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Arbuscular mycorrhizae of dominant plant species in Yungas forests, Argentina

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Abstract: In Argentina the Yungas forests are among the ecosystems most affected by human activity, with loss of biodiversity. To assess the arbuscular mycorrhizal (AM) colonization and the arbuscular mycorrhizal fungi (AMF) spore numbers in these ecosystems, the roots of the most dominant native plants (one tree, *Alnus acuminata*; three herbaceous, *Duchesnea indica*, *Oxalis conorrhiza*, *Trifolium* aff. *repens*; and one shrub, *Sambucus peruviana*) were studied throughout the year from two sites of Yungas forests. Assessments of mycorrhizal colonization (percent root length, intraradical structures) were made by washing and staining the roots. Soil samples of each plant species were pooled and subsamples were obtained to determine AM spore numbers. The herbaceous species formed both *Arum*- and *Paris*-type morphologies, whereas the tree and the shrub species formed respectively single structural types of *Arum*- and *Paris*-type. AM colonization, intraradical fungi structures and AMF spore numbers displayed variation in species, seasons and sites. *D. indica* showed the highest AM colonization, whereas the highest spore numbers was observed in the rhizosphere of *A. acuminata*. No correlation was observed between spore numbers and root length percentage colonized by AM fungi. Results of this study showed that *Alnus acuminata* is facultatively AM. The AM colonization, intraradical fungi structures and AMF spore numbers varied in species depending on phenological, climatic and edaphic conditions.

Key words: *Alnus* forests, AM intraradical structures, colonization-spore numbers correlation, montane cloud forest, seasonality

INTRODUCTION

Yungas forests have become some of the most widely affected ecosystems due to human activity, which has resulted in loss of biodiversity. To protect biodiversity, not only is it necessary to identify areas with large diversity of species, but it also is required that other areas should be preserved to maintain genetic and environmental variation (Brown et al 1993).

The montane cloud forest is one of the environmental units in Yungas forests. In this unit one of the most important plant communities is *Alnus acuminata* Kunth (Betulaceae) forests (Cabrera 1976). *Alnus acuminata* is tolerant to infertile soils, hence its ability to form ectomycorrhizal (ECM) (Becerra et al 2002, 2005a, b, c, d), arbuscular mycorrhizal (AM) (Becerra and Cabello 2007, Becerra et al 2007a) and actinorrhizal relationships with *Frankia* (Carú et al 2000), thus enabling it to fix atmospheric nitrogen in natural and disturbed soils (Cervantes and Rodríguez Barrueco 1992).

Alnus acuminata forests are monospecific as regards the tree stratum, with a high dominance of 95% (Bell 1991), whereas 186 species of shrubs and herbs compose the understory (Giusti et al 1996). Most of these plant species present symbiotic associations with arbuscular mycorrhizal fungi (AMF) (Becerra et al 2007b). These symbiotic associations, important for plant growth, succession and rehabilitation of deforested lands, are determined by features of the host plant and mycorrhizal fungus and regulated by soil and environmental factors (Janos 1996, Siqueira and Saggin-Júnior 2001). AM colonization usually promotes plant growth in natural ecosystems (Merryweather and Fitter 1995, Klironomos 2003) and AMF communities influence a number of important ecosystem processes, including plant productivity, plant diversity and soil structure (van der Heijden et al 1998, 2006; Vogelsang et al 2006).

Colonization rates are influenced by the placement and density of AM fungi propagules in soil, edaphic factors such as soil type and fertility, climatic factors

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such as soil temperature and moisture availability, host factors such as plant species and age of roots and fungal species identity (Cade-Menun et al 1991, Sanders and Fitter 1992, Sanders 1993). Differences in AMF communities have been found among plant species, ecosystems, locations and seasons (Bever et al 2001, Husband et al 2002, Öpik et al 2006) and also among parts of the root system.

Mycorrhizal diversity is considered an important factor in the establishment, survival and maintenance of plant community diversity (van der Heijden et al 1998, Smith et al 1999). Despite the important role played by mycorrhizal diversity in natural plant communities, little information is available on the AM dynamics in Yungas forests. The aim of this research was to study the AM colonization and AMF spore numbers of most dominant plant species from two sites of Yungas forests, analyze their morphological variation in fungal colonization and observe the presence of seasonal changes. This study will let us improve our knowledge on the ecology of Yungas forests and the mycorrhizal status of their dominant native plants.

