Mycorrhizal Dependency of Alcaparro (*Senna pistaciifolia* Kunth) at Three Concentrations of Soil Solution Phosphorus

Dependencia Micorrizal de la Alcaparra (*Senna pistaciifolia* Kunth) bajo Tres Concentraciones de Fósforo en la Solución del Suelo

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Abstract. A greenhouse bioassay was carried out to determine the mycorrhizal dependency of alcaparro (Senna pistaciifolia Kunth). A randomized complete experimental design was employed, with six treatments in a factorial arrangement 3x2; the treatments consisted of three levels in soil solution phosphorus (P) (0.002, 0.02, and 0.2 mg L^{-1}) combined with two levels of mycorrhizal inoculation either uninoculated or inoculated with Glomus agreggatum. The variables studied were leaf P content as a function of time, shoot dry matter, shoot P content, mycorrhizal colonization of roots, and the mycorrhizal dependency (MD). The results indicate that the leaf P content increased significantly with the mycorrhizal inoculation in alcaparro to 0.02 mg L^{-1} after the second sampling days, but not in the other soil P levels. Likewise, shoot dry weight increased significantly at 0.02 mg L^{-1}. On the other hand, the total plant P content increase at all levels of soil available P. The mycorrhizal colonization in alcaparro roots was 11, 23, and 0% at 0.02, 0.002 and 0.2 mg of P L^{-1}, respectively. The MD for alcaparro was 32%, which allow classify this specie as moderately dependent on the mycorrhizal association.

Key words: *Glomus aggregatum,* phosphate, plant nutrition, soil restoration.

Resumen. Se realizó un bioensayo en invernadero para determinar la dependencia micorrizal de alcaparro (Senna pistaciifolia Kunth). Se utilizó un diseño experimental completamente randomizado. Los tratamientos se arreglaron en un factorial 3x2, estos consistieron en la combinación de tres niveles de P en la solución del suelo (0.002, 0.02 y 0.2 mg L^{-1}) y 2 niveles de inoculación micorrizal (inoculado y no inoculado) con el hongo Glomus agreggatum. Se emplearon como variables respuesta el contenido de P foliar en función del tiempo; al momento de la cosecha, se determinaron la masa seca aérea, el P total en la parte aérea y la colonización micorrizal. Los resultados indican que el contenido de P foliar a 0.02 mg L^{-1} en el segundo muestreo aumentó significativamente con la inoculación micorrizal, pero no en los otros niveles de P. La masa seca aérea también creció significativamente a 0.02 mg L^{-1}. De otro lado, el contenido de P total absorbido se incrementó significativamente en todos los niveles de P. La colonización micorrizal en las raíces del alcaparro fue de 11, 23 y 0 % para los niveles de 0.002, 0.02 y 0.2 mg L^{-1}, respectivamente. La dependencia micorrizal fue del 32 %, por lo que esta especie se clasifica como moderadamente dependiente.

Palabras clave: *Glomus aggregatum*, fosfato, nutrición de plantas, recuperación de suelos.

One of the most successful strategies developed by plants to overcome the stress during the colonization of terrestrial ecosystems is the formation of symbiosis with arbuscular mycorrhizal fungi (Vosátka and Albrechtova, 2009). These fungi form mutualistic associations with the roots of plant species (Habte, 2006), which produces significant benefits for the plant host such as increase in water and nutrient uptake, improve in soil aggregate formation and stability, promote tolerance to plant pathogen, and favor positive interactions with other beneficial microorganisms (Mansfeld *et al.*, 2002; Barea *et al.*, 2002; Elsen *et al.*, 2003; Johansson *et al.*, 2004; Osorio and Habte, 2013). Although for some plant species the arbuscular mycorrhizal association is required for nutrient uptake and growth, the degree of mycorrhizal dependency (MD) varies among plant species. The MD was defined as the degree on which a plant species depends on the mycorrhizal association to reach a maximal growth and development (Plenchette *et al.*, 1983).

In the last years in Colombia, several studies have been conducted to determine the MD of plant species such as *Nageia rospigliosii* (Diez *et al.,* 2008), *Solanum quitoense* (Gonzalez and Osorio, 2008), *Coffea arabiga* (Jaramillo and Osorio 2009), *Ocotea* sp. (Sierra *et al.*, 2009), *Persea americana* (Montoya and Osorio, 2009), *Calophyllum brasiliense* (Sierra *et al.*, 2012), among others. These studies have confirmed the significance of mycorrhizal inoculation to promote plant nutrition and growth of agronomical and forestry plant species,

particularly in soils with low phosphorus (P) availability, a major limiting factor for plant performance in the tropics (Osorio and Habte, 2013).

