

# Ectomycorrhizal colonization of *Alnus acuminata* Kunth in northwestern Argentina in relation to season and soil parameters

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**Abstract** – The objective of this study was to determine patterns of ECM colonization of Andean alder 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. Twelve ECM morphotypes were found on *A. acuminata* roots. The ECM colonization varied among soil types and was affected positively by electrical conductivity. Multiple regression relationships among ECM colonization and edaphic properties variables showed no significant differences at two seasons and among soil types with respect to morphotype diversity values. Positive correlations were found between three morphotypes (*Cortinarius tucumanensis*, *Gyrodon monticola* and *Russula alnijorrulensis*) and soil types and two other morphotypes (*Naucoria escharoides* and *Lactarius* sp.) between two seasons. Results of this study provide evidence that ECM colonization of *A. acuminata* is affected by some chemical edaphic parameters and indicate that some ECM morphotypes are sensitive to changes in seasonality and soil parameters.

*Alnus acuminata* / ectomycorrhizal diversity / Andean forest / soil type

**Résumé** – Colonisation ectomycorrhizienne d'*Alnus acuminata* Kunth au nord-ouest de l'Argentine en relation avec la saison et quelques paramètres du sol. Le but de cette étude était de déterminer, au cours de deux différentes saisons (août et printemps), les modèles de colonisation de l'aulne andin dans deux forêts naturelles en relation avec quelques paramètres de sol. Les paramètres de sol étudiés étaient la capacité au champ, le pH, la conductivité électrique, le P disponible, le N total et la matière organique. Douze morphotypes de ECM ont été trouvés sur des racines de *A. acuminata*. La colonisation par les ECM varie en fonction des types de sols et est affectée positivement par la conductivité électrique. Les relations de régression multiple entre la colonisation de ECM et les variables de propriétés du sol n'ont montré aucune différence significative entre les deux saisons et entre les types de sol pour ce qui concerne des valeurs de diversité morphotypique. Des corrélations positives existent entre *Cortinarius tucumanensis*, *Gyrodon monticola* et *Russula alnijorrulensis* et les types de sol, et entre *Naucoria escharoides* et *Lactarius* sp. et les deux saisons. Les résultats de cette étude mettent en évidence que la colonisation de ECM de *A. acuminata* est affectée par quelques paramètres édaphiques chimiques et indiquent que quelques morphotypes de ECM sont sensibles aux changements des paramètres saisonniers et pédologiques.

*Alnus acuminata* / diversité ectomycorrhizienne / forêt Andine / type de sol

## 1. INTRODUCTION

*Alnus acuminata* Kunth (Andean alder) a member of the Betulaceae, is distributed along the Andes from Venezuela to latitude 28° S in northwestern Argentina [21]. Given its ability to form ectomycorrhizal (ECM), endomycorrhizal and actinorrhizal relationships [14], *A. acuminata* is tolerant to infertile soils. It grows rapidly and improves soil fertility by increasing soil nitrogen, organic matter, and cation-exchange capacity [21]. 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 [35].

From studies on ectomycorrhizae of alder species in North America, Europe and South America, it is known that ectomycorrhizal symbionts are dominant on *Alnus* sp. roots [9, 10, 31, 45, 46]. *A. acuminata* is associated with a number of ECM fungi belonging to the genera *Russula*, *Lactarius*, *Inocybe*, *Laccaria*, *Cortinarius*, *Naucoria*, *Alpova* [32, 47, 50]. Ectomycorrhizas are relatively specialized with a distinctive morphology and

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physiology [4]. Morphologically distinct ectomycorrhizas resulting from colonization by different fungi on the same host, may exhibit different physiological properties.

The importance of mycorrhizal fungi in the mineral nutrition of the host plant depends on the ability of the fungi to exploit sources of non-mobile nutrients in the soil. Factors such as root properties, soil or climate type, soil organisms, soil disturbance and host-fungus compatibility, may influence the occurrence and effectiveness of mycorrhizal associations [13]. Ectomycorrhizal activities have been reported to occur both in organic matter and in mineral horizons, at least to a depth of 35 cm [33].

