SCIENTIFIC PAPER

Response of Leucaena leucocephala *cv. Peru to the application of different doses of agricultural MicoFert*

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ABSTRACT: A study was conducted at the Livestock Production Enterprise El Tablón (Cumanayagua, Cienfuegos province, Cuba), in order to evaluate the effect exerted by the application of different doses of agricultural MicoFert on the dry matter (DM) production and the leaf phosphorus content in *Leucaena leucocephala* cv. Peru. The design was randomized blocks, with six treatments and three replications. The treatments were constituted by four doses of MicoFert (250, 500, 750 and 1 000 g m^{-1}), a variant at a rate of 25 kg of N ha^{-1}, and the control. The experiment lasted two years; four cuttings per year were made, with a frequency of 90 days, at a height of 25 cm over the soil. The application of agricultural MicoFert increased the DM yield between 13 and 40 %, proportionally to the applied doses, with regards to the control. The highest DM yield (18,44 t ha^{-1}) was obtained with the application of nitrogen, and significantly differed from the other treatments. The effect exerted by the inoculation with the biofertilizer on the leaf phosphorus content was evident, showing significant differences among the variants with MicoFert and the control. The colonization of rootlets by the arbuscular mycorrhizal fungi (AMF) was proportional to the increase of the biofertilizer doses, with values of 34, 38, 44 and 49 %, respectively. The fertilization with nitrogen and the control showed 28 and 26 % of mycorrhizal colonization, which indicated the presence of native AMF.

Key words: vesicular arbuscular mycorrhizae, *Leucaena leucocephala*

INTRODUCTION

Intensive agriculture is characterized by the use of high fertilizer doses, mainly nitrogen and phosphorus (Verbruggen and Kiers, 2010). The agricultural management causes several environmental problems, among which the excessive chemical fertilization prevails, contaminating the bodies of water and causing their eutrophication (Cuenca *et al*., 2007). Likewise, tillage can modify the physical, chemical and biological properties of the soil (Beauregard *et al*., 2010), as well as cause the mycorrhizal diversity at family level to decrease (Jansa *et al*., 2002).

According to Jeffries and Barea (2001), sustainable agriculture is a system integrated by plant and animal production practices that should, at long term: satisfy the human needs of fiber and food, improve the environmental quality and the basis of natural resources on which the agricultural economy depends, make an efficient use of non-renewable resources, support the economic viability of agricultural activities, and contribute to the increase of the quality of life of farmers and the society as a whole.

Among the conservationist managements is the use of biofertilizers, which include arbuscular mycorrhizal fungi (AMF); they form mutualistic symbiotic associations with most agricultural crops, and offer a wide perspective of use in the different farming systems (Castillo, 2012).

MicoFert (trademark registered at the Cuban Office of Industrial Property –OCPI, for its initials in Spanish– and at the National Institute of Defense of the Competition and of Protection of the Intellectual Property -Indecopi–, Peru) is a biofertilizer produced by the Institute of Ecology and Systematics –IES– (Ministry of Science, Technology and Environment, Cuba), through a technology developed since 1993 (Ferrer *et al*., 2004; Ley-Rivas *et al*., 2009). It is constituted by a mixture of different AMF strains, from the Cuban collection of mycorrhizal fungi –CCAMF– (Herrera-Peraza *et al*., 2011), which are associated to the rootlets of plants and significantly increase the absorption surface of the root.

The objective of this research was to evaluate, the effect exerted by the application of different doses of agricultural MicoFert on the DM production and the leaf phosphorus content in *L. leucocephala* cv. Peru, protein forage that could increase the productive indicators (García *et al*., 2008; Ruiz *et al*., 2008).

MATERIALS AND METHODS

The study, which lasted two years, was conducted on a Greyish Brown soil (Hernández *et al*., 1999), which showed the following agrochemical characteristics: pH (KCl): 4,19; P_2O_5: 1,94; K_2O: 9,86 mg 100 g^{-1} of soil; and organic matter: 1,34 %. A randomized block design was used, with three replications and 16-m^2 plots; which were planted with *L. leucocephala* cv. Peru destined for forage, because this plant has excellent adaptation to the edaphoclimatic conditions of the territory and occupies more than 70 % in the forage areas.

