

ARBUSCULAR MYCORRHIZAL COLONIZATION OF *Alnus acuminata* KUNTH IN NORTHWESTERN ARGENTINA IN RELATION TO SEASON AND SOIL PARAMETERS

ALEJANDRA GABRIELA BECERRA^{1*}; NILDA MARTA ARRIGO²; NORBERTO BARTOLONI³; LAURA SUSANA DOMÍNGUEZ¹ & MARÍA NOELIA COFRÉ¹

¹Instituto Multidisciplinario de Biología Vegetal (CONICET). C.C. 495. 5000 Córdoba. República Argentina.

*abecerra@efn.uncor.edu; ²Cátedra de Edafología, Facultad de Agronomía, UBA;

³Cátedra de Métodos Cuantitativos Aplicados, Facultad de Agronomía, UBA.

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ABSTRACT

The objective of this study was to determine patterns of arbuscular mycorrhizal (AM) colonization of *Alnus acuminata* Kunth at two natural forests in relation to soil parameters at two different seasons (autumn and spring). The soil parameters studied were field capacity, pH, electrical conductivity, available P, total N and organic matter. The percentage of AM colonization was estimated and correlated to soil properties and to two different seasons. The results indicate that the percentage of AM colonization varied among soil types and was higher in spring than autumn. A significant positive correlation was found between AM colonization and electrical conductivity, organic matter and total Nitrogen. Results of this study provide evidence that AM colonization of *A. acuminata* can be affected by some soil parameters and seasonality.

Key words. *Alnus*, arbuscular mycorrhizal, seasonality, soil type, Yunga forest.

COLONIZACIÓN MICORRÍFICO ARBUSCULAR DE *Alnus acuminata* KUNTH EN EL NOROESTE ARGENTINO EN RELACIÓN A LA ESTACIONALIDAD Y A LOS PARÁMETROS EDÁFICOS

RESUMEN

El objetivo de este estudio fue determinar el patrón de colonización micorrízico arbuscular (MA) de *Alnus acuminata* Kunth en dos bosques del Noroeste Argentino, en relación a los parámetros edáficos y en dos estaciones del año (otoño y primavera). Los parámetros edáficos estudiados fueron: capacidad de campo, pH, conductividad eléctrica, fósforo disponible, N total y materia orgánica. Se cuantificó el porcentaje de colonización MA y se lo correlacionó con las variables estudiadas (parámetros edáficos y estaciones). Los resultados indican que el porcentaje de colonización MA varió entre los dos tipos de suelo y las estaciones, siendo mayor en primavera. Se observó correlación positiva significativa entre el porcentaje de colonización MA y la conductividad eléctrica, materia orgánica y N total. Los resultados de este estudio indican que la colonización MA de *A. acuminata* puede ser afectada por algunos parámetros edáficos y la estacionalidad.

Palabras clave. *Alnus*, micorrizas arbusculares, estacionalidad, tipo de suelo, bosque de las Yungas.

INTRODUCTION

In South America, *Alnus acuminata* Kunth (Andean alder) forests are distributed along the Andes Mountains between 400 and 3,000 m a.s.l. at latitude of 28° S in Northwestern Argentina (Grau, 1985). Andean alder is mainly harvested for firewood, pulp, and timber. It is an important species recommended for management in land

reclamation, watershed protection, agroforestry, and erosion control (National Academy of Sciences, 1984).

Alnus acuminata is tolerant to infertile soils given its ability to form ectomycorrhizal (ECM), arbuscular mycorrhizal (AM) and actinorrhizal relationships (Cervantes & Rodríguez Barrueco, 1992). All these symbionts are known to be beneficial to the host, con-

tributing to a better nutritional status and pathogen defense and thus enhancing the capacity for establishment of individual plants and plant populations.

Previous studies on ectomycorrhizas of alder species in North America, Europe and South America, have shown that ectomycorrhizal symbionts are dominant on *Alnus* spp. roots (Miller *et al.*, 1991; Pritsch *et al.*, 1997a; Pritsch *et al.*, 1997b; Becerra *et al.*, 2002; Becerra *et al.*, 2005a). AM have been found in *A. rubra* Bong (Rose, 1980), *A. glutinosa* (L.) Gaertn. (Rose, 1980; Beddiar, 1984), *A. crispa* (Ait.) Pursh. (Daft, 1983), *A. incana* (L.) Moench (Averby & Ulf, 1998), *A. japonica* S. et Z. (Chattarpaul *et al.*, 1989) and *A. acuminata* (Albornoz, 1991; Becerra, 2002). However AM were not found on *A. rubra* and *A. glutinosa* by Miller *et al.* (1992) and Pritsch *et al.* (1997b) respectively.