Ectomycorrhizal species composition and diversity react to changing soil conditions [46]. Studies that focus on the relationship between edaphic factors and mycorrhizas are lacking as stated by Swaty et al. [52], Newbery et al. [38], Moyersoen et al. [34] and El Karkouri et al. [16]. This work was carried out to determine the phenology and diversity of the ECM in natural forests of *A. acuminata* in relation to some soil parameters (field capacity, pH, electrical conductivity, available P, total N and organic matter) at two different seasons (spring and autumn). The soils of this study belong to the Ustorthent order which represents young soils with little depth, and no horizontal differentiation [43]. With these characteristics we expected to find poor levels of nutrients and an ECM colonization affected by these nutrient levels.

## 2. MATERIALS AND METHODS

### 2.1. Sampling sites

The field sites were located in the NW region of Argentina (NOA), namely: (1) Quebrada del Portugués, Tafí del Valle, (Tucumán Province), elevation 2 187 m; 26° 58' S 65° 45' W, average precipitation between 1200–1500 mm, the soil is classified as Lythic Ustorthent; and (2) Sierra de Narvárez, (Catamarca Province), elevation 1820 m; 27° 43' S 65° 54' W, average precipitation of 698 mm, the soil is classified as Typic Ustorthent. Mean annual temperatures range from 5.8 to 24 °C for both locations. The vegetation is a nearly homogeneous *A. acuminata* forest (height 6–15 m, age 20–30 years) with few herbaceous understory plants such as *Duschesnea* sp., *Conyza* sp., *Axonopus* sp., *Selaginella* sp. and *Prunella* sp. [2].

### 2.2. Field collection and laboratory analysis

Twenty square plots (10 × 10 m) were established randomly at each site during spring 1999 and fall 2000. A mature tree (i.e. an individual producing female and male cones) with a trunk diameter of 10–25 cm was sampled inside each plot and one soil core of 15 × 15 cm<sup>2</sup> and 25 cm depth excavated at 15 to 50 cm distance from the tree. The majority of Andean alder roots occurred in the top 20 cm of the soil at both sites. The samples were placed in plastic bags and stored at 4 °C during transport to the laboratory.

### 2.3. Analysis of root samples

Every root sample was checked for ECM types and alder roots which were easy to identify due to their morphological appearance were separated. After mycorrhizae were cut off, they were sorted according to their morphological features (color, mantle layers, rhizomorphs, lactifers, etc.) under a Zeiss stereo microscope at × 10–40 magnification. For DNA-based identification, several tips of every

morphotype as well as small fruitbody pieces of potential mycorrhizal fungi were prepared for DNA extraction. For PCR, primers ITS1/ITS4 [58] were used and PCR conditions were as described by Henrion et al. [25]. PCR-products were subsequently cleaved with the restriction endonucleases *TaqI*, *HinfI* and *EcoRI*. Restriction patterns were compared visually, and for identical patterns fragment lengths were determined [9, 10]. For those morphotypes where no matches were found within the ITS-PCR/RFLP patterns, ITS-PCR products were sequenced in duplicate using ITS1 and ITS4 as the sequencing primers. The resulting sequences were aligned and the respective resulting consensus sequence was compared to the NCBI database using BlastN [Becerra et al., unpublished]. Unidentified mycorrhizas were termed according to Agerer [3] using the genus of the tree species completed by “rhiza” and a describing epithet. Twelve ECM types could be characterized in this way and they have been described in detail [8]. A brief description of their most prominent morphological and anatomical features is given in Table I.

### 2.4. Quantification

The percentage of root tips colonized by ECM was determined as described by Gehring and Whitham [19]. ECM roots were distinguished from non ECM roots by the occurrence of a fungal mantle. The roots in each sample were divided for operative reasons into three subsamples due to the large number of root tips per sample (200–400 tips). The roots of each subsample were randomly distributed on a tray of 54 equal compartments each measuring 2.5 × 2.5 cm and all the roots within the compartments were counted. Percentage ECM colonization was calculated as the number of ECM root tips divided by the total number of root tips [19]. Percent colonization for each ECM morphotype was calculated for each sampled tree by dividing the number of root tips of each ECM type by the total number of root tips, and multiplying by 100 [24].