MATERIALS AND METHODS

Research sites.—This study was carried out at two field sites in the Yungas in the northwestern region of Argentina (NWA), namely Quebrada del Portugués, Tafí del Valle (Tucumán Province) and Narváez Range (Catamarca Province). Details of the two study sites and phytosociological aspects have been reported by Becerra et al (2005d, 2007a). Results estimated from the Braun-Blanquet (1965) method, with an abundance scale of + to 5, allowed the selection of the plant to be studied. *Alnus acuminata* Kunth was the dominant tree species studied (Braun-Blanquet: 4/5). *Duchesnea indica* (Andrews) Focke (Rosaceae) (Braun-Blanquet: 2), *Oxalis conorrhiza* Jacq. (Oxalidaceae) (Braun-Blanquet: 2) and *Trifolium* aff. *repens* L. (Fabaceae) (Braun-Blanquet: 2) were the dominant herbaceous understory plants studied, whereas *Sambucus peruviana* Kunth (Caprifoliaceae) (Braun-Blanquet: 1) was the dominant shrub sampled.

Sampling of soil and roots.—Sampling of soil and roots was conducted in both locations in autumn (May 2001, dry season), winter (Jul 2002, dry season), spring (Nov 2002, rainy season) and summer (Mar 2002, rainy season). In each zone (Quebrada del Portugués and Narváez Range) one homogeneous site (30 × 30 m) was selected. In each site and during each season 10 samples of the dominant plant species were sampled. The root systems of all plants were carefully excavated to confirm connection between roots and shoots. The size of samples for *A. acuminata* trees and *S. peruviana* shrubs was 15 × 15 × 25 (depth) cm because the majority of *A. acuminata* and *S. peruviana* roots occurred in the top 20 cm soil (Becerra et al 2005d). For herbs (*D. indica*, *O. conorrhiza* and *T.* aff. *repens*) the whole root system was sampled. The samples were placed in plastic bags and stored at 4 C.

Soil analysis.—Soil samples were air-dried and sieved (2 mm grid) and the ≤ 2 mm fraction was analyzed as follows. Electrical conductivity of a saturation extract was measured at 25 C following Bower and Wilcox (1965). Field capacity was determined in a previously saturated sample of soil (1 cm thick), after being subjected to a centrifugal force of 1000 × *g* 30 min (Veihmeyer and 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 with the method of Bray and Kurtz I (Jackson 1964) by relating spectral and standard absorbance of the sample. Organic matter content was determined following the method of Nelson and Sommers (1982). Total nitrogen was determined with the micro-Kjeldhal method (Bremner and Mulvaney 1982).

Arbuscular mycorrhizal colonization.—Ten roots samples of each species were washed to remove soil and adhering organic particles. The root system of each plant was preserved with FAA and cleared and stained for observation (Phillips and Hayman 1970). Roots were cleared with 10% KOH (15 min at 90 C). Dark roots of *A. acuminata* and *S. peruviana* were bleached with 30% H_2O_2 (5 min, room temperature). The roots were acidified with 1% HCl (1 min, room temperature) and stained in 0.05% trypan blue.

Root samples (ca. 25–30, 1 cm long) for *A. acuminata* were mounted on slides and viewed under a compound microscope at 400× (McGonigle et al 1990) due to the presence of an ectomycorrhizal mantle. The presence of AM fungal structures for each season and site 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 were expressed as colonized intersects/total number of intersects × 100.

In the case of herbs and shrubs 10 samples of 100 root segments (1 cm long each) were analyzed for each season and site. Frequency of colonization and percentage of root length colonized (percent RLC) were quantified according to the grid-line intercept method (Giovannetti and Mosse 1980) under stereoscope-magnified Leica M 420. Ten colonized root fragments from herbs and shrubs of each sample (n = 100) were placed on slides, and the fungal intraradical structure, such as % arbuscules, number of vesicles and entry points, was estimated according to Ocampo et al (1980). Quantification of AM root colonization was classified in these categories: very high (>80%), high (60–79%), medium (40–59%), low (20–39%) and very low (1–19%), following Zangaro et al (2002).

