Journal MVZ Cordoba
2019; 24(2):7256-7261.
https://doi.org/10.21897/rmvz.1703

Research article

Native arbuscular mycorrhizal fungi effectiveness in soils with different agricultural uses

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Received: July 2018; Accepted: March 2019; Published: June 2019.

ABSTRACT

Objective. To determine the effectiveness of mycorrhizal-arbuscular fungi (AMF) of soils with different agricultural uses, in the middle valley of the Sinú river, Colombia . **Materials and methods**. The experiment was carried out under greenhouse conditions, where *Leucaena leucocephala* was used as the indicator plant, which was planted in masons with an Andisol soil (horizon B) used as a substrate. This substrate was inoculated separately with samples from seven soils (Ap horizon), as a source of AMF, from the Turipaná Research Center in Agrosavia. As controls we included inoculation with HMA *Glomus fasciculatum* and one non-inoculated one. The effect of mycorrhizal inoculation was evaluated by monitoring the foliar P content in *L. leucocephala* plants, as a function of time to 64 days, when the dry mass of its aerial part and mycorrhizal colonization was determined. **Results**. The growth of *L. leucocephala* and the foliar P content was significantly higher when the soil was inoculated with *G. fasciculatum*, in comparison to that observed with the other treatments. Likewise, mycorrhizal colonization was very high in the roots of *L. leucocephala* that grew in the soil inoculated with G*. fasciculatum* and lower in the other treatments. **Conclusions.** The results indicate that the native AMF of soils with different uses, from Turipaná, exhibited low potential to develop mycorrhizal symbiosis, which limited the growth and concentration of leaf P in the host plant.

Keywords: Arbuscular mycorrhiza, *Leucaena leucocephala,* phosphorus, soil inoculation (*Source: AGROVOC*).

RESUMEN

Objetivo. Determinar la efectividad de hongos micorrizo-arbusculares (HMA) de suelos con diferentes usos agropecuarios, en el valle medio del río Sinú, Colombia. **Materiales y métodos.** Bajo condiciones de invernadero se realizó el experimento, donde se utilizó como planta indicadora *Leucaena leucocephala*, la cual se sembró en materos con suelo de un Andisol (horizonte B) usado como sustrato. Este sustrato, se inoculó separadamente con muestras de siete suelos (horizonte Ap), como fuente de HMA, provenientes del Centro de Investigación Turipaná de Agrosavia. Como controles se incluyeron inoculación con HMA *Glomus fasciculatum* y uno no-inoculado El efecto de la inoculación micorrizal se evaluó mediante el monitoreo del contenido de P foliar en las plantas de *L. leucocephala,* en función del tiempo hasta 64 días cuando se determinó la masa seca de su parte aérea y la colonización micorrizal. **Resultados**. El crecimiento de *L. leucocephala* y el contenido de P foliar fue significativamente superior cuando el suelo se inoculó con *G. fasciculatum,* en comparación a aquel observado con los demás tratamientos. De igual forma, la colonización micorrizal fue muy alta en las raíces de *L. leucocephala* que crecieron en el suelo inoculado con *G. fasciculatum* e inferior en los otros tratamientos. **Conclusiones.** Los resultados indican que los HMA nativos de suelos con diferentes usos, provenientes del C.I Turipaná de Agrosavia, exhibieron bajo potencial para desarrollar simbiosis micorrizal, lo que limitó el crecimiento y la concentración de P foliar en la planta hospedera.

Palabras clave: Fósforo, inoculación del suelo, *Leucaena leucocephala*, micorrizas arbusculares (*Fuente: AGROVOC*).