Diversity of mycorrhizal morphotypes was calculated by Simpson's dominance index (SR) [49] using the mean relative percentage of each morphotype associated with each tree. Relative colonization of morphotype *t* on a root system was calculated by dividing the percentage of morphotype *t* by the total percentage:

$$SR = \left( \sum_{t=1}^m p_t^2 \right)^{-1}$$

where  $p_t$  is the relative colonization of ECM morphotype *t* and *m* is the number of ECM morphotypes. Simpson's diversity index tends to be less sensitive to sample size and minor species compared with other diversity indexes [23].

### 2.5. Soil analysis

Soil samples were air-dried and sieved (2 mm) and the ≤ 2 mm fraction was analyzed as follows. Field capacity was determined in a previously saturated sample of soil (1 cm thick), after being subjected to a centrifugal force of 1000 times gravity for 30 min [55]. Soil pH was determined with a glass electrode in soil water relation 1:2.5 (w/w) [40]. Electrical conductivity of a saturation extract was measured at 25 °C following Bower and Wilcox [11]. Available phosphorus was determined using the method Bray and Kurtz I [26] by relating the spectral absorbance of the sample and that of a standard. Total nitrogen was determined using the micro-Kjeldhal method [12]. Organic matter content was determined following the method by Nelson and Sommers [36].

### 2.6. Statistical analysis

The influence of two treatments (sampling dates and study site) and six independent covariates (field capacity, pH, electrical conductivity,

**Table I.** Brief description of morphological and anatomical characters of 12 morphotypes of *Alnus acuminata*.

Name	Mantle colour, type and thickness <sup>a</sup>	Root morphology	Emanating elements (hyphae diameter)	Hartig net <sup>b</sup>	Differentiating features
<i>Cortinarius helodes</i>	White to beige mantle, plect. (219 µm)	Simple ramification, straight to tortuous tips	Numerous hyaline hyphae with smooth surface (2–11 µm)	Paraep.	Thick mantle
<i>Cortinarius tucumanensis</i>	Silvery, whitish mantle, plect. (101 µm)	Simple ramification, tortuous tips	Numerous hyaline hyphae with smooth surface (3–8 µm)	Periep.	Tips with / without mantle
<i>Alnirhiza metalicans</i>	Silvery, whitish mantle, plect. (72 µm)	Simple ramification, straight to tortuous tips	Numerous hyaline hyphae with smooth surface (2–7 µm)	Paraep.	Many soil particles
<i>Lactarius omphaliformis</i>	Yellow to red brown mantle, pseud. (40 µm)	Simple to irregular pinnate, straight tips	Sparse hyaline hyphae with smooth surface (2 µm)	Periep.	Laticifers in the mantle
<i>Gyrodon monticola</i>	Yellow to light brown mantle, plect. (57 µm)	Monopodial to irregular pinnate, tortuous tips	Numerous hyaline hyphae (4 µm), brown (5 µm) with smooth surface	Paraep.	Brown cystidia on the mantle
<i>Tomentella</i> sp. 1	Brown mantle, plect. (51 µm)	Simple to irregular pinnate, straight tips	Numerous brown hyphae with smooth surface (2–6 µm)	Periep.	Acute tips
<i>Naucoria escharoides</i>	Yellowish to brown mantle, plect. (57 µm)	Simple to monopodial, straight tips	Numerous hyaline hyphae with smooth surface (2–5 µm)	Periep.	Usually tips without mantle
<i>Tomentella</i> sp. 2	Dark brown to black mantle, pseud. (70 µm)	Simple to irregular pinnate, flexuous tips	Sparse brown hyphae with smooth surface (2–5 µm)	Periep.	Abundant cystidia <sup>c</sup>
<i>Russula alnijorullensis</i>	Nacar to light brown mantle, pseud. (61 µm)	Simple to irregular pinnate, tortuous tips	Sparse hyaline hyphae with smooth surface (2–4 µm)	Paraep.	Laticifers in the mantle
<i>Tomentella</i> sp. 3	Yellowish to grayish mantle, plect. (60 µm)	Monopodial to irregular pinnate, tortuous tips	Numerous hyaline hyphae smooth surface (2–5 µm)	Periep.	Sparse hyphal bundles
<i>Lactarius</i> sp.	Yellow mantle, pseud. (35 µm)	Simple to monopodial pinnate, tortuous tips	Sparse hyaline hyphae with smooth surface (1–3 µm)	Periep.	Latex cells in the mantle
<i>Alnirhiza amarella</i>	Yellow to beige mantle, plect. (52 µm)	Simple to irregular pinnate, flexuous tips	Numerous hyaline hyphae with smooth surface (1–3 µm)	Periep.	Acute tips