AMF spore numbers.—Ten individual soil samples of plant species were pooled and thoroughly mixed. Three aliquots of 100 g soil were obtained for every plant species. Spores were extracted from soil samples by wet sieving and decanting (Gerdemann and Nicolson 1963), followed by centrifugation in water and in 80% sucrose solution (Walker et al 1982). A fine sieve (38 μm) was used to collect the spores, and the coarse material remaining on the top sieve (500 μm) also was checked for sporocarps and large spores. Only apparently healthy spores were counted under

TABLE I. Soil properties of the two sites, Quebrada del Portugués (QP, Tucumán) and Narvaéz Range (NR, Catamarca), as analyzed from soil profiles taken during field work

Parameters[a]	Quebrada del Portugués	Narvaez Range
Soil type	Epileptic Regosol Eutric	Haplic Regosol Eutric
FC (percent dry weight)	21.51 ± 2.12	25.83 ± 0.12*
pH 1: 2.5	5.65 ± 0.38	5.66 ± 0.60
EC (dS m^{-1})	0.30 ± 0.24	0.35 ± 0.30
P (mg kg^{-1})	13.75 ± 4.98*	9.73 ± 3.00
OM (%)	0.21 ± 0.08	0.37 ± 0.07*
N (%)	2.22 ± 0.49	3.65 ± 0.79*
Texture	Sandy loam	Loam

[a] Mean values of 20 samples. EC: electrical conductivity, FC: field capacity, P: available phosphorus, OM: organic matter, N: total nitrogen. Significance according to Tukey test indicated as * ($P < 0.05$).

stereomicroscope directly and recorded as mean spores per 100 g soil. Permanent slides of all spores were prepared by placing them in polyvinyl-lacto-glycerol (PVLG) and PVLG + Melzer's reagent. Spores were cracked open under the cover slip for observation of spore wall and inner wall characteristics.

Statistical analysis.—Data distribution of AM colonization and spore numbers was not normal (Kolmogorov-Smirnov and Shapiro-Wilks normatility tests), and variances were not homogeneously distributed (Levene test). Thus AM colonization percentages and AMF spore numbers were respectively arcsine and logarithmically transformed. ANOVA was performed to test the influence of the seasons and sites (Quebrada del Portugués and Narváez Range) on the AM root colonization and spore numbers in the studied plant species. ANOVA generated 40 mean values (5 hosts × 4 seasons × 2 site treatments), which was followed by the application of DGC multiple comparison Test (Di Rienzo, Guzmán and Casanoves test) to compare the means of the data (Di Rienzo et al 2002).

Variables of AM colonization (percent arbuscules, number of vesicles and entry points) were non-normal and the variances were not homogeneous. These variables were transformed into ranks and analyzed statistically by ANOVA, the equivalent to the nonparametric analyses (Zar 1999). DGC multiple comparison tests were applied (di Rienzo et al 2002). The relationships among AM colonization, intraradical AM fungal structures and AMF spore numbers for all plants, seasons and sites were analyzed by Pearson correlations.

RESULTS

Sites characterization.—Soils of Quebrada del Portugués (QP) are epileptic regosol eutric and Narváez Range (NR) are haplic regosol eutric (IUSS Working Group WRB 2006). Both soils were slightly acidic with low electrical conductivity but differed in texture and nutrient content (TABLE I). The soils from NR present higher contents of organic matter, total N and field capacity than QP, which had slightly higher levels of P. The site at NR presented a lower mean annual precipitation than that at QP (698 and 1350 mm respectively). Mean annual temperatures were similar at both locations, with 12 C, 10 C, 17 C and 19 C for autumn, winter, spring and summer respectively.

Root colonization and spore numbers.—Dominant plant species presented AMF structures in their roots. The herb roots presented terminal arbuscules, intra- and intercellular aseptate hyphae, coils and oval to rectangular intra- and intercellular vesicles (*Arum*- and *Paris*-morphological type). *Alnus acuminata* presented intercellular aseptate hyphae and intra- and intercellular vesicles (*Arum*-type), while *S. peruviana* showed only intracellular hyphae and vesicles (*Paris*-type).

