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Ectomycorrhizal and arbuscular mycorrhizal colonization of *Alnus acuminata* from Calilegua National Park (Argentina)

Received: 8 June 2004 / Accepted: 25 February 2005 / Published online: 21 July 2005
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Abstract The objective of this study was to determine patterns of ectomycorrhizas (ECM) and arbuscular mycorrhizas (AM) colonization associated with *Alnus acuminata* (Andean alder), in relation to soil parameters (electrical conductivity, field H₂O holding capacity, pH, available P, organic matter, and total N) at two different seasons (autumn and spring). The study was conducted in natural forests of *A. acuminata* situated in Calilegua National Park (Jujuy, Argentina). Nine ECM morphotypes were found on *A. acuminata* roots. The ECM colonization was affected by seasonality and associated positively with field H₂O holding capacity, pH, and total N and negatively associated with organic matter. Two morphotypes (*Russula alnijorullensis* and *Tomentella* sp. 3) showed significant differences between seasons. Positive and negative correlations were found between five morphotypes (*Alnirhiza silkacea*, *Lactarius omphaliformis*, *Tomentella* sp. 1, *Tomentella* sp. 3, and *Lactarius* sp.) and soil parameters (total N, pH, and P). A significant negative correlation was found between field H₂O holding capacity and organic matter with AM

colonization. Results of this study provide evidence that ECM and AM colonization of *A. acuminata* can be affected by some soil chemical edaphic parameters and indicate that some ECM morphotypes are sensitive to changes in seasonality and soil parameters.

Keywords *Alnus* · Arbuscular mycorrhizas · Ectomycorrhizas · Soil parameters

Introduction

Alnus spp., (Betulaceae), are used in highland areas as cattle forage and as a firewood source in South and Central America, and they quickly spread over previously deforested areas (Dawson 1990). *Alnus acuminata* Kunth (Andean alder) is distributed along the Andes from Venezuela to 28°S latitude in NW Argentina; it is the southernmost species of the genus, growing between 400 and 3,000 m a.s.l. (Grau 1985; Halloy 1991).

Alder roots are associated with ectomycorrhizal (ECM), arbuscular mycorrhizal (AM), and actinorrhizal symbionts (Trappe 1962; Baker and Mullin 1992; Cervantes and Rodríguez Barrueco 1992). All of these symbionts are known to be beneficial to the host, contributing to a better nutritional status and pathogen defense and thus enhancing the capacity for establishment of individual plants and plant populations.

From studies on ectomycorrhizas of alder species in North America, Europe, and South America, it is known that ectomycorrhizal symbionts are dominant on *Alnus* spp. roots (Miller et al. 1991; Pritsch et al. 1997a,b; Becerra et al. 2002, 2005a). AM have been observed from *Alnus rubra* Bong. (Red alder) (Rose 1980), *A. glutinosa* (L.) Gaertn. (Hall et al. 1979; Rose 1980; Beddiar 1984), *Alnus crispa* (Ait.) Pursh. (Daft 1983), *A. incana* (L.) Moench (Chatarpaul et al. 1989; Averby and Ulf 1998), *A. japonica* S. et Z. (Chatarpaul et al. 1989), and *A. acuminata* (Albornoz 1991; Becerra 2002). However, AM infection was not found on *A. rubra* and *A. glutinosa* by Miller et al. (1992) and Pritsch et al. (1997b), respectively.

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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. 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 (Brundett 1991).

Ectomycorrhizal species composition and diversity reacts to changing soil conditions and thus is an important ecological parameter for the performance of a tree species (Pritsch et al. 1997b). AM fungi are sensitive to physical, chemical and biological soil conditions (Bowen 1987; Wilson and Tommerup 1992; 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 Swaty et al. 1998; Moyersoen et al. 2001; and El Karkouri et al. 2002), with emphasis in South America.

This work was carried out to determine the phenology of the ECM and AM in the *A. acuminata* mountain forest of Calilegua National Park (Argentina) in relation to some soil parameters (electrical conductivity, field H₂O holding capacity, pH, available P, organic matter, and total N) at two different seasons (spring and autumn).

Materials and methods

Sampling sites

The field site was in an area of Calilegua National Park rain forest (elevation of 1,700 m, 23°40'35"S 64°53'53"W). Mean annual temperature is between 10 and 15°C and mean annual rainfall is between 800 and 1,000 mm (Ramadori et al. 1996). Plant communities are dominated by *A. acuminata* Kunth (Betulaceae), *Acacia aroma* (Leguminosae), *Cedrela angustifolia* Sesse et Mocino ex DC. (Meliaceae), *Podocarpus parlatorei* Pilg. (Podocarpaceae), and *Ocotea puberula* (Nees. & Mart.) Nees. (Lauraceae). At the highest elevation of the forest, there are mono-specific or mixed forests of *A. acuminata*.