In northwestern Argentina (Catamarca and Tucumán provinces) ectomycorrhizal colonization of *A. acuminata* ranged from 30 to 94% (Becerra *et al.*, 2005b). Meanwhile, in Calilegua National Park (Jujuy province, Argentina) dual colonization of *A. acuminata* was consistently found to be low for AM (0-8%) and high for ECM (23-96%) (Becerra *et al.*, 2005c). Based on these studies, *A. acuminata* is predominantly ectomycorrhizal and slightly arbuscular mycorrhizal. Although a low AM colonization might provide high benefits to plants an increased colonization could increase the cost of carbohydrates to plants (Berg *et al.*, 2001).

The importance of mycorrhizal fungi in the mineral nutrition of the host plant depends on the ability of the fungi to exploit sources of nonmobile nutrients in the soil. Root colonization by AM fungi is a dynamic process that may be influenced by the placement and density of their propagules in soil (spores, infected root fragments, extraradical hyphae), soil properties (soil type, fertility) or climate type (temperature, moisture), host factors (species, root longevity) and the fungal species (Amijee *et al.*, 1989; Smith & Read, 1997).

AM fungi are sensitive to physical, chemical and biological conditions (Hamel *et al.*, 1997). Studies on the distribution of AM fungi, quantification, identification, and biodiversity are important to understand the plant-fungi-soil interaction. However, there is a lack of knowledge on edaphic factors influencing mycorrhizae (as stated by Moyersoen *et al.*, 2001) with emphasis in South America.

Based on our previous studies (Becerra *et al.*, 2005b), we considered important to continue with the analysis of these under-studied environments, so aiming to help in the completion of a basic knowledge for the region. Thus, our objective for this work was to study the phenology of AM on *A. acuminata* in northwestern

Argentina sites in relation to some soil parameters (electrical conductivity, field capacity, pH, available P, organic matter and total N) and at two different seasons (spring and autumn) -chosen on the basis of fungus seasonality- (Brundrett *et al.*, 1996). The study sites have soils that belong to the Ustorthent order (these soils are young, with little depth, and no difference in the horizons) (Pritchett & Fisher, 1987). With these characteristics we expected to find poor levels of nutrients and an AM colonization affected by these nutrient levels. In this work, we studied the phenology of AM in *A. acuminata* to improve our knowledge of the ecology of *Alnus* forests and the mycorrhizal biology of this native plant for future re-vegetation programs.

METHODS

Study sites

Study sites were located in the NW of Argentina namely (NWA): 1) Quebrada del Portugués, Tafí del Valle, Tucumán Province, 26° 58' S 65° 45' W, with an elevation of 2187m; average annual precipitation ranges 1,200-1,500 mm, the soil is classified as Epileptic Regosol Eutric; and 2) Sierra de Narváez, Catamarca Province, 27° 43' S 65° 54' W, with an elevation of 1820 m, mean annual precipitation 698 mm, the soil is classified as Haplic Regosol Eutric (IUSS Working Group WRB, 2006). Mean annual temperatures range from 5.8 to 24 °C for NWA. The vegetation is a nearly homogeneous *A. acuminata* forest (height: 6-15 m, age: 20-30 years) with few herbaceous understory species such as *Duchesnea* sp., *Conyza* sp., *Axonopus* sp., *Selaginella* sp. and *Prunella* sp. (Aceñolaza, 1995).

Root and soil sampling

Twenty square plots (10 x 10 m) were established randomly within a homogeneous area (100 x 50 m) in each site during spring (November 1999) and autumn (May 2000). A mature tree (i.e. an individual producing female and male cone) with a trunk diameter of 10-25 cm was sampled inside each plot and one soil core of 15 x 15 cm² and 25 cm depth excavated inside the canopy at 15 to 50 cm distance from the tree. The majority of Andean alder roots occurred in the top 35 cm of the soil with a maximum distance of the stem of 50 cm. The samples were placed in plastic bags and stored at 4 °C during transport to the laboratory.