The evaluated treatments were:

1. Agricultural MicoFert: 250 g m^{-1}
2. Agricultural MicoFert: 500 g m^{-1}
3. Agricultural MicoFert: 750 g m^{-1}
4. Agricultural MicoFert: 1 000 g m^{-1}
5. Nitrogen: 25 kg ha^{-1} $year^{1}$
6. Control

MicoFert was placed on the bottom of the row, during planting. Nitrogen (46 % urea as fertilizer source) was first applied 25 days after the plants had emerged and at the beginning of the second year, in doses of 25 kg $ha^{-1}year^{1}$. Leucaena was sown at a depth of 2-3 cm, at a distance of 0,70 m between rows and 0,05 m between plants, guaranteeing a density of 28 571 plants ha^{-1}. The number of plants per plot was 457; from them, 257 corresponded to the evaluable area. Before sowing, the seeds were soaked in tap water during twelve hours; when sowing concluded, irrigation was applied to guarantee the soil moisture.

The cut of establishment was made at 6,5 months, when the plants reached an average height of 143 cm. Eight cuttings (four per year) were performed, with a frequency of 90 days; cuttings 1, 4, 5 and 8 corresponded to the dry season; and cuttings 2, 3, 6 and 7, to the rainy season. In each one the green mass was measured, in an area of 9 m^2 (after eliminating the edge effect); samples were taken with a framework of 1 m^2 (29 plants), in which the dry matter (DM) and the phosphorus content by digestion with H_2SO_4 + Se were determined, through the molybdovanadate method (Ríos *et al*., 1982). The establishment cutting was not considered within the analyses.

After cutting, the mycorrhizal variables were analyzed in rootlet samples. From the plants comprised in 1 m^2 of evaluable area the root systems and the associated rhizospheric soil (depth of 0-10 cm) were taken, which were air dried and stored in plastic bags until their processing in the laboratory. The rootlets with a diameter lower than 2 mm were washed, cut (1 cm of length) and were dyed with trypan blue, according to the method proposed by Phillips and Hayman (1970). The percentage of arbuscular mycorrhizal colonization was quantified according to the methodology suggested by Giovannetti and Mosse (1980), which consisted in distributing randomly, approximately, 1,5 g of dyed roots on a Petri dish of 8 cm diameter; on its bottom a grid was drawn with 0,5-inch (1,27 cm) squares, and 100 intersections of roots with the lines of this grid were counted. The dish was looked over three times for each sample, through displacements in straight parallel lines. The presence of mycorrhization in each intersection represented the mycorrhizal colonization of the root.

The mycorrhizal dependence (MD) was calculated through the following formula proposed by Siqueira and Franco (1988):

$$MD = \frac{\text{mycorrhized dry mass}}{\text{Dry mass of the non-mycorrhized plant}} \times 100$$

The results were analyzed through a simple ANOVA and the means were compared according to Duncan's multiple range test (1955).

RESULTS AND DISCUSSION

The DM yield was higher in the treatments inoculated with agricultural MicoFert (table 1) and increased between 13 and 40 %, proportionally to the increase of the doses. The highest DM content was obtained with the application of nitrogen, and differed significantly from the other treatments.

Sieverding and Barea (1991) stated that natural soils can have low as well as high concentrations of propagules of native AMF (<1mL^{-1}-50mL^{-1}), for which the fungi introduced through inoculation have to compete with the native ones. These authors also referred that when the inoculant is put under the seed it can increase its competition capacity. The inoculation method used in this study partly coincided with the previously proposed by then, because agricultural MicroFert was applied at the moment of planting, on the row bottom and under the seeds. The results indicated that the inoculation of MicoFert was effective, for which its form of production and application was validated.

The inoculation with mycorrhiza-forming fungi is more feasible when the quantity of AMF propagules in agricultural soils is very low, and where a non-

Table 1. DM yield of *L. leucocephala*. cv. Peru.