How to cite (Vancouver)
Martínez J, Osorio VN, Garrido PJ. Native arbuscular mycorrhizal fungi effectiveness in soils with different agricultural uses. Rev MVZ Cordoba. 2019; 24(2):7256-7261. DOI: https://doi.org/10.21897/rmvz.1703

ISSNe: 1909-0544

INTRODUCTION

Soil degradation affects 3600 million hectares in the world, which constitutes approximately a quarter of the entire land area (1). About 23% of the arable land in the world has been affected by degradation. Consequently, productivity levels have decreased. About 16% of the degraded lands are in Latin America, which is in third place, behind Asia and Africa (2). In Colombia, the agricultural productive activity has been accompanied by processes of deterioration of the natural environment, which affect its environmental and economic sustainability. The departments of the Caribbean Region are among the most eroded in the country. Most of them present erosion degradation figures between 78 and 100%, as a consequence of historical, socioeconomic and environmental circumstances (relief, climate, wind, among others) (3).

In Latin America, Colombia is the country with the highest percentage of land dedicated to grassland for livestock production (4). In the Caribbean Region, the bovine production system occupies 51% of its territory (5), this sector plays an important role in the country's economy, since it represents about 35% of the national herd, contributes 40% % of the volume of fresh milk and 38% of meat. However, despite the importance of this productive line in the national economy, the primary sector of this system faces constraints that influence the competitiveness and sustainability of productive indicators, particularly those related to soil and grassland degradation processes. This is reflected in the low biological and economic efficiency of the livestock systems, due to the low nutritional quality and supply of forage during the dry season of the year.

Due to the importance of livestock in the economy of the region, it is necessary to implement management alternatives that allow the conservation and / or, recovery of soils. The sensitivity of soil biological indicators allows observing the effects of management practices on the systems, as a simultaneous response to changes in the physical and chemical characteristics of the system. One of these indicators is the formation of the mycorrhizal association between soil fungi and their host plants.

The importance of arbuscular mycorrhizal fungi (AMF) in the nutrition of forage grasses and legumes, especially in the capture of phosphorus (P), ammonium and other elements that are not very mobile in the soil, has been the object of many studies (6). The increase in the volume of soil, explored thanks to the large number of fine AMF hyphae, seems to be, in large part, responsible for the considerable differences in survival, growth rate and yields between mycorrhized and non-mycorrhized plants, particularly in degraded soils. The effective symbiosis between grasses and AMF has been associated with the recovery of degraded soils thanks to the fact that the plant has a better nutrition due to the access to a greater volume of soil (7).

Therefore, this research sought to determine the effectiveness of HMA of soils with different uses (forest, transient crops of corn and cotton, degraded grassland and silvi-pastoral systems of different structural complexity) from the department of Córdoba.

MATERIALS AND METHODS

Location. An experiment was established in the greenhouse of the National University of Colombia, Medellín (6º15'N, 75º35'W and 1495 m altitude). Soil samples (horizon B) of an Andisol were used to fill plant pots at a rate of 690 g / plant pot. The soil was air dried, sieved at 4 mm, mixed in a 1: 1 ratio with quartz, and sterilized twice in an autoclave at 120 ºC and 0.1 MPa for one hour, with a period of 24 hours between each sterilization (8). The soil had a pH (1: 1, water) of 5.8 and an isotherm of P adsorption was made (9) to determine the amount of P required to obtain a P concentration in the soil solution of 0.02 mg L^{-1}; this concentration is considered optimal for mycorrhizal activity (10). At the time of sowing, P was applied at a rate of 588 mg kg^{-1}. KH_2PO_4 was used as source of P. Soil analysis of the substrate presented the following results : organic matter 5.5% (Walkley & Black); P 22 mg kg^{-1} (Bray II); Ca, Mg and K 5.2, 0.9 and 0.31 cmolc kg^{-1} (Ammonium acetate), respectively.

