos y Fertilizantes Escambray, Barajagua, Cienfuegos, Cuba*
E-mail: ljojeda@ucf.edu.cu

ABSTRACT: On a Grayish Brown soil, a study was conducted to determine the presence of arbuscular mycorrhizae associated to the roots of the species: *Leucaena leucocephala* cv. Perú, *Centrosema pubescens* IH-129, *Centrosema pubescens* CIAT-482, *Centrosema macrocarpum* CIAT-5434, *Canavalia ensiformis* and *Desmodium* sp., in natural areas of the dairy laboratory farm No. 3 of the Livestock Production Enterprise El Tablón (Cumanayagua, Cienfuegos province, Cuba). An efficiency test was also made on the native arbuscular mycorrhizal fungi (AMF), under semicontrolled conditions (glass house), and with a randomized block design for each species, two treatments and three replications. Under these conditions, the forage legumes were naturally colonized by mycorrhizae, but with low colonization: between 21 and 34 % in the first sampling, and between 14 and 20 % in the second one. When the native AMF were inoculated in an indicator crop (forage sorghum) there were no significant differences regarding the leaf content of phosphorus and the colonization; while the DM yield was slightly benefited in *Desmodium* sp. in the two variants; therefore, it cannot be ascribed to the effects of AMF. Such results showed the need to test commercial AMF strains in legume species under these conditions and soil type.

Key words: forage legumes, vesicular arbuscular mycorhizae, phosphorus

INTRODUCTION

The effective management of rhizosphere microorganisms can constitute an economically viable ecological alternative to increase the productivity, persistence and nutritional value of forage legumes, and to reduce the use of chemical fertilizers (Nascimento *et al*., 2008; Corbera and Nápoles, 2011). Historically, fungi have played an important function in the establishment and maintenance of ecosystems. Around 80 % of the plant species and 92 % of the families of land plants are mycorrhizal, while the arbuscular type is the most common and ancestral of these associations (Wang and Qiu, 2006).

It is known that the presence –or not– of arbuscular mycorrhizal fungi (AMF) can affect the productivity, diversity and dynamics of plants (Van der Heijden and Horton, 2009); specifically, feedback mechanisms between them and such microorganisms, which control the composition of plant communities, have been identified (Sanon *et al.,* 2009).

Despite the crucial role these fungi play in the functioning of the ecosystem (Hart and Klironomos, 2002), some natural areas –especially in the tropics− remain unexplored with regards to the determination of the presence of AMF and their ability to form arbuscular mycorrhizae. One of the main functions of AMF is to extend the absorption surface of the roots in the soil trough their external hyphae, which stimulates the colonized plants to increase the capture of mineral nutrients, particularly phosphorus (Smith and Read, 2008), and little mobile elements in the soil, like copper, zinc, among others.

The objective of this research was to determine the presence of AMF in forage legumes introduced in the Cienfuegos province.

MATERIALS AND METHODS

The research was conducted, during two years, in the dairy laboratory farm No. 3 of the Research Station Escambray, Livestock Production Enterprise El Tablón (Cumanayagua municipality, Cienfuegos province, Cuba), located at N: 591-260 and E: 259-250 on the map sheet Barajagua 1: 25 000; the soil is classified as Grayish Brown (Hernández *et al*., 1999), and in it the pH, P_2O_5, K_2O and OM were determined.

The presence– or not– of AMF was defined in the legumes: *Leucaena leucocephala* (Lam.) de Wit

cv. Perú, *Centrosema pubescens* Benth. IH-129, *Centrosema pubescens* Benth. CIAT-482, *Centrosema macrocarpum* CIAT-5434, *Canavalia ensiformis* (L.) Dc. and *Desmodium* sp.

The evaluation of the species was carried out under natural field conditions. The samplings were made in June: the first one when the plants had six months of establishment (they were not subject to any animal management or to cuttings for forage or other activities,) and the second one, after two years of forage exploitation.

The areas covered by such species were diagonally walked, taking 15 samples of rhizosphere in a frame of 1 m^2. The evaluation criterion consisted only in determining whether there were associations with AMF on the roots of these legumes. The species were not compared to each other.

The test of the native AMF efficiency was conducted in each sampling, under glass house conditions, in a independent randomized block design for each species, with three replications.