Arbuscular mycorrhizae analysis

In the laboratory, the roots were washed to remove soil and roots from other plants. Alder roots were easy to identify from the others by the presence of actinorrhizal nodules and their morphological appearance. Non-ectomycorrhizal roots

(approximately 200 root tips) were randomly sampled. They were placed in a 50 ml beaker containing 5 ml 20% KOH solution (clearing agent) and maintained at room temperature (22 °C) for 24 h. After clearing, the roots were rinsed in the beaker with tap water and transferred to another 50 ml beaker contained 5 ml of 2% HCl for 4 min. Roots were then transferred to a 50 ml beaker containing 5 ml of 5% Aniline blue. The beakers were maintained at room temperature for 24 h (Grace & Stribley 1991). After staining, the roots were stored for two weeks in 50% glycerin until percent root length colonization could be estimated.

Multiple root samples (approximately 25-30, 1 cm long root) from each plant were mounted on slides and viewed under a compound microscope at 400x magnification (McGonigle *et al.*, 1990). The presence of AM fungal structures was scored for 100 intersections of root and reticle line per plant. An intersection was considered mycorrhizal if the reticle intersected an arbuscule, a coil, a vesicle or an internal hypha attached to one of these structures. The colonization percentages are expressed as colonized intersects/total number of intersects x 100.

Soil analysis

Soil samples were air-dried and sieved (2 mm) and the ≤ 2 mm fraction was analyzed as follows. Electrical conductivity of a saturation extract was measured at 25 °C following Bower & Wilcox (1965). Field capacity was determined in a previously saturated sample of soil (1 cm thick), after being subjected to a centrifugal force of 1,000 times gravity for 30 min (Veihmeyer & Hendrickson, 1931). Soil pH was determined with a glass electrode in soil water relation 1:2.5 (w w⁻¹) (Peech, 1965). Available phosphorus was determined using the method Bray and Kurtz I (Jackson, 1964) by relating the spectral absorbance of the sample

and that of a standard. Organic matter content was determined following the method by Nelson & Sommers (1982). Total nitrogen was determined using the micro-Kjeldhal method (Bremner & Mulvaney, 1982).

Statistical analysis

AM colonization was not normally distributed, and data transformation was not suitable for parametric analysis. 80 data points from two sites, two seasons and twenty samples each were analyzed by Mann-Whitney *U*-tests for comparisons between sites and seasons. For soil x season interaction we used a test for non parametric analysis (Patel & Hoel, 1973). Associations between AM colonization and soil parameters were determined using the Kendall Tau non parametric tests correlations.

RESULTS

Both soils were slightly acidic, with low electrical conductivity but differed in texture and in nutrient content (Table 1). Due to the higher clay content, soils from Sierra de Narváez had higher contents of organic matter (3.65-3.78%), total N (0.35-0.38%), and field capacity (25.75-25.92% of dry weight) in both seasons than soils from Quebrada del Portugués, which had slightly higher levels in P in both seasons. The site at Sierra de Narváez presents a lower mean annual precipitation than that at Quebrada del Portugués (698 and 1,350 mm

Table 1. Soil properties in the two sites (Quebrada del Portugués -QP, Tucumán- and Sierra de Narváez -SN, Catamarca-) and the two seasons (autumn and spring) as analyzed from soil profiles taken during field work. Mean and standart error are values of twenty trees.

Tabla 1. Propiedades edáficas de los sitios (Quebrada del Portugués -QP, Tucumán y Sierra de Narváez -SN, Catamarca-) y las estaciones (otoño y primavera) analizadas a partir de los perfiles del suelo. Los valores son la media y el desvío estándar de veinte árboles.

Parameters	Autumn		Spring	
	QP	SN	QP	SN
EC (dS m ⁻¹)	0.18 ± 0.08	0.16 ± 0.05	0.43 ± 0.28	0.54 ± 0.34
FC (% of dry weight)	23.00 ± 4.28	25.92 ± 2.48*	20.01 ± 3.64	25.75 ± 6.14*
pH 1: 2.5	5.94 ± 0.23	6.18 ± 0.26*	5.37 ± 0.28*	5.14 ± 0.33
P (mg kg ⁻¹)	14.01 ± 3.99*	11.15 ± 3.27	13.48 ± 5.88*	8.32 ± 1.89
OM (%)	2.48 ± 0.99	3.65 ± 0.83*	1.73 ± 0.55	3.78 ± 0.62*
N (%)	0.25 ± 0.04	0.38 ± 0.07*	0.19 ± 0.04	0.35 ± 0.08*
Texture	Sandy loam	Loam	Sandy loam	Loam

EC: electrical conductivity, FC: field capacity, P: available phosphorus, OM: organic matter, N: total nitrogen. Significance between sites for each season are indicated by *, Tukey's test at $P < 0.05$.

respectively), mean spring and autumn temperatures were similar at both locations, with 17°C and 10°C respectively.