Among native forestry species in Colombia, alcaparro (*Senna pistaciifolia* Kunth) outstands because its use for restoration of degraded soils in Andean highlands, as a living fence, and as an ornamental plant (Alzate *et al*., 2012). Unfortunately, as it occurs with other plant species its growth and development is very slow, which impaired its use in reforestation and other uses, which has been associate to nutritional disorders particularly P deficiency (Haselwandter and Bowen, 1996; Rilling, 2004; Osorio *et al*., 2008; Diez 2011). Currently, there are not studies on nutritional requirements and the MD of alcaparro, which would allow land restorers and forestry managers to have a more effective use of this plant species. Then, the aim of this study was to determine its MD at three concentrations of soil solution phosphate.

MATERIALS AND METHODS

Site and soil preparation. This study was conducted in the greenhouse of the Universidad Católica de Oriente (6°9´15.2" N, 75°22´10.4" W, altitude 2.112 masl) (Rionegro, Antioquia - Colombia). The site had a mean air temperature of 17 °C, an annual rainfall of 1.700 mm, which correspond to ecological zone life of lower montane wet forest (Holdridge, 1967).

The soil in which plants grew was a sample of subsuperficial horizon (Bw, 30-50 cm) collected from and Andisol at the Forestry Station Piedras Blancas (6°15´38" N, 75°30´23" W, altitude 2.484 masl). This soil sample was used to reduce the influence of organic P on the level of soil solution P. The soil sample was air-dried, sieved at 4 mm, and then analyzed in the soil testing laboratory of the Universidad Nacional de Colombia at Medellín, Colombia. The results of these analyses were: sand 92%, silt 4%, clay 4%, soil texture sandy (Bouyucos), soil pH 5.7 (water, 1:1, V:V), soil organic matter 11% (Walkley and Black), calcium, magnesium, and potassium: 1.7, 0.1, and 0.05 $cmol_c$ kg^{-1} (1 M ammonium acetate), respectively; phosphorus 1 mg kg^{-1} (Bray II); iron, manganese, copper, and zinc 64, 1, 1, and 1 mg kg^{-1} (Olsen – EDTA); boron non-detectable (<0.1 mg kg^{-1}) (hot water); nitrate 3 mg kg^{-1} (0.025 M aluminum sulfate), and ammonium 18 mg kg^{-1} (1 M KCl). Details about the methods are available in Westerman (1990).

The soil pH was adjusted to 5.9 with the addition of $CaCO_3$ (2 g kg^{-1}) to improve calcium availability and optimize soil solution P. The lime requirement was determined by a lime incubation method (Uchida and Hue, 2000). Then, the soil was steamed at 80 °C for one hour. Soil fertilization was carried out with: urea 0.17 mg kg^{-1}, $CaSO_4$ $2H_2O$ 0.73 mg kg^{-1}, $MgSO_4.7H_2O$ 0.93 mg kg^{-1}, and K_2SO_4 0.21 mg kg^{-1}. Each planted bag received biweekly 25 mL of a P-free nutrient Hogland solution: (in mg L^{-1}) N 50, K 132, Mg 106, S 204, Zn 10, Cu 5, B 0.8, and Mo 0.5. The soil moisture content was maintained between 39 and 47%, which corresponded to 50-60% of the maximal holding water capacity, respectively.

Host plant. The seeds of alcaparro were collected from mature and healthy trees planted in LLanogrande region at the Rionegro municipality (Antioquia, Colombia). These seeds were rinsed with distilled water and disinfected with 1% sodium hypochlorite solution and then transferred to germinate in sterile sand (Pedroza y Tupaz, 2008). Once germinated, each seedling was transplanted into plastic pots with sterile sand until reach 10 cm of height and finally transplanted in the plastic bags with the respective treatment.

Treatments. In addition to the soil fertilization described above, P was added as KH_2PO_4 in order to achieve three concentrations of soil solution P: 0.002, 0.02 and 0.2 mg L^{-1}, according to Habte and Manjunath (1991) method for MD studies. To this purpose, an isotherm of P sorption was conducted with the Fox and Kamprath (1970) method (Figure 1). The amounts of KH_2PO_4 added were 0.0, 3.8, and 17.6 g kg^{-1}, respectively. The sterile and fertilized soil was then transferred into plastic bags (diameter 18 cm and high 29.5 cm) over a greenhouse bench.