Field collection and laboratory analysis

Roots and soil were sampled in 12 plots (10×10 m) at the highest elevation of the park, where a mono-specific forest of *A. acuminata* grows. At each plot, one tree was sampled during spring (1999) and autumn (2000). One soil block of 15×15 cm to a depth of ca. 10 cm was taken from each of the 12 plots at each time. The samples were placed in plastic bags and stored at 4°C during transport to the laboratory.

Ectomycorrhizal analyses and quantification

Alder roots identified from the others by the presence of actinorrhizal nodules and ECM and AM roots were sorted based on their morphological appearance. After ectomycorrhizae were carefully cut off, they were further sorted according to their morphological features (color, mantle layers, rhizomorphs, lactifers, etc.) under a Zeiss stereo microscope at 10–40× magnification. Within every morphotype, several tips were prepared for DNA extraction, while others were subjected to comparative anatomical studies following Agerer's (1991) method. Unidentified ectomycorrhizas were termed according to Agerer (1991) using the genus of the tree species completed by "rhiza" and a describing epithet. Nine ECM types could be characterized in this way and they have been described in detail (Becerra 2002).

The percentage of root tips colonized by ECM fungi was determined as described by Gehring and Whitham (1994). Each sample was divided into three subsamples; the roots of each subsample were randomly distributed on a plastic plate grid of 9×6 compartments each measuring 2.5×2.5 cm. The percentage of ECM colonization was calculated as the number of ECM root tips divided by the total number of short roots (Gehring and Whitham 1994). Percent colonization for each ECM morphotype was calculated for each root system by dividing the number of root tips of each ECM type by the total number of root tips and by multiplying it by 100 (Helm et al. 1999).

Arbuscular mycorrhizae analysis

Nonectomycorrhizal roots were randomly sampled. They were placed in a 50-ml beaker containing 5 ml 20% KOH solution (clearing agent). The beakers were maintained at room temperature for 24 h. After clearing, the roots were washed and transferred to another 50-ml beaker containing 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 and Stribley 1991). After staining, the roots were stored in 50% glycerin until percent root length colonization could be estimated.

Five slides each with five to six randomly selected stained roots (approximately 25–30, 1-cm-long root) were prepared from every individual tree sample and mounted permanently in polyvinyl alcohol–lactic acid–glycerol (PVLG) (Koske and Tessier 1983). Quantification of AM root colonization was performed using the magnified intersection method (McGonigle et al. 1990) by inspecting intersections between the microscope eyepiece cross hair and roots at 400× magnification. A total of 100 intersects per sampling site were examined with a compound mi-

croscopically, recording the presence or absence of arbuscules, vesicles, intraradical and extraradical aseptate hyphae.

Soil analyses

The soils were classified as Inceptisols. Main soil physical and chemical characteristics are described in Vargas Gil and Bianchi (1981). For both seasons, soil samples were air-dried and sieved through a 2-mm sieve, and the fraction that was ≤ 2 mm was analyzed. The following variables were measured within each sample: electrical conductivity (measured in the extract) (Bower and Wilcox 1965), field H_2O holding capacity (Veihmeyer and Hendrickson 1931), soil pH using the soil water paste technique (1:2.5 soil: water) (Peech 1965), available phosphorus (Bray-Kurtz method 1, Jackson 1964), organic matter (Nelson and Sommers 1982), and total N (micro-Kjedhal method).

Statistical analyses

The influence of sampling dates (autumn and spring) and six independent covariates (electrical conductivity, field capacity, pH, P, organic matter, and total N) 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 ECM colonization as response variable (StatSoft Inc. 1995) and soil parameters. The normality assumption was tested through the Shapiro-Wilk test. No multicollinearity was detected among the independent variables. Associations between soil parameters, seasonality, and morphotype percentage colonization data were determined using the Spearman rank-order correlation coefficient (StatSoft Inc. 1995).

AM colonization was not normally distributed, and data transformation was not suitable for parametric analysis application. All data were analyzed statistically by Kruskal-Wallis. Associations between AM colonization and soil properties were determined using Spearman rank-order correlation coefficient (StatSoft Inc. 1995).

Results

Soils from the Calilegua National Park were acidic, with a sandy-loam texture, high content of organic matter, N and P, and high levels of field H_2O holding capacity and electrical conductivity (Table 1). There were no significant differences of soil characteristics between seasons.