Treatment	DM (t ha^{-1})			Increase (%)
	DS	RS	Total	
Control	3,05^f	7,45^f	10,51^f	-
250 g m^{-1}	3,25^e	8,62^e	11,88^e	13
500 g m^{-1}	3,84^d	8,96^d	12,80^d	22
750 g m^{-1}	4,47^c	9,68^c	14,16^c	35
1 000 g m^{-1}	4,66^b	10,07^b	14,75^b	40
Nitrogen, 25 kg ha^{-1} year^{-1}	6,28^a	12,16^a	18,44^a	–
SE (±)	0,019**	0,014**	0,016**	

DS: dry season, RS: rainy season
a, b, c, d, e, f: values with different superscripts differ at $p < 0,05$; $p \leq 0,01$**

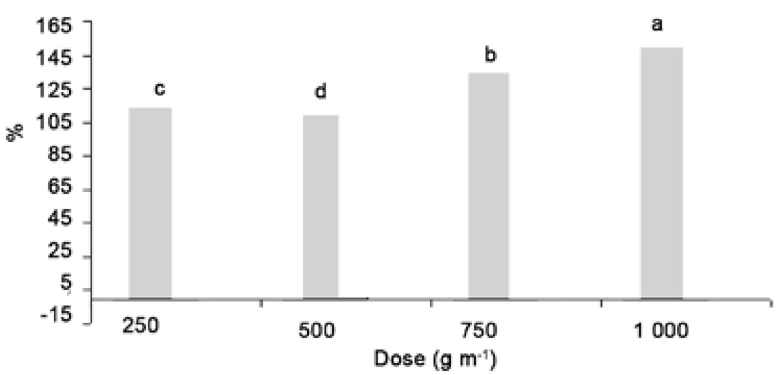
mycotrophic crop has previously existed and/or the autochthonous populations are neither sufficiently aggressive when colonizing the plants to promote plant growth. Ojeda *et al.* (1994) found the presence of AMF in grasslands established for more than five years (*Megathyrsus maximus*, *Brachiaria decumbens* and *Cynodon nlemfuensis*), under similar conditions as the ones in this study; but with a relatively low number of spores in 100 g of soil (383, 195 and 321, respectively), which could corroborate the satisfactory response of yield to the inoculation of agricultural MicoFert.

Likewise, several abiotic factors, such as agronomic management, soil erosion and pesticide use significantly influence the permanence and colonization of the mycorrhizal community of the soil. Among the variables with higher impact are: level of phosphorus and nitrogen fertility of the soil, organic matter content, acidity and season (Chaus, 2007), which did not undergo modifications; only rainfall varied, and its effect on the yield was evident when comparing the two seasons of the year.

The response to inoculation was well defined, not only among the different doses, but also in the control and in the treatment with mineral fertilization. In this sense, the favorable response of yield in the inoculated variants is important, indicating that MicoFert could last in time. This coincides with the report by Smith and Read (2008), who found a late effect of the inoculations with AMF in different species of legumes and grasses, under natural conditions.

In addition, it is known that the natural role of AMF can be minimized by the practice of intensive agriculture (Curaqueo *et al.*, 2011; Mirás-Avalos *et al.*, 2011), because the microbial communities in the conventional agricultural systems are modified by the field plowing and the application of high doses of inorganic fertilizers, herbicides and pesticides.

The mycorrhizal dependence was directly related to the dry biomass yield (fig. 1). In general, leucaena showed dependence on the inoculation



a, b, c, d: values with different superscripts differ at $p < 0,05$.
Figure 1. Mycorrhizal dependence of *L. leucocephala* cv. Peru.

with AMF, which increased proportionally to the increase of the biofertilizer dose

There are highly "mycorrhiza-dependent" plants, in which mycorrhiza could be considered as an alternative to fertilizers. In this sense, Sheng *et al*. (2008), Abbaspour (2010), Smith *et al*. (2010) and Doubková *et al*. (2013) showed that the artificial inoculation with AMF in agricultural species increases nutrition and plant growth, and allows to overcome situations of biotic and abiotic stress. In addition, they observed that 1 cm of root without AMF explores 1-2 cm^3 of soil, while when inoculating AMF this volume increases between 5 and 200 times; normally, the infested soil volume is 12-15 cm^3, and exceptionally it has been up to 200 cm^3.