AM colonization and AMF spore numbers were similar in both sites ($P = 0.404$ and $P = 0.889$ respectively) although significant differences were found among seasons ($P < 0.00001$ and $P < 0.000001$ respectively) and hosts ($P < 0.00001$ and $P < 0.003$ respectively) (TABLE II). Significant tripartite interaction was observed, seasons × hosts × sites, for AM colonization and AMF spore numbers ($P < 0.0001$ and $P < 0.00001$ respectively), indicating that these variables fluctuated significantly between hosts among the two sites and the four seasons.

AM colonization percentages ranged from very low to medium. For NR they varied 3–37% and 5–44% at QP (TABLE III). With respect to plant species, in *A. acuminata* the AM colonization was very low (3–12%); herbs presented different ranges of AM colonization, being low to medium (13–44%) for *D. indica*; very low to low (12–37%, 15–29%, 13–28%) for *O. Conorrhiza*, *T.* aff. *repens* and *S. peruviana* respectively (TABLE III). The highest AM colonization was observed in autumn, spring and summer (TABLE III). In both sites *O. conorrhiza* showed high AM colonization levels in autumn whereas *A. acuminata* showed high AM colonization levels in autumn and spring. In QP the highest AM colonization was observed in *D. indica* and in *T.* aff. *repens* in autumn. In NR the highest AM

TABLE II. Percentage of AM colonization and AMF spore numbers of the studied species in both sites (Quebrada del Portugués and Narváez Range) in four seasons

	Percentage of AM colonization[a]	AMF spore density (spore/g dry soil)
Alnus acuminata[b]	7.19 ± 8.10 c	529.62 ± 362.43 a
Duchesnea indica	25.71 ± 12.71 a	364.62 ± 171.10 b
Oxalis conorrhiza	23.89 ± 10.81 a	371.21 ± 182.63 b
Trifolium aff. *repens*	22.39 ± 8.39 a	433.25 ± 296.93 b
Sambucus peruviana	20.72 ± 9.92 b	511.54 ± 451.90 b
Quebrada del Portugués[c]	19.73 ± 11.89 a	392.62 ± 169.40 a
Narváez Range	20.23 ± 12.26 a	491.48 ± 406.85 a
Autumn (dry season)[d]	26.18 ± 14.46 a	498.46 ± 272.90 b
Winter (dry season)	15.01 ± 8.19 d	241.17 ± 81.48 c
Spring (rainy season)	21.12 ± 10.48 b	782.27 ± 336.44 a
Summer (rainy season)	17.63 ± 11.40 c	246.3 ± 80.88 c

[a] Values are mean ± SE.
[b] Mean of 80 samples and 24 samples for spore density.
[c] Mean of 200 samples for AM colonization and 60 samples for spore density.
[d] Mean of 100 samples for AM colonization and 30 samples for spore density. Values within columns followed by the same letter were not significantly different ($P < 0.05$).

colonization was observed during summer in *D. indica* and during autumn, winter and spring in *T.* aff. *repens*. *D. indica* was the herbaceous vegetation with the highest AM colonization. In NR, *S. peruviana* showed the highest AM colonization in spring and in QP during autumn and spring.

AMF spore numbers were 107–1329 per 100 g dry soil at NR site and 215–737 per 100 g dry soil at QP (TABLE III). In *A. acuminata* spore numbers were 199–1329 per 100 g dry soil, in *D. indica* 220–737 per 100 g dry soil, in *O. conorrhiza* 158–653 per 100 g dry soil, in *T.* aff. *repens* 208–1125 per 100 g dry soil and in *S. peruviana* 107–1256 per 100 g dry soil (TABLE III). The highest AMF spore numbers were observed in autumn and spring (TABLE III). At both sites *D. indica* showed the highest AMF spore numbers in spring, whereas *A. acuminata* and *T.* aff. *repens* in autumn and spring. In *O. conorrhiza* and *S. peruviana* rhizospheres significantly higher AMF spore numbers were observed in autumn and spring in NR; however in QP higher AMF spore numbers were observed in spring.