Ectomycorrhizal colonization of *A. acuminata* in autumn was 85.6% [standard error (SE) 0.23] with a range from 70.4 to 95.6%; in spring, it was 62.0% (SE 3.96) with a range from 23 to 90%. The ECM colonization on roots was significantly affected by the two sampling dates ($P < 0.0001$) and soil parameters (electrical conductivity, field capacity, pH, available P, organic matter, and total N) used as covariates ($P < 0.001$).

Table 1 Soil properties for both seasons studied at Calilegua National Park

Parameters	Autumn	Spring
Soil type	Inceptisol	Inceptisol
Electrical conductivity (dS m^{-1})	0.50 \pm 0.25	0.37 \pm 0.13
Field capacity (%)	28.45 \pm 8.46	30.79 \pm 5.39
pH 1:2.5	4.18 \pm 0.29	4.38 \pm 0.59
Available phosphorus (mg kg^{-1})	20.40 \pm 8.01	25.55 \pm 12.67
Organic matter (%)	4.82 \pm 1.62	4.99 \pm 1.32
Total N (%)	0.32 \pm 0.17	0.42 \pm 0.29
Texture	Sandy loam	Sandy loam

No significant differences between seasons were found. Values represent the mean for 12 samples

The polynomial function estimated by the multiple regression analysis showed that 72% ($R^2=0.7224$) of the overall variation in percentage of ECM colonization may be explained through the variation in the independent variables (soil parameters). ECM colonization for all morphotypes together with *A. acuminata* was positively correlated with field H_2O holding capacity ($\beta=0.524$, $t=2.834$, $P<0.05$), pH ($\beta=0.567$, $t=2.859$, $P<0.05$), and total N ($\beta=0.771$, $t=3.787$, $P<0.01$) and negatively correlated with organic matter ($\beta=-0.693$, $t=-4.042$, $P<0.001$).

While nine ECM morphotypes were common in the soil at both sampling dates with no significant differences in their frequency, some morphotypes showed significant reactions to the site conditions (Tables 2, 3). The morphotypes *Russula alnijorullensis* (Sing.) Sing. and *Tomentella* sp. 3 presented a significantly different degree of colonization between sampling dates (Table 2). Variation in the percentage of ECM morphotypes was associated with some soil variables (Table 3). *Lactarius omphaliformis* Romagn. and *Lactarius* sp. percentages were associated positively with high total N and negatively with pH, while *Tomentella* sp. 1 was associated positively with pH and negatively with total N. *Alnirhiza silkacea* was associated positively with available P and *Tomentella* sp. 3 was associated negatively with pH.

Table 2 Ectomycorrhizal colonization (%) by fungi (morphotypes) in *Alnus acuminata* for both seasons at Calilegua National Park

Morphotypes	Seasons	
	Autumn	Spring
<i>Alnirhiza silkacea</i>	1.49 \pm 2.19	7.03 \pm 8.23
<i>Cortinarius helodes</i>	0.03 \pm 0.09	0.72 \pm 2.48
<i>Cortinarius tucumanensis</i>	0.70 \pm 1.12	1.71 \pm 2.21
<i>Lactarius</i> sp.	4.59 \pm 7.17	2.03 \pm 5.46
<i>Lactarius omphaliformis</i>	10.24 \pm 13.67	7.11 \pm 10.93
<i>Russula alnijorullensis</i>	0.00 \pm 0.00	7.99 \pm 18.17*
<i>Tomentella</i> sp. 1	24.76 \pm 31.54	12.52 \pm 18.96
<i>Tomentella</i> sp. 2	0.54 \pm 1.87	2.57 \pm 5.71
<i>Tomentella</i> sp. 3	44.04 \pm 25.64	17.86 \pm 18.07*

Significance between seasons are indicated as * $P < 0.05$. Values are means of 12 trees for each season

Table 3 Associations between ECM morphotype colonization in *Alnus acuminata* and soil parameters for both seasons

Morphotypes	E.C. ^a	F.C. ^a	pH ^a	P ^a	OM ^a	Nt ^a
<i>Alnirhiza silkacea</i>	NS	NS	NS	+	NS	NS
<i>Cortinarius helodes</i>	NS	NS	NS	NS	NS	NS
<i>Cortinarius tucumanensis</i>	NS	NS	NS	NS	NS	NS
<i>Lactarius</i> sp.	NS	NS	—****	NS	NS	+
<i>Lactarius omphaliformis</i>	NS	NS	—****	NS	NS	+
<i>Russula alnijorullensis</i>	NS	NS	NS	NS	NS	NS
<i>Tomentella</i> sp. 1	NS	NS	+	NS	NS	—
<i>Tomentella</i> sp. 2	NS	NS	NS	NS	NS	NS
<i>Tomentella</i> sp. 3	NS	NS	—**	NS	NS	NS