<sup>a</sup> Plect.: plectenchymatous (hyphae of mantle recognizable as individual hyphae), pseud.: pseudoparenchymatous (hyphae of mantle simulating true parenchyma).

<sup>b</sup> Hartig net: Paraep.: Paraepidermal (penetrating only to the depth of the transverse walls of the epidermal cells), Periep.: Periepidermal (hyphae entirely encircle the epidermal cells) follows Godbout and Fortin [20].

<sup>c</sup> Urtical like cystidia, Type C [3].

P, total N and organic matter) upon the ectomycorrhizal colonization was first analyzed through an Analysis of Covariance (ANCOVA).

Multiple regression analysis (linear model) was used to examine the relationships between percentage ECM colonization as response variable [48], soil type and sampling dates. The normality assumption was tested through the Shapiro-Wilk test. No multicollinearity was detected among the independent variables. Additionally, inter-site and intra-site regression relationships between soil properties and ECM colonization were analyzed.

Kruskal-Wallis ANOVA test for ranks and  $\chi^2$  median tests were used to test for differences in the percentage of each morphotype as influenced by soil types and sampling dates, since most data did not follow the assumptions of analysis of variance (ANOVA) even after various transformations.

### 3. RESULTS

Both soils were slightly acidic, but differed in texture and in nutrient content (Tab. II). Due to the higher clay content, soils from Sierra de Narváez (Catamarca province) had higher con-

tents of organic matter and N, a higher field capacity and a higher electrical conductivity than soils from Quebrada del Portugués (Tucumán province), which had slightly higher levels in P. The site at Sierra de Narváez presents a lower mean annual precipitation than that at Quebrada del Portugués (698 and 1350 mm respectively). The values of soil water potential for autumn and spring were  $-0.025$  MPa and  $-0.018$  MPa for the Sierra de Narváez site and  $-0.017$  and  $-0.023$  respectively for the Quebrada del Portugués site, while mean spring and autumn temperatures were similar at both locations, with 17 °C and 10 °C respectively.

Ectomycorrhizal colonization of *A. acuminata* ranged from 30.3 to 94%. The ECM colonization on roots was not significantly affected by the two sites or the two sampling dates (Tab. III). There was only a slight interaction site x season effect. These results indicate that ectomycorrhizal colonization was not affected by sites or sampling dates. However, soil parameters (covariates) (field capacity, pH, electrical conductivity,

**Table II.** Soil properties of the two sites Quebrada del Portugués (Tucumán) and Sierra de Narváez (Catamarca) as analyzed from soil profiles taken during field work. Mean values of 20 trees. Significance indicated as \* ( $P < 0.05$ ).

Parameters	Quebrada del Portugués	Sierra de Narváez
Soil type	Lythic Ustorthent	Typic Ustorthent
Field Capacity (dry weight)	21.51 ± 2.12	25.83 ± 0.12*
pH 1: 2.5	5.20 ± 0.00	5.15 ± 0.00
Electrical conductivity (dS m <sup>-1</sup> )	0.11 ± 0.00	0.61 ± 0.59*
Available phosphorus (mg kg <sup>-1</sup> )	16.08 ± 1.19*	15.83 ± 2.94
Total nitrogen (%)	0.22 ± 0.04	0.36 ± 0.02*
Organic matter (%)	2.58 ± 0.93	4.53 ± 0.26*
Texture	Sandy loam	Loam

**Table III.** Results of ANCOVA of data from the Quebrada del Portugués and Sierra de Narváez sites and seasons.

Variable	Source of variation								
	Sites (Z)			Seasons (S)			Interaction (ZxS)		
	F	d.f.	P	F	d.f.	P	F	d.f.	P
Ectomycorrhizal colonization	0.816	1	0.369	0.497	1	0.482	3.405	1	0.069

**Table IV.** Results of ANCOVA within cells-regression (site and season combination) of data from the six soil properties studied.