The treatments were: 1) inoculation of the soil collected in the rhizosphere of the species; 2) control without inoculation.

Ten grams of soil collected in the rhizosphere of each species were taken, which were mixed with the typical soil of the area (previously sterilized at 1,5 atmospheres in autoclave, during one hour) and forage sorghum was planted as indicator crop. After 100 days the sorghum plants were extracted and the DM yield, the content of leaf phosphorus and the mycorrhizal colonization of the indicator crop were determined. The results were analyzed through an ANOVA and when *F* resulted significantly different, the means were compared according to Duncan's multiple range test (1955).

Rootlet samplings of each species were taken to determine the mycorrhizal variables in the two research phases, from the extraction of the root systems and the associated rhizosphere soil, at a depth of 0-10 cm. Then, they were air-dried and stored in plastic bags until their processing in the laboratory. The rootlets with less than 2 mm of diameter were washed, cut (1 cm of length, approximately) and were dyed with trypan blue, according to the method proposed by Phillips and Hayman (1970).

The percentage of arbuscular mycorrhizal colonization (percentage of MC) was quantified, according to the methodology of Giovannetti and Mosse (1980). The procedure consisted in distributing randomly around 1,5 g of dyed roots on a Petri dish of 8 cm of diameter, on whose bottom a square grid of 0,5 inches (1,27 cm) was drawn, and 100 intersections of roots with the lines of this grid were counted. The Petri dish was looked over three times for every sample, through displacements in parallel straight lines. The presence of AMF in each intersection represented the mycorrhizal colonization of the root. The scale to value the percentage of visual density (VD) was the following: 0: absence of AMF; 1: 1 %; 2: 2,5 %; 3: 15,5 %; 4: 35,5 %; 5: 47,5 % (Herrera *et al*., 2004).

RESULTS AND DISCUSSION

According to the components of soil fertility, the acidity and low contents of phosphorus and OM shown by the area where the research was conducted can be appreciated (table 1).

It is important to consider that the low content of phosphorus is an aspect of great interest to measure the effectiveness of the arbuscular mycorrhizal symbiosis per host plant, because the AMF can improve the absorption capacity of the

Table1. Components of soil fertility.

Species	Sampling No. 1				Sampling No. 2			
	pH	P_2O_5 mg/100 g	K_2O mg/100 g	OM %	pH	P_2O_5 mg/100 g	K_2O mg/100 g	OM %
L. leucocephala cv. Perú	4,10	2,62	4,44	1,46	4,20	2,36	4,15	1,52
C. pubescens IH-129	4,05	2,14	5,61	1,44	4,11	2,17	5,40	1,47
C. pubescens CIAT-482	4,40	3,10	4,60	1,77	4,35	3,30	4,42	1,64
C. macrocarpum CIAT-5434	4,15	2,62	5,60	1,46	4,09	2,52	5,70	1,50
C. ensiformis	4,10	2,50	6,60	1,52	4,25	2,42	6,49	1,46
Desmodium sp.	4,10	3,00	4,60	1,62	4,15	3,10	4,50	1,55
$\bar{x}$	4,15	2,66	5,24	1,54	4,19	2,64	5,11	1,52

roots and mobilize the assimilable phosphorus from the deepest levels of the soil (Herrera *et al*., 2004).

Regarding the arbuscular mycorrhizal symbiosis (table 2), it can be observed that the legumes were colonized. However, in both samplings there were colonization values lower than 50 %, and a general average of 22,1 % was reached; which is considered low, according to the criterion expressed by Read *et al*. (1976). These authors also refer that in acid-pH soils, there is generally a predominance of the AMF communities with regards to the other microorganisms, which does not coincide with the colonization values obtained. However, other authors consider that colonization percentages between 20 and 45 % are acceptable (Herrera *et al*., 2004; Fernández *et al*., 2006; Plana *et al*., 2008).

According to Van der Heijden e*t al*. (2008), the soil microbiota plays a key role in the regulation of the land ecosystems, because it influences the productivity, diversity and structure of plant communities. These authors indicate that the OM is decomposed by the activity of different species of bacteria and fungi that release nutrients to the soil, and thus they remain available to be absorbed by the plants again. The absorption can be direct, through the roots; or indirect, via the microorganisms that form symbiosis with the roots, such as AMF. They are considered an active and diverse biological community that facilitates the maintenance of agroecosystems, and represent the symbiosis of higher relevance in agroecological systems. In this sense, it is important to determine the AMF in natural pastures, taking into account their role in the plant and the contribution to the soil of the so-called mycorrhizosphere.