^aE.C. (dS m⁻¹), F.C. field H₂O holding capacity (%), pH (1:2.5), P (mg kg⁻¹), OM (%), Nt (%)

NS Not significant, + positive association, — negative association

**P* < 0.05

***P* < 0.01

****P* < 0.001

*****P* < 0.0001

Arbuscular mycorrhizal colonization of *A. acuminata* in autumn was 4.68% [standard error (SE) 2.23] with a range from 1 to 8.5%; in spring, it was 1.98% (SE 2.68) with a range from 0 to 8%. AM colonization differed between seasons (*K* 6.937, *P* < 0.01), and AM colonization was negatively associated with only two edaphic variables, field H₂O holding capacity (*R* −0.481, *P* < 0.05) and organic matter (*R* −0.468, *P* < 0.05).

Discussion

The very few studies that have focused on the belowground ectomycorrhizal community of *Alnus* reported low numbers of ectomycorrhizal types. Miller et al. (1991) defined 11 ectomycorrhizal types on *A. rubra* Bong., and Pritsch et al. (1997b) distinguished 16 ectomycorrhizal types on *A. glutinosa*. Nine morphotypes were observed on *Alnus sinuata* (Helm et al. 1996). We found nine morphotypes (in 12 samples) associated with *A. acuminata* in contrast to Becerra et al. (2005b), who found 12 morphotypes in *A. acuminata* (in 24 samples), although the same symbionts were found in both studies. These numbers are generally lower than those reported for coniferous trees, such as *Pinus* sp., *Picea* sp., which present high ECM morphotypes (Taylor and Bruns 1999; Dahlberg et al. 1997; Jonsson et al. 1999). Possible reasons for our low number of ECM morphotypes may relate to limited fungal associates with *Alnus* and inadequate sampling. Most studies that have analyzed species richness based on ectomycorrhizal root-tip data have not sampled enough to adequately capture all of the species in a stand (Horton and Bruns 2001). However, *Alnus* is known to associate with a low number of host-specific fungi (Molina et al. 1992), especially when compared to a host such as Douglas fir (*Pseudotsuga menziesii*), which can associate with some 2,000 species of fungi, most of which show a broad host range (Trappe and Fogel 1977). We therefore feel that the

low species richness observed here relates more to specificity phenomena rather than an artefact of low sample size.

Some soil parameters and seasonality affected ECM diversity and ECM and AM colonization in *A. acuminata*. At the two seasons of sampling, an influence on the percentage of ECM colonization was observed. Seasonal variation in temperature, soil moisture, physiological and phenological changes in the host plant affected both symbionts (Marx et al. 1970; Bowen 1970; Theodorou and Bowen 1971; Harvey et al. 1978; Swaty et al. 1998). In this study, we observed a higher ECM colonization in autumn than in spring. In autumn, labile forms of organic N like amino acids reach their zenith in soil (Abuarghub and Read 1988), and this may have contributed to the increased level of ECM colonization reported here.

It is known that mycorrhizal formation, in general, depends on the soil conditions (Baar 1995). In our work, ECM colonization was positively associated with field H₂O holding capacity, pH, and total N and negatively associated with organic matter.

Soil moisture is a very important soil parameter for ECM formation (Slankis 1974; Harvey et al. 1986). In our study, ECM colonization was positively associated with soil moisture. This is in concordance with other studies since drought has been shown to have a negative effect on mycorrhizal colonization (Harvey et al. 1978; Read and Boyd 1986; Lanzac et al. 1995; Nilsen et al. 1998). Higher values of field H₂O holding capacity were obtained during autumn, and the ECM fungi appeared to respond with higher levels of root colonization.

ECM fungi are generally considered to be acidophilous and tolerate a range of pH from 3 to 5 (Marks and Kozłowski 1973; Read 1991; Paul and Clark 1996). pH is an important soil parameter for the efficiency and distribution of ectomycorrhizal fungi (Danielson and Visser 1989; Erland and Söderström 1990). The pH range in this study was between 4.18 and 4.38, with a maximum ECM percentage at pH 4.18. Lee (1981) and Becerra (2002) observed a positive correlation between the highest values of pH and ECM colonization on *Pinus* spp. and *A. acuminata*. In the present work, ECM fungi were adapted to acidic soils.