Variable	Source of variation					
	Soil parameters			Error		
	F	d.f.	P	F	d.f.	P
Ectomycorrhizal colonization	2.639	6	0.022	0.497	70	0.482

available P, total N and organic matter) significantly influenced ectomycorrhizal colonization (Tab. IV).

Three out of the 12 morphotypes found on *A. acuminata* roots, showed significant differences in their percentage of occurrence as related to soil types (Tab. V). The ECM morphotypes *Cortinarius tucumanensis* Mos. and *Gyrodon monticola* Sing. were more common in the Typic Ustorthents than in Lythic Ustorthents, while *Russula alnijorullensis* (Sing.) Sing. was observed primarily in Lythic Ustorthents. The morphotypes *Naucoria escharoides* (Fr.:Fr.) Kummer and *Lactarius* sp. presented a different degree of colonization between sampling dates (Tab. VI). The morphotypes *Tomentella* sp. 1 and *Tomentella* sp. 3 were two of the ECM morphotypes regularly occurring at all investigated plots with an estimated proportion of 65% of all detected morphotypes.

Diversity (Simpson's diversity index) was not significantly different at the two seasons (H: 0.38;  $P$ : 0.5412) and the two types of soil (for spring, H: 0.84;  $P$ : 0.3644; for autumn, H: 0.61;  $P$ : 0.4396).

The polynomial function estimated by the multiple regression analysis showed that 18% ( $R^2 = 0.1845$ ;  $P < 0.05$ ) of the

**Table V.** Ectomycorrhizal colonization (%) by morphotypes in both soil types. Significance indicated as \*  $P < 0.05$ , \*\*  $P < 0.0001$ . Values are means of 40 trees for each type of soil at both seasons.

Morphotypes	Site	
	Quebrada del Portugués	Sierra de Narváez
<i>Cortinarius helodes</i>	1.237	0.352
<i>Cortinarius tucumanensis</i>	1.029	0.447*
<i>Alnijorhiza silkacea</i>	2.208	2.091
<i>Lactarius omphaliformis</i>	5.812	5.020
<i>Gyrodon monticola</i>	0.865	0.000*
<i>Tomentella</i> sp. 1	12.500	19.274
<i>Naucoria escharoides</i>	2.871	3.180
<i>Tomentella</i> sp. 2	5.335	1.964
<i>Russula alnijorullensis</i>	0.226	8.439**
<i>Tomentella</i> sp. 3	25.043	21.310
<i>Lactarius</i> sp.	0.067	1.647
<i>Alnijorhiza amarella</i>	0.542	0.000

overall variation in percentage may be explained through the variation in the independent variables (soil parameters, study sites and sampling dates). In both soil types, ECM colonization for all morphotypes together of *A. acuminata* was significantly affected only by electrical conductivity as indicated by partial regression ( $\beta = 0.378262$ ,  $t = 3.213958$ ,  $P < 0.05$ ).

The regression relationships among ECM colonization and edaphic properties at each combination of site and sampling

**Table VI.** Ectomycorrhizal colonization (%) by morphotypes in both seasons at both sites. Significance indicated as \*  $P < 0.05$ , \*\*  $P < 0.0001$ . Values are means of 40 trees for each season.