Before transplanting, the soil was inoculated (M) with 20 g kg^{-1} of a crude inoculum, which contained the mycorrhizal fungus *Glomus agreggatum* (Schenck and Smith modified by Koske) strain "UH-2002" suspended in a matrix soil: sand. The inoculum contained 8,500 infective mycorrhizal propagules per kg, which was determined by the most probable number technique (Porter, 1979). This fungus was originally supplied by M. Habte from the University of Hawaii (Honolulu, USA) and then multiplied in sorghum and kudzu roots following the method propossed by Habte and Osorio (2001). Its effectiveness to promote plant P uptake and growth has been extensively probed in a

series of studies (Habte and Manjunath, 1991; Osorio and Habte, 2001; Jaramillo *et al.*, 2004; Diez *et al.*, 2008; Sierra *et al.*, 2009, 2012) with native forestry species. The uninoculated soil received 20 g kg $^{-1}$ of

the autoclaved crude inoculum and 20 mL of filtrates from a 10% suspension made with the crude after removing mycorrhizal propagules fungal Whatman No. 1 filter paper.
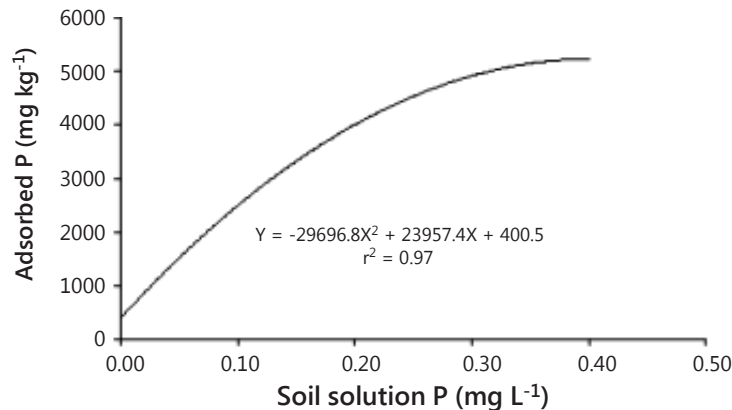

The plot shows the curve with equation:

$$Y = -29696.8X^2 + 23957.4X + 400.5$$
$$r^2 = 0.97$$

with x-axis "Soil solution P (mg L^{-1})" and y-axis "Adsorbed P (mg kg^{-1})".

Figure. 1. Isotherm of P sorption for the soil from Piedras Blancas Forestry Station.

Measured variables. Leaf P content was monitored as a function of time in the youngest mature leaf following using the non-destructive sampling method developed by Aziz and Habte (1987). To this purpose, a leaf-disk (6 mm of diameter) was collected from this leaf, leaf samples were collected at 148, 179, 213, and 227 days after planting; samples were then ashed in a muffle furnace at 500 °C for three hours. Then, the ashes were dissolved in distilled water and the solution P concentration was measured by the blue-molybdate method (Murphy and Riley, 1962; Habte and Osorio, 2001). At harvesting (227 days after planting), the shoot dry weight (SDW) was determined after oven-dry the samples at 60 ºC for 72 h. Shoot P content (SPC) was measured as the product of shoot dry weight and the leaf P concentration at harvest. To determine root colonization by the fungus, 100 root fragment (1 cm) from fine roots were collected from each replicate and then were separately rinsed with water, cleared by immersion in 10% KOH solution for 24 h (Phillips and Hayman, 1970) and stained with fucshin acid (Kormanik *et al.*, 1980) for 24 h. To quantify the presence of mycorrhizal structures we used the method of grid line intersection (Giovannetti and Mosse, 1980) in the 100 root fragments per replicate. The number of grid-line intercepts observed per sample was *ca*. 150.

The MD was evaluated as proposed by Plenchette *et al.* (1983) as the difference between SDW of inoculated and uninoculated plants, expressed as a percentage

of SDW of inoculated plants. To this purpose data of SDW were used at each level of soil solution P level.

The mean value of MD obtained at the soil solution P of 0.02 mg L^{-1} was compared with the categories proposed by Habte and Manjunath (1991).

$$MD = \frac{SDW_{inoculated\ plants} - SDW_{inoculated\ plants}}{SDW_{inoculated\ plants}} \times 100$$

Experimental design and data analyses. The experimental design was completely randomized; treatments consisted of a factorial arrangement 3x2. This is, three levels of soil solution P: 0.002, 0.02, and 0.2 mg L^{-1} and two levels of mycorrhizal inoculation; each treatment had five replicates; this means that we had 30 experimental units. Data were subjected to analyses of variance and the Fisher´s least significant difference (LSD) tests for mean separation at each level of soil solution P. Both tests were conducted with a significance level (*P*) 0.05 in the statistical software StatGraphics Plus version 4.0 (Statpoint, Inc., Herdon, Virginia, USA).