Siqueira and Franco (1988), when evaluating the effect of the inoculation with AMF in grasses under field conditions and in annual legumes at the moment of sowing, found dependence on mycorrhization in rye (36-167 %), millet (30-60 %), soybean (21-50 %), cowpea (20-50 %), common beans (10-39 %) and red clover (20-150 %). The yield increase (5-45 %) in this study, with regards to the absolute control, is within the range cited by these authors; who also stated that the application of AMF in forage legumes was feasible when they were used intercropped with grasses or in protein banks.

The above-mentioned authors also referred to the fact that with the application of 200 g m^{-1} (4 t ha^{-1}) in a soil with low phosphorus content a fast plant establishment, higher biomass production and effectiveness of the inoculant in time (residual effect) of 6-9 months,were achieved, which allowed to perform three cuttings per year. These results coincide with the ones in this research, although in this case the residual effect was higher, nine cuttings were made (including the establishment one) and the presence of AMF was detected.

In annual crops, the benefits of symbiosis are expressed 20-30 days after biofertilization; while in perennial crops, until after three months. In the South Pacific (states of Chiapas, Guerrero and Oaxaca) nine validation sites were established on lands of farmers, planted with beans (*Phaseolus vulgaris*), and yields of 910 kg ha^{-1} in the control and 1 335 kg ha^{-1} when applying *Glomus intraradices* (Aguirre-Medina, 2009).

The mycorrhizal biofertilizers that are commercialized at present have different forms of presentation (liquid or solid); they commonly contain soil as basis and show fine grind dust consistency, which can be easily incorporated to the seeds or applied to the plant material before sowing, such as the rooted stakes or the stolons of some tropical forage plants. The increasing demand of these biopreparations is due to the costs of inorganic (chemical) fertilizers and to the need of producing foodstuffs with a lower negative residual effect for the soils (Aguirre-Cadena, 2011). With the utilization of agricultural MicoFert satisfactory results can be obtained.

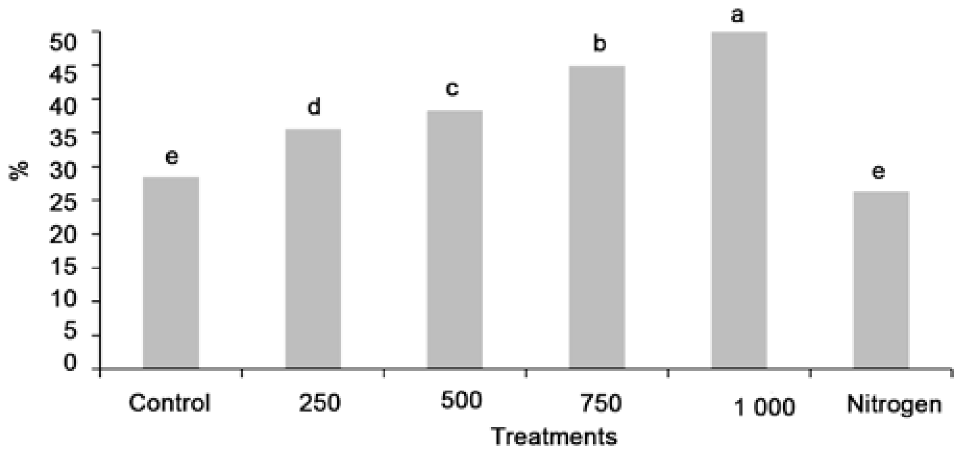
In this research, the colonization of rootlets increased proportionally to the rise of the biofertilizer doses (fig. 2). Likewise, there was colonization by AMF in the treatment with nitrogen and in the control, although with lower values, which implies the presence of native mycorrhizae. On the other hand, it is significant that after two years of applying MicoFert a high colonization of rootlets was found; this suggests that the AMF contained in it were established in the soil and were effective for the host plant, in spite of the time passed. However, in Cuba there are no references of the dosed application of agricultural MicoFert in production areas of forage legumes.