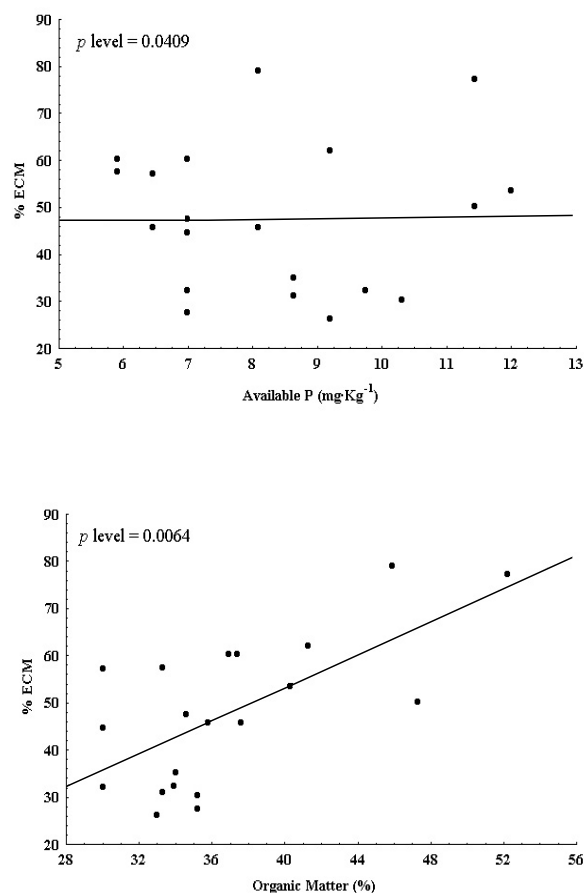
Morphotypes	Seasons	
	Autumn	Spring
<i>Cortinarius helodes</i>	0.869	0.720
<i>Cortinarius tucumanensis</i>	0.485	0.991
<i>Alnirrhiza silkacea</i>	1.707	2.591
<i>Lactarius omphaliformis</i>	4.691	6.141
<i>Gyrodon monticola</i>	0.527	0.337
<i>Tomentella</i> sp. 1	16.644	15.130
<i>Naucoria escharoides</i>	5.299	0.752**
<i>Tomentella</i> sp. 2	3.910	3.230
<i>Russula alnijorullensis</i>	4.371	4.295
<i>Tomentella</i> sp. 3	26.418	19.935
<i>Lactarius</i> sp.	1.715	0.000**
<i>Alnirrhiza amarella</i>	0.542	0.000

dates showed some significant differences. At Sierra de Narv ez (spring), the observed ECM colonization could be explained with a probability of 69% ( $R^2 = 0.6926$ ;  $P < 0.001$ ) to be slightly positively dependent on P and positively on organic matter (Fig. 1). At Quebrada del Portugu es (autumn), the observed ECM colonization could be explained with a probability of 65% ( $R^2 = 0.6597$ ;  $P < 0.05$ ) to be positively dependent on field capacity, pH and electrical conductivity, but highly significantly negatively dependent on P and negatively on total nitrogen (Fig. 2). No differences were detected between ECM colonization and Sierra de Narv ez in autumn ( $R^2 = 0.2084$ ;  $P: 0.747$ ), and ECM colonization and Quebrada del Portugu es in spring ( $R^2 = 0.2864$ ;  $P: 0.542$ ).

#### 4. DISCUSSION

The results of this study revealed a significant influence of some soil parameters on ECM colonization of *A. acuminata* forests in Argentina.

There have been few reports on the level of ECM colonization in *Alnus* roots. In this study ECM colonization of *A. acuminata* ranged from 30.3 to 94%, in contrast with the findings of other authors for tree genera such as *Picea* sp., *Betula* sp., *Populus* sp., which present high ECM colonization (> 85%) [6, 54, 56]. However, our low results are similar to those of Helm et al. [23], which observed 30–60% of ECM colonization in *A. sinuata* forests, but no further discussion on this is reported. On the other hand, Pritsch [44] found a high presence of ECM colonization in *A. glutinosa* forests, with values of 90%. A possible reason for this variation, and for our low percentage of colonization, could be the dual presence of ectomycorrhizal/endomycorrhizal symbiosis on *A. acuminata* roots, what may bring some competition effects. However, some authors have found that in roots of some *Acacia* and *Eucalyptus* spp. both fungal symbionts can coexist without competition [18, 27], what clearly shows that further analysis may be needed on this.