Some abiotic factors, like agronomic management, soil erosion and use of pesticides, significantly influence the permanence and colonization of the mycorrhizal community; among the variables with higher impact are: the soil fertility level in phosphorus and nitrogen, temperature, humidity, OM, acidity and season (Chaus, 2007). Although some of these variables were not measured in this research, the season in which both samplings were made was the same and, however, the response of the indicator plant was different, which can suggest a spatial variation in the colonization of AMF.

The low mycorrhizal colonization can be ascribed to the effect of the previous application of nitrogen fertilizer (400 kg/ha/year) on the soil during several years –to achieve the suitable pasture yield–, together with animal grazing and the systematic use of agricultural machinery. According to Jansa *et al.* (2003), these factors can negatively influence the AMF communities. It is also known that an intensive agriculture can affect the natural function of these fungi (Kabir, 2005; Curaqueo *et al*., 2011; Mirás-Avalos *et al*., 2011); thus, in conventional agricultural systems the microbial communities are modified, due to the plowing of the fields and the application of high doses of inorganic fertilizers, herbicides and pesticides. All these factors could influence the soil deterioration, which was manifested in the disturbance of its microbiological balance, as well as in the erosion processes and the loss of OM.

Parodi and Pezzani (2011) did not find a significant relationship in the mycorrhization, in grazed and non-grazed areas, when studying the interactions between two native grasses (*Nassella neesiana*, C3, of winter cycle; and *Coelorhachis selloana*, C4, of summer cycle) and the native AMF that colonized their roots; although *C. selloana*

Table 2. Mycotrophy in forage legume species at six months of establishment and after two years

Species	Presence of AM		Initial sampling		Final sampling	
	Yes	No	Col. (%)	VD (%)	Col. (%)	VD(%)
L. leucocephala cv. Perú	x	–	24	2,64	19	1,69
C. pubescens IH-129	x	–	28	2,06	20	1,70
C. pubescens CIAT-482	x	–	34	2,16	14	1,54
C.macrocarpum CIAT-5434	x	–	26	2,43	18	1,26
C. ensiformis	x	–	24	2,60	13	1,14
Desmodium sp.	x	–	21	2,17	19	1,17
Mean value			26,1	2,34	17,1	1,41

Col.: rootlet colonization, VD: virtual density.

showed a trend to a higher colonization by AMF under grazing conditions.

On the other hand, in studies of AMF characterization in pasturelands of the Tolú municipality (Colombia), on moderately-acid soils, Peroza (2003) found a density of 353 to 2 176 spores per 100 g of soil (average: 931,8 spores), which can be considered low; while the colonization of the roots oscillated between 19 and 76 % (mean: 41,28 %) and can be classified as moderate. The author also reported the isolation of 25 native morphotypes of AMF, of the genera *Gigaspora* and *Glomus*.

In this research, the mean value of the colonization was 26,1 %; while Ojeda *et al*. (1994), under the same conditions of Grayish Brown soil, but in pasturelands of grasses established for more than five years (Guinea grass cv. Likoni, king grass, *Brachiaria decumbens* and Jamaican star grass), found AMF in all the species (383, 3 399, 195 and 321 spores per 100 g of soil, respectively), and an average level of rootlet colonization of 55,4 %. At that moment 11 species of AMF were identified, belonging to the genera *Glomus, Sclerocystis* and *Scutellospora*. Likewise, the level of rootlet colonization by the AMF was higher in the forage grasses than in the legumes.

When referring to the ubiquity of the symbiosis, Pérez (2003) pointed out that, although there is not specificity in the soil-fungus relation, some results indicate the preference of certain fungi for certain soil types. In this sense, the influence of some physical-chemical properties, such as pH, clay and OM, is fundamental. This author reported that the colonization by mycorrhizae was lower in sandy soils, which coincides with the soil type evaluated in this research.

In a survey made to quantify the colonization percentage of rootlets and the distribution of AMF in tropical forage legumes cultivated in southern Florida, Richardson *et al*. (2009) found that the colonization percentage oscillated from 3 to 41 %. In the present study the colonization did not exceed 28 % in any of the species, which suggests the existence of a disturbed soil.