ECM fungi are intimately associated with the litter layers for providing access to both inorganic and organic N compounds (Dames et al. 1999). N soil availability is the best predictor of ECM community effects because of its known direct effect on ECM growth (Wallander 1995; Lilleskov et al. 2001). In general, the ECM mycelium, which is the main functional part of the fungal biomass, can be affected by increased N availability. In this work, ECM colonization was positively associated with total N. In contrast, Lee (1981) found a negative correlation between total N and ECM colonization on *Pinus* spp. This can be explained by the broad physiological potential of ectomycorrhizas for N uptake and supplying this N to the plant host (Smith and Read 1997). Species of ECM fungi have been shown to vary in their response to soil N (Lilleskov et al. 2002).

ECM colonization decreased with higher amounts of organic matter. Our results are in concordance with Marx et al. (1977) and Lee (1981), who found that high amounts of organic matter in the soil suppressed the ECM colonization in *Pinus* spp. This decreased ECM colonization suggests that a number of interacting factors may be present including availability of nutrients, water relations, physical constraints on root growth, and chemical leaching from litter (Michelsen et al. 1995).

The percentage of colonization of morphotypes *R. alnijorullensis* and *Tomentella* sp. 3 varied by seasons with a higher rate of colonization during spring and autumn for each morphotype, respectively. This could be associated with periods of greatest root growth and mycorrhizal activity (production of mycorrhizal fruit bodies and mycelial growth) during spring and autumn (Leake and Read 1997). The dynamics of mycorrhiza formation by individual fungi depends on the growth of hyphae, the intrinsic rate of infection from propagules, and the capacity of fungi to use carbon substrates from host roots (Tommerup 1983; Wilson 1984; Nadarajah and Nawawi 1987; Pearson and Jakobsen 1993).

Correlations between morphotypes and soil parameters could be due to different species of fungi exhibiting different physiological properties (Mejstrik and Dominik 1969). They colonize the same substrate, but they may extract, adapt, or react to different components of the substrate (Erland and Taylor 2002). As Ogawa (1985) suggested, the floras of higher fungi in forests are decided by plant species composition, soil properties, and soil microbial floras and also vary continuously following the development of forest ecosystem.

The quantity of mycorrhizal root colonized by AM fungi within a soil can change throughout the season (Rosendahl et al. 1989). The arbuscular mycorrhizal colonization differed between seasons, with the higher rate occurring during autumn. These results are in agreement with Brundrett and Kendrick (1990), who obtained greatest root mycorrhizal activity in autumn and winter. In contrast, Becerra (2002) found higher colonization during spring for *A. acuminata*. These results could be the result of climate factors, soil moisture, nutrient pulse, or host phenology affecting AM colonization (Abbott and Robson 1991; Cade-Menun et al. 1991; Sanders and Fitter 1992; Eissenstat et al. 1993; Sanders 1993), but we can neither discard the possibility of a small sample size.

AM colonization was affected negatively by organic matter and field H₂O holding capacity. These results are in agreement with Becerra (2002), who found negative correlation between soil variables and AM colonization with the same host. Low AM colonization with high soil fertility (Mejstrik 1973; Hayman et al. 1976) may be due to low AM spore germination and/or reduced carbon allocation to mycorrhizal roots by the plants (Linderman 1997; Smith and Read 1997). With respect to soil moisture, Mejstrik (1965), Redhead (1971), Kahn (1972), Trinick (1977), and Cade-Menun et al. (1991) report similar results where high soil moisture reduced AM colonization.

This could be due to the low spore abundance and germination occurring at higher soil moisture (Daniels and Trappe 1980; Sylvia and Schenk 1983; Anderson et al. 1984). Competition with ECM fungi in soils with high fertility and soil H₂O capacity may also contribute to low AM colonization.

Although we only measured some soil parameters, the present results suggest that future research should focus on other soil parameters and seasonal variations that permit a complete comprehension of the ECM-AM-*A. acuminata* soil complex. In this study, we explain how ECM diversity and ECM and AM colonization is affected in two seasons by some soil parameters. This knowledge can be used in forest management and reforestation practices with *A. acuminata*.

Acknowledgements This work was partially supported by funding from PROYUNGAS (1999, 2001) to A.B. A.B. is grateful to FOMEC and CONICET for the fellowship provided. M.R.Z. is grateful to CONICET for the fellowship provided. This project was also supported in part from the US Forest Service and the NRI Competitive Grants Program/USDA award 99-35107-7843 to T.R.H.

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