Intraradical fungal structures.—Percentage of arbuscules differed significantly between sites, seasons and hosts. Significant interaction was observed among seasons and hosts ($P < 0.001$). In *D. indica* the highest percentage of arbuscules was observed in autumn and summer. Percent of arbuscules in *O. conorrhiza* and *T.* aff. *repens* respectively were higher in winter and autumn. No arbuscules were observed in *A. acuminata* and *S. peruviana* (TABLE IV). The number of vesicles differed significantly according to seasons, hosts and sites. Significant interaction was observed among hosts, seasons and sites ($P < 0.01$). In NR the number of vesicles was higher during autumn in *A. acuminata*, during winter and summer in *D. indica*, during summer in *O. conorrhiza*, during winter and autumn in *T.* aff. *repens* and during winter in *S. peruviana*. For QP the number of vesicles was higher during autumn in *A. acuminata* and *T.* aff. *repens*, during autumn and winter in *D. indica* and during winter in *O. conorrhiza*; no significant differences for *S. peruviana* were observed in any season (TABLE IV). The number of entry points also differed significantly according to sites, seasons and hosts. Significant interactions were observed among hosts and sites ($P < 0.001$) and seasons and hosts ($P < 0.001$). In *D. indica* the number of entry points was higher in spring and autumn, in *O. conorrhiza* during winter, spring and summer, in *T.* aff. *repens* during winter and spring and in *S. peruviana* during spring (TABLE IV).

Root colonization vs. spore number.—A positive correlation was observed between percentage of AM colonization and percentage of arbuscules ($r = 0.49$; $P < 0.001$), percentage of AM colonization and entry points ($r = 0.59$; $P < 0.001$) and percentage of arbuscules and number of vesicles ($r = 0.41$; $P < 0.01$). No relationship was found between the percentage of AM colonization and spore density ($r = 0.06$; $P = 0.76$).

DISCUSSION

Soil characteristics of these forests were similar to Aceñolaza's (1995) results for *A. acuminata* forests in Tucumán Province and to observations for genus *Alnus* (Tarrant and Trappe 1971).

TABLE III. Percentage of AM colonization (percentage AMC) and AMF spore number (AMF) for each host, sites (Quebrada del Portugués, QP; Narváez Range, NR) and seasons. Values are mean of 10 samples (for percentage AMC) and three samples (for AMF) ± SE. Values within a row followed by the same letter were not significantly different for each host among seasons ($P < 0.05$)

Host		Sites	Autumn (dry season)	Winter (dry season)	Spring (rainy season)	Summer (rainy season)
Alnus acuminata	%AMC	QP	12 ± 14.2 a	5 ± 4 b	12 ± 12.3 a	7 ± 4.3 b
		NR	7 ± 3.4 a	3 ± 6.3 b	8 ± 4.0 a	3 ± 3.2 b
	AMF	QP	611.33 ± 66.52 a	286.67 ± 40.53 b	604.33 ± 73.32 a	298 ± 73.08 b
		NR	673.33 ± 114.15 a	199.67 ± 26.31 b	1329.67 ± 10.69 a	234 ± 84.33 b
Duchesnea indica	%AMC	QP	43.58 ± 10.5 a	16.33 ± 8.4 c	28.73 ± 8.6 b	22.98 ± 10.5 b
		NR	21.34 ± 9.7 b	13.72 ± 3.7 c	25.22 ± 7.3 b	33.51 ± 13.7 a
	AMF	QP	315 ± 28.83 b	270.67 ± 57.85 b	737 ± 72.64 a	220.33 ± 68.70 b
		NR	256 ± 32 b	248 ± 39.68 b	518.67 ± 61.17 b	264 ± 21.17 b
Oxalis conorrhiza	%AMC	QP	36.13 ± 11.57 a	19.81 ± 4.19 b	24.63 ± 4.03 b	12.55 ± 4.66 c
		NR	36.46 ± 12.01 a	18.89 ± 5.78 b	25.22 ± 5.57 b	17.42 ± 6.89 c
	AMF	QP	420.33 ± 108.03 b	378.67 ± 57.85 b	653.33 ± 150.05 a	307.67 ± 41.58 b
		NR	358.33 ± 45.08 a	158.67 ± 17.95 b	561.67 ± 54.24 a	218.33 ± 145.91 b
Trifolium aff. *repens*	%AMC	QP	28.87 ± 9.13 a	16.07 ± 5.37 b	15.89 ± 5.78 b	26.85 ± 6.49 a
		NR	27.78 ± 6.16 a	23.29 ± 6.24 a	22.57 ± 8.71 a	17.83 ± 7.83 b
	AMF	QP	374 ± 91.24 a	256.33 ± 33.56 b	435.33 ± 46.97 a	288.33 ± 150.22 b
		NR	538 ± 59.92 a	239.67 ± 52.72 b	1125.67 ± 140.74 a	208.67 ± 48.05 b
Sambucus peruviana	%AMC	QP	26.93 ± 8.2 a	13.37 ± 4.1 b	20.93 ± 5.0 a	15.63 ± 8.4 b
		NR	21.04 ± 13.1 a	20.54 ± 5.7 a	27.64 ± 15.7 a	19.71 ± 6.8 a
	AMF	QP	313 ± 49.24 b	265.67 ± 70.12 b	600.67 ± 41.86 a	215.67 ± 52.17 b
		NR	1125.33 ± 351.58 a	107.67 ± 53.89 b	1256.33 ± 362.44 a	208 ± 75.42 b