González *et al*. (2012), when evaluating the colonization by AMF in roots of legumes cultivated under field conditions, found that it increased rapidly with time (34 and 56 % 30 days after planting and up to 14 months later, respectively). These authors observed significant changes in the dry biomass yield, mainly due to causes that disturbed the soil.

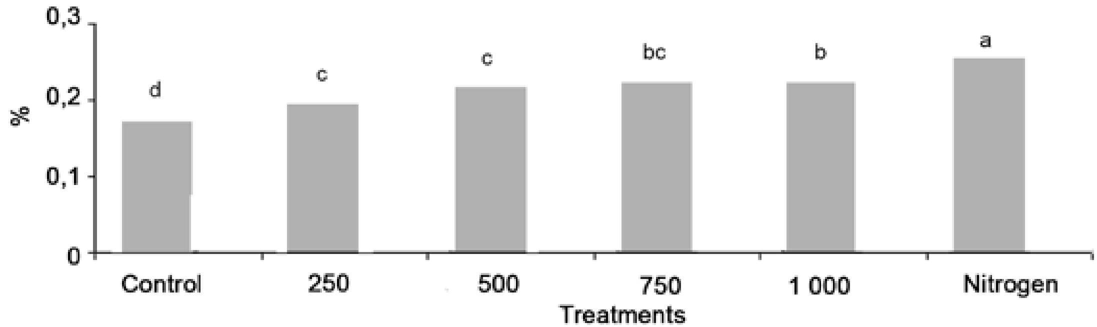
In a mesophilous evergreen forest of Sierra del Rosario, Herrera-Peraza *et al*. (2004) reported colonization percentages between 10 and 100, in grasses, shrubs and trees, similar values to the ones obtained in this study.

On the other hand, the leaf phosphorus content was significantly higher in the plants inoculated with the biofertilizer with regards to the control (fig. 3). This favorable effect of inoculation can be considered as an indicator of the efficiency of MicoFert.

Sieverding and Barea (1991) evaluated the effect of the inoculation of *L. leucocephala* cv. Campina Grande with *Glomus etunicatum* (currently *Claroideoglomus etunicatum* (W. N. Becker & Gerd.) C. Walker & A. Schüssler), *Acaulospora muricata*, *Gigaspora margarita* (W. N. Becker & I. R. Hall) and *Scutellospora heterogama* (currently *Dentiscutata heterogam*a (T. H. Nicolson & Gerd.) Sieverd., F. A. Souza & Oehl), and stated that all the indicators (DM, phosphorus and nitrogen content in plant) increased. These authors correlated the response to inoculation with the low phosphorus



a, b, c, d, e: values with different superscripts at $p < 0,05$.
Figure 2. Effect of the inoculation with AMF on rootlet colonization.



a, b, c, d: values with different superscripts differ at $p < 0,05$.
Figure 3. Influence of the inoculation with agricultural Micofert on the leaf phosphorus content.

content in the soil, which coincides with the findings in this study, because the soil used had 1,94 mg of P_2O_5 (low value, according to the agrochemical indicators).

When increasing the dose of the AMF inoculant a lower capture of phosphorus by the plant occurred, with which benefits were obtained in the absorption of this element, of low mobility in the soil. In that sense, it has been reported about the capacity of mycorrhized plants of capturing nutrients (nitrogen, calcium and boron) of the soil, through the network of hyphae and rootlets (Talbot and Treseder, 2010; Whiteside *et al*., 2012).

It is concluded that the application of agricultural MicoFert in forage areas of *L. leucocephala* cv. Peru increased the DM yield, with a mycorrhizal dependence between 13 and 40 %, proportionally to the increase of the doses. In addition, the favorable effect exerted by inoculation on the leaf phosphorus content was evident. The treatment with nitrogen and the control showed mycorrhizal colonization, which indicated the presence of native AMF.

Received: January 27, 2015

Accepted: April 06, 2015