The soil of the plant pots was inoculated separately with 30 g/plant pot of surface soil samples taken from the first 10 cm depth (Ap horizon) from the Turipaná Research Center of -Agrosavia (8 ° 50'N, 75 ° 47 'W and 15 m altitude) located in the municipality of Cereté (Córdoba, Colombia). The soils were subjected to different uses and management as follows: (i) degraded grassland (Pd) with *Dichanthium aristatum* under intensive farming for more than 15 years, (ii) intensive system of corn-cotton (MA) in alternation, (iii) 14 years old secondary forest (Bs), (iv) grassland (Pr) with rotation between *Dichanthium aristatum* and *Panicum maximum*, (v) silvi-pastoral system constituted by a grassland of *Dichantium aristatum* and *P. maximum* with tree species (Pr + A), (vi) silvi-pastoral system constituted by pastures of D. aristatum and P. maximum with arboreal and shrub species (Pr + A + a), (vii) silvi-pastoral system constituted by pastures of *D. aristatum* and *P. maximum* with arboreal, shrub and timber species (Pr + A + a + M). As a positive control, a crude inoculum of *Glomus fasciculatum* (viii) from the collection of the Microbiology Laboratory of the National University of Colombia, Medellin Headquarters was used; this inoculum contained 40 infective propagules (spores, extraradical mycelium and colonized roots) per gram, which was determined by the most probable number technique (11). Additionally, a non-inoculated treatment was included as a negative control (ix).

Three germinated seeds of *Leucaena leucocephala* were sown in each pot, which were previously scarified with sulfuric acid for 30 minutes and washed six times with distilled water. This was used as an indicator plant due to its very high mycorrhizal dependence and fast growth (10). The pots were randomly distributed and received water to maintain the soil between 50-60% of the maximum water retention capacity. Once a week, 25 cm^3 of the P-free Hoagland solution was applied (10).

Inoculation effect. To evaluate the effect of mycorrhizal inoculation, the foliar P content in the fourth pinnule of the most developed young leaf of *L. leucocephala* plants (10) was monitored as a function of time (20, 31, 42, 53 and 64 days after sowing). At 64 days, the dry mass of the aerial part of *L. leucocephala* plants was determined after drying in an oven (60ºC) for 72 hours.

Colonization. Mycorrhizal root colonization was determined after clarification with KOH (10%) (12) and staining with acid fuchsin (13) and then determining the extent of colonization by the intercept method of the grid (14).

Experimental design. A completely randomized experimental design was used. The treatments consisted of separate inoculations with seven samples of the soils described above, inoculation with *G. fasciculatum* and the non-inoculated control, for a total of nine treatments. Each treatment had four repetitions, for a total of 36 experimental units.

The data were subjected to analysis of variance (ANOVA) and the comparison of means was made with the minimum significant difference test (LSD), with a level of significance (P) of 0.05. Analyzes were performed with the statistical package SAS version 8.

RESULTS

The content of foliar P in *L. leucocephala* plants was significantly higher when the soil was inoculated with *G. fasciculatum* compared to that observed with the other treatments (Figure 1). The favorable effects of inoculation with this mycorrhizal fungus were detected from day 42 after sowing; this effect was maintained until the end of the evaluation period (64 days) (Table 1).

Table 1. Levels of significance of the analysis of variance and coefficients of variation (CV) for the variables under study.

Variable	P20	P31	P42	P53	P64	MSA	CM
P-value	NS	NS	0.01	0.0001	<0.0001	<0.0001	<0.0001
CV (%)	22.2	20.8	27.1	14.2	20.1	19.3	22.4
LSD	0.76	0.69	0.89	0.39	0.56	0.18	2.61

P20-64: P foliar content on sampling days; MSA: aerial dry mass; CM: mycorrhizal colonization.

In the first two sampling dates (20 and 31 days), the content of foliar P was not significantly influenced by the source of inoculum used. The inoculation with the soil samples did not significantly increase the foliar P content with respect to the non-inoculated control in the different sampling dates (Figure 1).

The effect of the sources of the inoculi used was also easily observed in the aerial dry mass records of *L. leucocephala* (Figure 2) and corroborate the results indicated above. The *L. leucocephala* plants that grew in soil inoculated with *G. fasciculatum* exhibited an aerial dry mass significantly superior to that of the other treatments (Table 1). When the soil was inoculated with the other sources of inoculi, the aerial dry mass did not increase above that detected with the negative control treatment (non-inoculated).