Root colonization and spore numbers.—The present study reports the presence of AM colonization and AMF spore numbers in the rhizosphere of four dominant plant species in the Yungas forest, being also the first report for AM spore numbers for *A. acuminata* rhizosphere. Symbiotic associations with AMF were found in *A. acuminata* (Betulaceae), *D. indica* (Rosaceae), *O. conorrhiza* (Oxalidaceae), *T.* aff. *repens* (Fabaceae) and *S. peruviana* (Caprifoliaceae) in the Yungas. These species belong to families that are cited as mycorrhizal (Harley and Harley 1987, Wang and Qiu 2006). Based on findings by Carling and Brown (1982) and Yamato and Iwasaki (2002), these herb families can present *Arum-Paris* morphological types as well as other species of the same genus (Yamato 2004). The AM morphological type of *A. acuminata* is in accordance with results reported for these forests (Becerra et al 2007a).

The very low to medium percentages of AM colonization can be explained by high soil fertility observed in both sites. High soil fertility could indicate a low ability of the AM fungi to colonize the host species in accordance with Mejstrik (1973) and Hayman et al (1976). Nevertheless low AM colonization could reflect a low mycotrophic nature of the plant species analyzed (Ingleby et al 1997).

The range of AM colonization found in the plants (from 3% in *A. acuminata* to 44% in *D. indica*) is in agreement with Brundrett and Kendrick (1988), Smith and Read (1997) and van der Heijden et al (1998), who suggested that plants can differ in AM colonization. Very low AM colonization was found in *A. acuminata*, which then could be categorized as facultatively mycorrhizal (<25% *sensu* Brundrett and Kendrick 1988). This condition can be explained by soil nutrient concentrations, the radical system and the physiology of the plant (Brundrett 1991, 2004). On the other hand, as stated by Berg et al (2001), low AM colonization might provide significant benefit to plants whereas increased colonization could increase the cost of carbohydrates to plants. Moreover the root length colonized by AM does not represent the quantity of living or active fungal structures in the nutrient transfer (Smith and Gianinazzi-Pearson 1990).