The AMF that naturally cohabit in the soil are greatly important to establish management policies regarding the communities of native AMF and the plants that will be used. That is why in the second phase of the experiment the native endophytes of the soil where the species grew were evaluated, using forage sorghum as indicator crop. Table 3 shows that the inoculation was not effective on any of the measured indicators; hence sorghum did not show dependence on the inoculation with the native AMF.

The inoculation with native AMF showed a low colonization potential, which, like the leaf phosphorus content, did not differ statistically. This potential depends not only on the genome of the fungi and of the host plant, but also on different biotic and abiotic factors, which can influence and determine the increase of DM yield and phosphorus content of the plant due to the AMF. This could indicate that the agroecosystem shows a low incidence of effective propagules; however, although most tropical pastures have a high mycorrhizal dependence, some factors related to the pasture species, its regime of exploitation, the efficiency of AMF strains –native or inoculated– and the soil conditions influence the response to symbiosis (Grigera and Oesterheld, 2004). The dry matter yield among the species showed significant

Table 3. Response of sorghum to the inoculation with the native endophyte of the soil.

Species	DM yield (g/plant)		Leaf phosphorus (%)		Colonization (%)	
	Inoculated	Without inoculation	Inoculated	Without inoculation	Inoculated	Without inoculation
L. leucocephala cv. Perú	2,61^{c}	2,68^{c}	0,13	0,13	16,2	12,0
C. pubescens IH-129	3,01^{b}	3,03^{bc}	0,17	0,15	13,5	12,7
C. pubescens CIAT-482	3,10^{b}	3,04^{bc}	0,20	0,17	14,2	10,7
C. macrocarpum CIAT-5434	2,7^{bc}	2,90^{c}	0,12	0,15	16,7	15,7
C. ensiformis	3,11^{b}	3,20^{b}	0,12	0,13	17,5	14,5
Desmodium sp.	3,97^{a}	3,84^{a}	0,14	0,15	13,5	14,5
SE ±	0,1794*	0,1993*	0,0771	0,0911	0,1115	0,1312

a, b, c: values with non-common superscripts differ at $p < 0,05$; $p \leq 0,05$*

differences only in *Desmodium* sp., but not in relation to the inoculum, which indicates that there was not mycorrhizal dependence and the response to the native AMF was not apparent.

The response to the inoculation with AMF of native legumes is different with respect to the introduced ones, because it is known that the interaction between both participants in the symbiosis can be important in the success of the introduced plants through diverse mechanisms, as stated by Pringle *et al*. (2009) and Shah *et al*. (2009). Among these mechanisms are: the great benefits that plants receive thanks to this association (Harner *et al*., 2010), the reduction of the dependence of the plants on AMF (Seifert *et al*., 2009; Vogelsang and Bever, 2009), as well as disturbances in the communities of these fungi in the soil (Zhang *et al*., 2010; Busby *et al*., 2013). Such results could constitute starting points for further research about AMF associated to introduced legumes, as in the case of this research.

The microbial populations in the soil are involved in an interaction that can influence plant growth, because they participate in processes that ensure the stability and productivity of agroecosystems and of natural ecosystems. In strategic and applied research interest has been shown in some microbial cooperation activities that can be exploited as a low-cost technology, and thus contribute to sustainable and environment-friendly agrotechnological practices; to which the use of a microorganism complex would be adapted in the search for improving the agronomic quality of pastures, forages and food crops. In general, the ecological, synergical and physiological indicators, as well as the biochemical processes of microorganisms in the environment, are determining and act jointly with the different crops. This allows to consider the results of the analyzed species in order to make proposals of new methodologies for biofertilization of forage legumes (Pedraza *et al.,* 2010).

CONCLUSIONS

The forage legumes evaluated under conditions of Grayish-Brown soil were naturally colonized, but with low colonization levels (between 21 and 34 % in the first sampling, and between 14 and 20 % in the second one). The inoculation in an indicator crop (forage sorghum) with native AMF did not produce significant differences with regards to the leaf phosphorus content and the colonization; while the DM yield was slightly favored in *Desmodium* sp. in the two variants, therefore it cannot be ascribed to the presence of the applied AMF. It is necessary to test commercial AMF strains in legume species, under the conditions of this study.

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