The colonization of *L. leucocephala* roots by AMF was significantly different depending on the sources of inoculi (Table 1, Figure 3). Mycorrhizal colonization was very high in the *L. leucocephala* roots that grew in the soil inoculated with *G. fasciculatum* (63%). No mycorrhizal colonization was detected in the plant roots of the non-inoculated control. The mycorrhizal colonization of the roots with the other treatment sources of inoculi was very low or zero (≤ 4%).

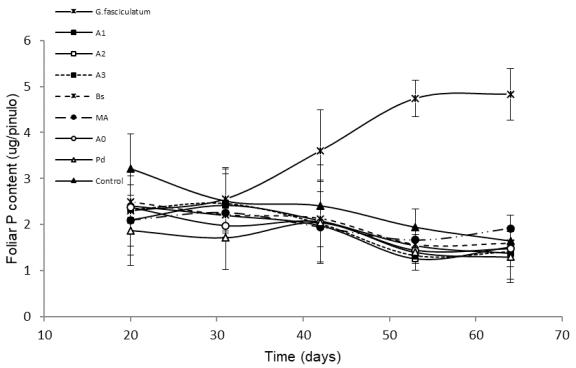

Figure 1. Content of foliar P in leucaena pínules depending on the inoculation with samples of soils under different use and management from the middle valley of Sinú, through the sampling time. The data of the first two dates did not differ according to the treatments. The bars represent the least significant difference (LSD) for p=0.05 between means.

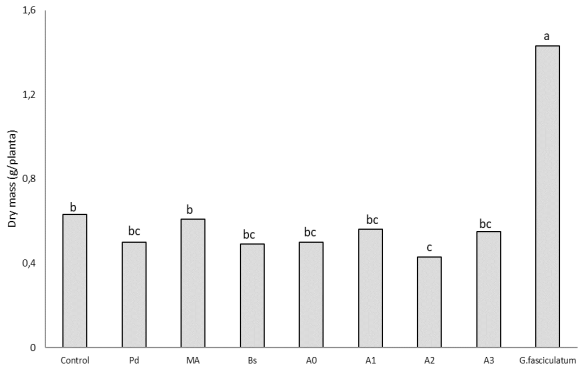

Figure 2. Aerial dry mass of *Leucaena* depending on the inoculation with soil samples under different use and management from the middle valley of Sinú.

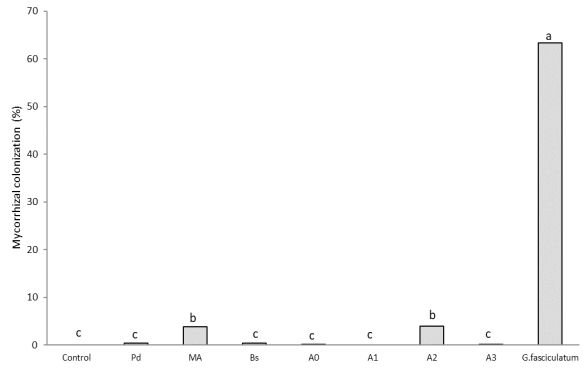

Figure 3. Mycorrhizal colonization of *Leucaena* roots as a function of inoculation with soil samples under different use and management from the middle valley of Sinú.

DISCUSSION

The results do not support the hypothesis that the effectiveness of AMF of the soils is a function of the type of coverage and management of the soil and that it can change according to the degree of complexity of the systems where the fungi are present. The soils used as a source of mycorrhizal inoculum exhibited a low mycorrhizal effectiveness and did not surpass the non-inoculated control. This low mycorrhizal effectiveness may be due to the physical-chemical conditions of the soils studied. Its fine fraction is dominated by clays of type 2: 1, which exhibit a high capacity for expansion and contraction depending on the level of soil moisture and given the marked seasonality of the rainy season and drought this is an important factor in the physical dynamics of these soils (15,16,17,18).