Mycorrhizas are three-way interactions of plants, fungi and soil (Brundrett 1991), thus environmental and edaphic factors are expected to affect their structure and function. The highest percentages of AM colonization were observed in autumn, spring and summer. These are the seasons where the greatest increase in root growth and mycorrhizal activity has been reported due to higher soil temperatures and adequate soil moisture (Brundrett and Abbott 1994). The level of AM intraradical colonization of a root segment can vary over days or weeks depending on factors influencing both fungal

TABLE IV. Intraradical structures variation among host species, seasons and sites (Quebrada del Portugués, QP; Narváez Range; NR). Values are mean of 10 samples ± SE. Values within a column followed by the same letter were not significantly different for each host among seasons ($P < 0.05$)

		Percent arbuscules		Vesicles		Entry points	
Host	Seasons	QP	NR	QP	NR	QP	NR
Alnus acuminata	Autumn (dry season)	0	0	5.65 ± 6.90 a	2.85 ± 1.33 a	0	0
	Winter (dry season)	0	0	1.99 ± 1.93 b	1.53 ± 3.08 b	0	0
	Spring (rainy season)	0	0	3.52 ± 4.50 b	2.04 ± 2.84 b	0	0
	Summer (rainy season)	0	0	2.02 ± 1.87 b	1.11 ± 1.35 b	0	0
Duchesnea indica	Autumn (dry season)	36.1 ± 27.42 a	22.4 ± 17.88 a	4.26 ± 4.27 a	5.83 ± 9.51 b	7.19 ± 2.48 a	5.35 ± 1.36 a
	Winter (dry season)	2.82 ± 4.72 b	15.25 ± 15.92 b	12.24 ± 9.63 a	13.24 ± 9.50 a	5.9 ± 1.52 b	3.52 ± 1.50 b
	Spring (rainy season)	5.52 ± 4.62 b	16.35 ± 15.37 b	1.07 ± 0.88 b	1.23 ± 1.06 b	7.41 ± 1.90 a	6.07 ± 0.98 a
	Summer (rainy season)	26.85 ± 27.25 a	49.7 ± 27.99 a	2.39 ± 2.56 b	14.9 ± 0.85 a	7.09 ± 1.76 b	4.28 ± 1.34 b
Oxalis conorrhiza	Autumn (dry season)	4.1 ± 6.22 b	8.24 ± 21.43 b	3.14 ± 3.24 b	0.96 ± 1.99 b	4.92 ± 1.30 b	3.5 ± 1.29 b
	Winter (dry season)	7.4 ± 10.03 a	15.7 ± 18.75 a	5.33 ± 6.12 a	1.47 ± 1.97 b	6.35 ± 1.29 a	6.43 ± 1.34 a
	Spring (rainy season)	3.75 ± 4.42 b	5.3 ± 6.55 b	2 ± 1.62 b	0.46 ± 0.59 b	6.88 ± 1.50 a	5.33 ± 1.39 a
	Summer (rainy season)	0.75 ± 2.37 c	2.1 ± 4.65 c	1.11 ± 2.08 b	5.59 ± 7.68 a	6.69 ± 1.53 a	5.85 ± 1.48 a
Trifolium aff. *repens*	Autumn (dry season)	1.85 ± 4.16 a	4.3 ± 8.42 a	7.72 ± 9.33 a	6.08 ± 4.23 a	6.33 ± 1.45 b	4.55 ± 1.41 b
	Winter (dry season)	0.2 ± 0.63 b	1.5 ± 4.74 b	2.15 ± 3.25 b	8.27 ± 8.75 a	6.97 ± 1.05 a	5.38 ± 2.22 a
	Spring (rainy season)	0.25 ± 0.79 b	0.05 ± 0.16 b	0.21 ± 0.21 b	2.23 ± 5.87 b	9.49 ± 1.55 a	4.93 ± 1.26 a
	Summer (rainy season)	0 b	0 b	0.46 ± 0.43 b	4.86 ± 9.69 b	5.84 ± 2.20 b	4.14 ± 1.34 b
Sambucus peruviana	Autumn (dry season)	0	0	4.29 ± 2.76 a	5.04 ± 9.34 b	2.93 ± 0.96 c	2.67 ± 0.97 c
	Winter (dry season)	0	0	4.84 ± 3.81 a	3.66 ± 3.62 a	3.93 ± 0.96 b	4.49 ± 1.35 b
	Spring (rainy season)	0	0	3.77 ± 3.74 a	2.97 ± 2.86 b	4.96 ± 1.11 a	5.29 ± 0.73 a
	Summer (rainy season)	0	0	6.04 ± 7.23 a	0.71 ± 0.88 b	4.39 ± 1.16 b	3.96 ± 0.98 b

growth and root dynamics (Miller and Kling 2000). In this study (temperate region) host species present lower AM colonization levels, usually found in sites with high P. In general a good development of AM fungi is associated with a low concentration of soil nutrients (Gehring and Connell 2006).