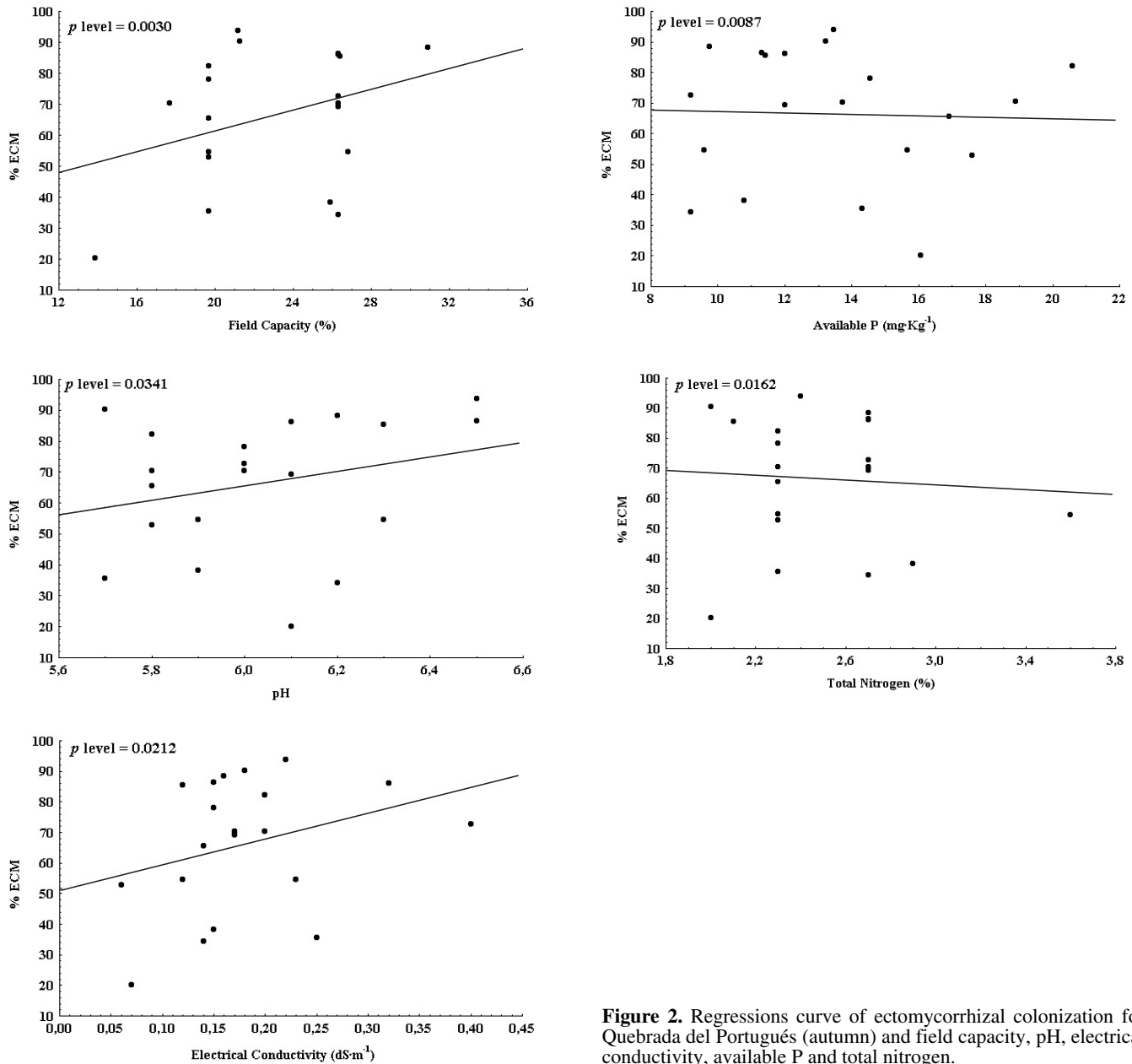


**Figure 1.** Regressions curve of ectomycorrhizal colonization for Sierra de Narv ez (spring) and available P and organic matter.