During the rainy season when the clay is expanded the circulation of oxygen in the soil can be limited, which would affect the AMF as they are aerobic. On the other hand, during the dry season the contraction of the material could generate strangulation of plant roots and eventually rupture of the mycorrhizal fungal hyphae. Several authors (19,20,21,22,23) have reported that by mechanically disturbing the soil, the hyphae of AMF are broken and, consequently, its viability and infectivity are reduced. Similarly, soils that were sources of mycorrhizal inoculi are exposed to periodic flooding and can remain saturated for up to three consecutive months a year. Such anaerobic conditions restrict the access of these fungi to oxygen, which obviously is unfavorable for their development (16).

Subsequent tests allowed to determine that the number of AMF spores was very low (11-22 spores/g) in the soil samples used as a source of mycorrhizal inoculum (Table 2), lower than values reported by studies on pastures of *Bothriochloa pertusa (L)* A. Camus from cattle farms in the municipality of Corozal in the department of Sucre, with records of 15 - 60 spores / g (24). It can be inferred that the spores present in the soils used as source of mycorrhizal inoculum in our study have lost their viability and are not capable of forming the mycorrhizal association. The values of colonization found in this study were less than 5%, this would explain the low mycorrhizal effectiveness detected in these soils; These results are in contrast with those observed in *D. aristatum* grasslands in farms of the coastal zone of the department of Sucre, where colonization exhibited values higher than 20% (25)., Thich is attributable to differences between environments and physical-chemical characteristics of soils.

The soils evaluate, before the establishment of the current production systems were under intensive livestock farming for not less than 15 years (26). Given the conditions of deficient internal and external drainage, these soils were compacted (27). It is considered that at the time, the plants of the silvi-pastoral systems were established because the soil is chemically very fertile and this allowed them to tolerate these physical-chemical and atmospheric conditions in some unfavorable periods (26). The plants of *L. leucocephala* have a very high dependence on the mycorrhizal association to absorb P (10). This is consistent with the results of the present study. If there is not enough viable mycorrhizal inoculum, the plants that depend on the AMF can disappear from the system, as has happened with *L. leucocephala* within the silvi-pastoral systems evaluated in this study.

Additionally, analysis of the concentration of soluble P in the soil ($CaCl_2$ 0.01M) allowed to detect that the availability of P in these soils is relatively high (Table 2) and is above the optimum level (0.02 mg L^{-1}) for the mycorrhizal activity. High concentrations of P in the soil solution can decrease the activity and colonization of AMF in soils (10, 28).

Table 2. Number of AMF spores and concentration of P in the soil solution of samples used as source of inoculum.

Soil/Cover	Spores AMF* (# g^{-1})	P concentration in the solution** (mg L^{-1})
Pr+A	11	0.17
Pr+A+a	12	0.19
Pr+A+a+M	14	0.23
Bs	17	0.15
MA	13	0.11
Pr	20	0.35
Pd	22	0.13
G.fasciculatum	25	0.02
Control	0	0.02

* *Wet sieving method*
** *0.01 M $CaCl_2$ y determination by phosphomolybdate*

The mycorrhizal symbiotic potential of a soil is the result of soil type, soil use and management practices (agricultural practices, mining), physical,-chemical, environmental and microbiological conditions, and the presence of plant species that may favor or disfavor the mycorrhizal association (29-32). The former explain the low symbiotic effectiveness of mycorrhizal populations in the studied soils. These results are comparable to those obtained (33) when inoculating a growth substrate with samples of degraded soils (as a source of inoculum); the substrate exhibited a low number of infective mycorrhizal propagules and there was no good mycorrhizal colonization. Therefore, there was no beneficial effect on the absorption of P and the plant growth of *L. leucocephala* plants.

In conclusion, the results indicate that the native AMF of soils with different evaluated uses, showed low potential to develop mycorrhizal symbiosis, which limits the growth and the concentration of foliar P in the host plant.

Interest conflict

The authors declare no conflicts of interest

Acknowledgments

This work was carried out with the financial support of the Ministry of Agriculture and Rural Development within the project 2007O5209-866 / 1466, led by la Corporación Colombiana de investigación-Agrosavia. The evaluations were carried out at the facilities of the Greenhouse and the Laboratory of Soil Microbiology of the National University of Colombia, Medellín.

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