AMF spore number in soils is variable (Smith and Read 1997). For both sites the total number of AMF spores was similar to those reported for tropical plantations (Cuenca and Meneses 1996) and tropical rain forests (Zhao et al 2001, Shi et al 2006). However, as Morton and Bentivenga (1994) suggest, a low AM fungal spore number does not mean absence of AM fungi. In tropical forests hyphae and root fragments colonized are the principal propagules in soils whereas AMF spores are the dormant state of these fungi (Janos 1996). The high humidity and fertility of Yungas soils might explain the low AMF sporulation, and the AM colonization could depend more on root fragments colonized and on the extraradical mycelium than on AM spores (Barea et al 1991).

In this study the higher AMF spore numbers observed during autumn and spring in all host species for both sites are in accordance with Clapp et al (1995) and Douds and Millner (1999) in forests sites, where a higher number of AMF spore number was observed during the period of maximum root growth. Ecological factors, such as seasonality, soil factors, host-dependence, age of the host plant, sporulation ability of AMF species in soils, could affect the development and distribution of AM fungi in the rhizosphere (Zhao 1999, Greipson and El-Mayas 2000, Lovelock et al 2003). Seasonal differences in AMF spore numbers probably reflect seasonal differences in spore formation, as Pringle and Bever (2002) stated.

Alnus acuminata's rhizosphere showed the highest AMF spore number and the lowest AM colonization. This could be explained by the fact that plant roots in forests are connected and therefore Glomalean spores might be found at some distance from the roots where they originated (Merryweather and Fitter 1998). The dominant understory species studied with a very low to medium AM colonization could influence the AMF sporulation of *A. acuminata* with a very low AM colonization, hence the higher sporulation observed in *A. acuminata* rhizosphere.

Intraradical fungal structures.—Intraradical fungal structures varied according to different seasons, hosts and sites. As stated by Ruotsalainen et al (2002) species-specific features as well as edaphic conditions might influence the development of AM colonization.

A clear AM colonization development—entry points-hyphae-arbuscules-vesicles—was not observed in this study. Instead of the highest occurrence of arbuscules found in spring, autumn and summer, the highest occurrence of vesicles and entry points in most plants was observed in cold dry seasons (autumn and winter), which confirms that the root system colonization by AMF is a dynamic process influenced not only by the growth and formation of infection units (spores, root fragments, hyphal networks) but also by the growth of the root system (Smith and Read 1997). The partitioning of fungal growth between intra- and extraradical structures is probably influenced by environmental or edaphic factors (e.g. soil temperature, soil moisture and soil fertility) as well as host responses to these factors (Brundrett 1991, Merryweather and Fitter 1998, Helgason et al 1999).

Colonization and AM spore number correlation.—No correlation between spore numbers and percentage of AM colonization, and the wide range of spore numbers in the rhizosphere of studied plants were in line with results obtained by Zhao et al (2001), Liu and Wang (2003) and Zhang et al (2004). There are two possible explanations for our results. First, the roots associated with AM fungi might have decayed before sampling and, second, it is likely that some spores included in the counts were not viable or were present in clusters that functioned as one (inseparable) ineffective propagule in field soil (Jansa et al 2002). The relationship between AMF spore number and percentage of AM colonization is complicated, as Stutz and Morton (1996) suggested, and might be influenced by many environmental and biological factors (AM fungal species, plant host and soil nutrients).

In conclusion this first published report on the AM colonization and AMF spore number dynamics of dominant plant species of Yungas forests shows that these plants are very low to medium colonized; *Alnus acuminata* is facultatively AM (while presenting a high ectomycorrhizal colonization, Becerra et al 2005d); the AM colonization, intraradical fungi structures and AMF spore numbers in the plants vary depending on phenological, climatic and edaphic conditions. Further long-term studies are necessary to elucidate the ecological role of AM fungi in Yungas forests of Argentina.

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