RESULTS AND DISCUSSION

The mycorrhizal inoculation significantly increased the SDW of alcaparro seedlings, however, this effect varied with the level of soil soluble P (Figure 2A); for instance, at 0.002, 0.02, and 0.2 mg L^{-1} the magnitude

of the increase of SDW respect to non-inoculated plants was 38, 47, and 218%, respectively. However, the value of SDW was significantly lower at the highest level of soil soluble P (0.2 mg L^{-1}) in comparison with that at the 0.002 and 0.02 mg L^{-1}. It is worth to mention that the soil pH was adjusted to 5.9, which favors the solubility of P ions (H$_2$PO$_4^-$) (Osorio, 2012) and guaranteed the presence of soil soluble P in this soil.

The SPC was significantly increased by the mycorrhizal inoculation, but the magnitude of this effect also varied with the level of soluble P (Figure 2B). Thus, at 0.002, 0.02, and 0.2 mg L^{-1} the increase in SPC respect to the non-inoculated plants was 59, 96, and 225%, respectively. However, the value of SPC was lower at 0.2 mg L^{-1} than at the other soil P levels (Figure 2B).
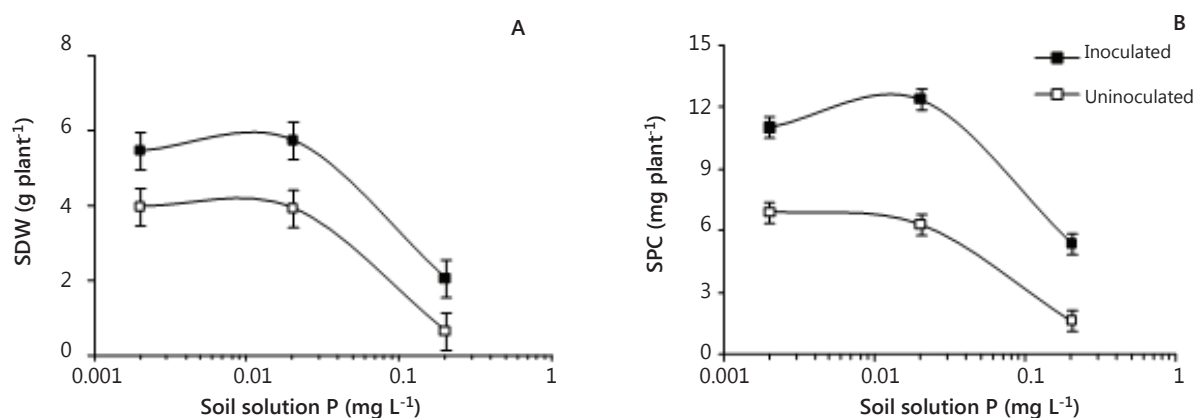

Figure 2. (A) Shoot dry weight (SDW) and (B) shoot P content (SPC) of alcaparro seedlings (*Senna pistaciifolia* Kunth) as a function of the soil solution P concentration and the mycorrhizal inoculation.

Previous studies conducted with *Leucaena* species (Fabaceae) inoculated with *G. agreggatum* have produced contrasting results; for instance, *L. leucocephala* and *L. diversifolia* had high levels of mycorrizal colonization in their roots even at high soil P levels (0.2 mg L^{-1}), while *L. retusa* and *L. trichodes* had lower levels of mycorrhizal colonization particularly at high soil P levels (Habte and Osorio, 2001), a tendency observed with alcaparro in the current study. Thus, the mycorrhizal colonization of alcaparro roots was 10.7, 22.9, and 0% at the soil soluble P levels of 0.002, 0.02, and 0.2 mg L^{-1}, respectively (Table 1). No mycorrhizal structures

Table 1. Mycorrhizal colonization in the roots of alcaparro seedlings (*Senna pistaciifolia* Kunth) as a function of the soil solution P concentration and the mycorrhizal inoculation.

Mycorrhizal colonization (%)					
0.002 mg L^{-1}		0.02 mg L^{-1}		0.2 mg L^{-1}	
Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
0	10.7	0	22.9	0	0

were detected in uninoculated plants. Although the mean foliar P content was higher in inoculated plants than uninoculated plants, at least at 0.002 and 0.02 mg L^{-1}, these differences were not significant, except at day 179 and at 0.02 mg L^{-1} (Table 2).