Arbuscular mycorrhizal fungal colonization on *A. acuminata* was characterized by inter- and intra-cellular oval vesicles, 16-26 µm diameter, walls smooth; inter- and intracellular hyphae, 2-12 µm diameter. We also observed simple and terminal arbuscules during autumn. Based on these morphological characteristics AM colonization in *A. acuminata* was *Arum-type* (Smith & Smith, 1997).

The colonization significantly differed between the two seasons (Mann-Whitney *U*-test: 463.00; $P < 0.01$). Percentage of AM colonization in autumn was 2.40% (Standard Error, S.E.= 3.52), meanwhile in spring was 6.15% (S.E.= 6.57) (Table 2).

For each type of soil, AM colonization was significantly different (Mann-Whitney *U*-test: 495.50; $P < 0.01$). The mean general level of mycorrhizal colonization in Quebrada del Portugués was higher than in Sierra de Narváez. Percentage of AM colonization in Quebrada del Portugués was 5.73% (S.E.= 6.0) with a range from 0 to 25%, meanwhile in Sierra de Narváez was 2.81% (S.E.= 4.72) with a range from 0 to 18% (Table 2). There was not a significant interaction of type soils x season ($P = 0.102$).

For Sierra de Narváez there was a very highly significant difference between seasons for AM colonization (Mann-Whitney *U*-test: 73.5; $P < 0.001$) (Table 2). Percentage of AM colonization was higher in spring than in autumn. There was not a significant difference between seasons for AM colonization for Quebrada del Portugués (Mann-Whitney *U*-test: 146.5; $P = 0.1479$) (Table 2).

Significant positive correlation was found between AM colonization and electrical conductivity, organic matter and total nitrogen in Sierra de Narváez during spring (Table 3). No significant correlation in Quebrada del Portugués were detected (Table 3).

DISCUSSION

The results of this study explain the influence of some soil parameters and differences between seasons on AM colonization of *A. acuminata* mountain forest in the northwestern of Argentina.

There have been few reports on the level of AM colonization in *Alnus* roots. In this study AM colonization of *A. acuminata* ranged from 0 to 25%. These results are in agreement with Becerra *et al.* (2005c) who obtained lower colonization on *A. acuminata* in Calilegua National Park. A possible reason for this low percentage of colonization could be the dual presence of ectomycorrhizal/arbuscular mycorrhizal symbiosis on *A. acuminata* roots, what may bring some competition effects. If ectomycorrhizal fungi colonize first the root, a physical barrier to AM penetration is established. However, some authors have found that in roots of some *Acacia* and *Eucalyptus* spp. both fungal symbionts can coexist without competition (Lapeyrie & Chilvers, 1985; Founoune *et al.*, 2002), what clearly shows that further analysis may be needed on this.

A. acuminata belongs to the Betulaceae family (Furrow, 1979). For this family few reports exist regarding their arbuscular mycorrhizal type. The mycorrhizal colonization of *A. acuminata* observed resembles the typical *Arum-type* (Smith & Smith, 1997) in their entirely inter- and intracellular spread of the hyphae and vesicles, and the arbuscules were always simple and terminal. Similar results were observed by Maremmani *et al.* (2003) in *Alnus glutinosa* (L.) Gaertn. roots.

At the two seasons of sampling, influence on the percentage of AM colonization was observed with the highest AM colonization in spring (Table 2). In contrast Becerra *et al.* (2005c) found higher colonization during autumn for *A. acuminata* in Calilegua National Park

Table 2. Percentage of AM colonization between seasons and sites (QP: Quebrada del Portugués, Tucumán province; SN: Sierra de Narváez, Catamarca province).

Tabla 2. Porcentaje de colonización MA entre estaciones y sitios (QP: Quebrada del Portugués, provincia de Tucumán; SN: Sierra de Narváez, provincia de Catamarca).

Variable	Autumn		Spring	
	QP	SN	QP	SN
% AM	3.94 ± 4.11 *	0.86 ± 1.90	7.54 ± 7.09 *	4.77 ± 5.85

% AM: Percentage of AM colonization. Significance between sites for each season are indicated as *, Mann Whitney non parametric test $P < 0.05$.