Few studies have focused on the ectomycorrhizal community of *Alnus* and these studies have reported low numbers of ectomycorrhizal types [5, 9, 10, 23, 31, 45, 46]. In this study, the morphotypes *Tomentella* sp. 1 and *Tomentella* sp. 3 were abundant (65% of all colonization). Taylor and Bruns [53] have stated that it “is clearly and excellent competitor in mature forest settings”, what would somehow explain its conspicuous presence also among *A. acuminata* forests.

We found twelve morphotypes associated with *A. acuminata*. The higher percentages of morphotypes *Cortinarius tucumanensis* Mos. and *Gyrodon monticola* Sing. in the Typic Ustorthents of Catamarca Province than in the Lythic Ustorthents of Tucum n Province, is probably due to the higher organic matter content in the Typic Ustorthent soil type (Tab. II). Soil organic matter provides nutrients and retains moisture, thereby contributing to ECM activity [17, 57]. This has also been suggested by Ogawa [39], who states that *Cortinarius* sp. as well as some Boletales (*Suillus* sp., *Gyrodon* sp.) grow in O (organic) or A (humus) horizons, indicating a preference of these fungi for horizons with higher organic matter content.

On the other hand, a higher occurrence of the morphotype *Russula alnijorullensis* (Sing.) Sing. was observed in Lythic



**Figure 2.** Regressions curve of ectomycorrhizal colonization for Quebrada del Portugués (autumn) and field capacity, pH, electrical conductivity, available P and total nitrogen.

Ustorthents. This result is consistent with those of Menge et al. [30] and Lee [29]. These authors found that mycorrhizae on *Pinus* sp. roots were promoted by decreasing amounts of organic matter in contrast with the results found by Ogawa [39], who describes the genus *Russula* in horizons of fertile soils rich in organic matter.

Sampling dates differences in morphotypes *Naucoria escharoides* (Fr.:Fr.) Kummer and *Lactarius* sp., are probably due to the sensitivity of these fungi to changes in soil organic matter. Higher occurrences of these fungi in the fall can be attributed to the carbon content (allocation) in mineral horizons which reaches its peak in this season [28]. This is normally

associated with the periods of greatest root growth and mycorrhizal activity (production of mycorrhizal fruit bodies and mycelial growth) [28]. In deciduous forests such as *A. acuminata* this corresponds to the stage of leaf senescence, when fresh organic substrates are deposited in the litter layer [28]. The results obtained in this work coincide with those of Persson [41] where mycorrhizal roots of conifers like *Pinus sylvestris* attain peak of mycorrhizal activity in late autumn, at the time when concentrations of labile forms of organic nitrogen such as amino acids are greatest in the soil [1].

Diversity (Simpson's diversity index) of ECM morphotypes in the two sampling dates and the two sites studied did not differ

significantly. Lack of differences in ECM diversity is probably due to the fact that both soils are the adequate substratum for the growth of the symbionts.

Soils in the present study have low electrical conductivity (Tab. II). The ECM colonization was positively influenced by the higher electrical conductivity of loamy stand (Sierra de Narváez), which may be related to a higher availability of mineral nutrients.

That only electrical conductivity affected ECM colonization in *A. acuminata*, which might be explained by the fact that the other soil parameters (field capacity, pH, available P, total N and organic matter) were not limiting factors for both, fungus and tree development. Although only some soil parameters were measured, others such as soil texture [7], bulk density [33, 51],  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^-$ , Al, Ca, Cu, Fe, K, Mg, Mn, Zn contents, CEC [5, 37] and soil microorganisms [15, 42, 51] could affect the ECM colonization.

At the two seasons of sampling, no influence on the percentage of ECM colonization was observed, in contrast to other studies, where seasonal variation in temperature, soil moisture, physiological and phenological changes in the host plant affected both symbionts [22, 52]. In this study climatic differences between the seasons were minimal (spring and autumn) [2]; which may be the reason for similar ECM colonization.

This study partially explains how ECM colonization and ECM diversity of *A. acuminata* is affected by some soil parameters and seasonal changes. Further long term studies with higher sampling frequencies are necessary to elucidate further aspects of ECM fungi, eventually some clues of their ecological relationships in the NW forests of Argentina.

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