The results indicate that seedlings of alcaparro exhibited a MD of 32%, accordingly to Plenchete *et al.* (1983) formula. This plant species can be classified as moderately dependent on the mycorrhizal association

(Habte and Manjunath, 1991). From the ecological point of view, alcaparro can be considered a pioneer plant species because it grows in deforested land or out of the forests. Other pioneer plants of the same genera *Senna* such *S. macranthera* and *S. spectabilis* have exhibited certain degree of mycorrhizal dependency, which favor their growth (Siqueira *et al.*, 1998; Zangaro *et al.,* 2005). However, Janos (1980a, 1980b) said that pioneer plants either form or not the mycorrhizal association.

Table 2. Foliar P content (µg) in the leaf-disks of alcaparro seedlings (*Senna pistaciifolia* Kunth) as a function of the soil solution P concentration and the mycorrhizal inoculation.

Time (days)	Soil Solution P / Mycorrhizal inoculation						LSD*
	0.002 mg L^{-1}		0.02 mg L^{-1}		0.2 mg L^{-1}		
	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	
148	2.2	4.0	2.7	3.9	4.2	3.3	2.0
179	2.8	3.3	2.6	3.9	3.4	3.4	0.9
213	2.9	3.5	2.9	3.5	3.7	3.4	0.7
227	3.4	4.2	3.4	4.2	5.0	4.5	1.0

* Fisher´s least significant difference at a *P*-value ≤0.05

The slight variation in shoot dry weight and shoot P content between the lowest and medium soil soluble P (0.002 and 0.02 mg L^{-1}) suggest that alcapparro is well adapted to soil with low availability of P. At low levels of soil soluble P the fungus reduce the synthesis of sugars and phospholipids, which may increase the cell wall permeability and tranference to the cytoplasm (Genre y Bonfante, 1998). On the other hand, the poor plant performance at the high concentration of soluble P (0.2 mg L^{-1}) seems to confirm this point. Also, it has been observed that at high levels of soil soluble P the root are more colonized by hyphae and less by arbuscules (the structures that exchange carbon from the plant to the fungus and nutrient, such P, from the fungus to the plant) (Osorio, 2012). High concentrations of soil available P can diminish the availability of some nutrients such as iron, manganese, copper, and zinc (Havlin *et al.,* 2004). This may explain the decrease in plant growth and P uptake observed (Figure 2A, 2B). Similar results have been observed with other native species of the Andean mountains such as Laurel (*Ocotea* sp.) (Sierra *et al.*, 2009), Barcino (*Calophyllum brasiliense*) (Sierra *et al.,* 2012), Sweet granadilla (*Passiflora ligularis*) and Banana passion fruit (*P. tripartita*) (Osorio, 2012). For these reasons, we are reconsidering the use of the high level of soil solution P (0.2 mg L^{-1}), at least for further studies of mycorrhizal dependency of forest species originated from high montane ecosystems. At these high levels of soil soluble P (0.2 mg L^{-1}), these species had shown visual symptoms such as leaf and root necrosis likely associated to physiological disorders. Also, at this level the mycorrhizal colonization is impaired, which has been reported by some authors (Allen, 1996; Smith and Read, 1997; Habte and Osorio, 2001, Diez *et al.*, 2008; Montoya and Osorio, 2009) and this current study.

From the practical point of view, these results suggest that the propagation of alcaparro seedlings at nursery should be accompanied by moderate application of P fertilizers (enough to reach a soil solution P concentration of 0.02 mg L^{-1}) and by mycorrhizal inoculation. This clearly will promote the plant growth and the chance for a good establishment at the field will be high. On the other hand, since the arbuscular-mycorrhizal association is not specific for any plant-fungus combination (Walker y Trappe, 1993), it is possible to use any kind of fungus (e.g., *G. aggregatum*). However, it is likely to find better results with certain fungi in some plant species, which must be studied in the future not only for alcaparro but also for other plant species. In general terms and for practical reasons the use of an inoculum containing multi-species of arbuscular mycorrhizal fungi may be satisfactory in commercial nurseries.

CONCLUSIONS

The leaf P content increased significantly with the mycorrhizal inoculation in alcaparro to 0.02 mg L^{-1} after the second sampling days, but not in the other soil P levels. Likewise, shoot dry weight increased significantly at 0.02 mg L^{-1}. On the other hand, the total plant P content increase at all levels of soil available P. The mycorrhizal colonization in alcaparro roots was 23, 11, and 0% at 0.02, 0.002 and 0.2 mg of P L^{-1}, respectively. The MD for alcaparro was 32%, which allow classify this specie as moderately dependent on the mycorrhizal association.

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