(Argentina). As Brundrett and Kendrick (1988) suggested for deciduous forest, arbuscular mycorrhizal plants show their root growth and mycorrhizal activity during spring season. This could be attributed to the low temperatures and the photoperiod during autumn and winter, which affect plant phenology and symbiotic activity (Brundrett & Abbott, 1994; Wilson & Hartnett, 1997). Factors such as soil moisture, nutrient pulse or host phenology can also affect AM colonization (Abbott & Robson, 1991; Sanders, 1993). Giovannetti (1985) found the highest AM colonization during the flowering period of *Ammophila arenaria* (L.) Link. *A. acuminata* flowered during spring and the highest AM colonization was found during this period. AM fungi are able to colonize roots without physical barriers formed by ectomycorrhizal fungi even in pine (Horton *et al.*, 1998), and uncolonized roots may be more abundant in the spring in our system.

Soils in the present study have low electrical conductivity (Table 1). The AM colonization was positively influenced by the higher electrical conductivity of loamy sand (Sierra de Narváez) (Table 3), which may be related to a higher availability of mineral nutrients. Similar results were obtained by Van Duin *et al.* (1989), Mendoza *et al.* (2000), and Hildebrandt *et al.* (2001).

AM colonization was affected positively by organic matter and total N in Sierra de Narváez (Table 3). These results are in contrast with Becerra *et al.* (2005c) who found negative correlation between organic matter and AM colonization with the same host. As Hayman (1982) suggested, some plants present high mycorrhizal colonization in soils with high levels of soil nutrients.

Trees (such as *Alnus acuminata*) add a lot of organic material each year to soils, and this organic matter may lead to higher mycorrhizal development. In general, AM is abundant in both poor and rich soils, which shows that low soil fertility is not a prerequisite for extensive mycorrhizal development (Hayman, 1982).

AM colonization was not correlated with P in the soil samples (Table 3). As Smith & Read (1997) stated, it is frequent that high P concentrations eliminate AM colonization, although it is well known that P availability influences percentage colonization more than its concentration.

Percentage of AM colonization was associated positively with electrical conductivity, organic matter and total N. Contrary to our expectations, the studied soils were not low in nutrients, but AM colonization was associated by these parameters. Although only some soil parameters were measured, others such as soil texture (Hamel *et al.*, 1997), bulk density (Hamel *et al.*, 1997), Ca, Cu, Fe, K, Mg, Mn, Zn (Cade-Menun *et al.*, 1991; Hamel *et al.*, 1997; Diagne *et al.*, 2001) and soil microorganisms (Haselwandter & Bowen, 1996) may affect the AM colonization.

This study partially explains how AM colonization of *A. acuminata* is affected by some soil parameters and seasonal changes. Future research could involve: 1) the dynamics of AM fungal diversity and density in the rhizosphere and their relationships between AM fungal spore production with soil parameters, 2) long term seasonal variations including winter and summer of the AM colonization on Andean alder and 3) relationships between AM, ECM and actinomycetes associated with

Table 3. Kendall-Tau coefficients of correlation between percentage of AM colonization, seasons and sites (QP: Quebrada del Portugués, Tucumán province; SN: Sierra de Narváez, Catamarca province).

Tabla 3. Coeficientes de correlación de Kendall-Tau entre los porcentajes de colonización MA, las estaciones y los sitios (QP: Quebrada del Portugués, provincia de Tucumán; SN: Sierra de Narváez, provincia de Catamarca).

Variables	Autumn		Spring	
	QP	SN	QP	SN
% AM vs. EC (dS m ⁻¹)	+ 0.273	+ 0.157	+ 0.177	+ 0.328*
% AM vs. FC (% of dry weight)	+ 0.229	- 0.115	- 0.167	+ 0.259
% AM vs. pH	- 0.139	+ 0.011	- 0.076	- 0.259
% AM vs. P (mg kg ⁻¹)	+ 0.042	+ 0.286	+ 0.162	- 0.105
% AM vs. OM (%)	- 0.080	- 0.132	- 0.266	+ 0.521*
% AM vs. N (%)	- 0.041	- 0.075	+ 0.060	+ 0.355*

% AM: Percentage of AM colonization, EC: electrical conductivity, FC: field capacity, P: available phosphorus, OM: organic matter, N: total nitrogen. Significance indicated as * $P < 0.05$.

A. acuminata. Further long-term studies are necessary to elucidate the ecological role of AM fungi in the forests of northwestern Argentina.

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