

Mycorrhizal status of plant species in the Chaco Serrano Woodland from central Argentina

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Abstract We examined the mycorrhizal type of 128 plant species in two patches of native vegetation of the Chaco Serrano Woodland, central Argentina, the largest dry forest area in South America. Of the 128 plant species investigated (belonging to 111 genera in 53 families), 114 were colonized by arbuscular mycorrhizal fungi (AM), orchid mycorrhizal associations were present in the five terrestrial orchid species analyzed, one ectomycorrhiza was only present in *Salix humboldtiana* Willd., and 96 harbored a dark septate endophyte (DSE) association. Co-occurrence of AM and DSE was observed in 88 plant species. We determine morphological types of arbuscular mycorrhizal fungi (*Arum*, *Paris*, and intermediate AM structures) and report the mycorrhizal status in 106 new species, 12 of which are endemic to central Argentina and two, *Aa achalensis* Schltr. and *Buddleja cordobensis* Griseb., are declared to be vulnerable species. Root colonization in the Chaco Serrano Woodland is widespread and should be considered in revegetation programs due to the deterioration of this particular ecosystem. Considering the predominance of AM and DSE associations and the various potential benefits that these associations may bring to plant

establishment, they should receive special attention in conservation and reforestation of these woodlands.

Keywords Mycorrhiza · Arbuscular mycorrhiza · AM morphology · *Paris* · *Arum* · Dark septate endophytes · Chaco Serrano woodland · Argentina

Introduction

The Sierras Chicas, a region located in the central-north of Córdoba province, Argentina, covers an area of about 1,400 km². At between 500 and 1,300 m altitude, these hills are covered by arboreal vegetation, the so-called Chaco Serrano Woodland, the most extensive dry forest in South America (Cabrera 1976). In the last 50 years, the Chaco Serrano Woodland has been reduced and is now confined to several patches of different sizes; this has resulted in a reduction in species richness of plant communities (Cagnolo et al. 2006). Aerial photographs of the Sierras Chicas show that there was a 40% decrease in the woodland area between 1970 and 1997. Agricultural activities and tourism are the main causes of its deterioration (Gavir and Bucher 2004; Zak and Cabido 2004; Verzino et al. 2005). These causes have led to changes in the native vegetation and an increase in invasive exotic species such as *Gleditsia triacanthos* L. (C.K. Schneid), *Ligustrum lucidum* Aiton, *Pyracantha angustifolia* (Franch.) C.K. Schneid., *Ulmus pumila* L., *Morus alba* L., and *Melia azedarach* L. (Marco and Páez 2000).

The native flora in the Province of Córdoba has 300 endemic species (Barboza et al. 2006). Several of these species, including *Flourensia oolepis* S.F. Blake, *Gomphrena pulchella* (Griseb.) Pedersen, *Acanthocalycium spiniflorum* (K.Schumann) Backeberg, *Gymnocalycium monvillei* (Lem.)

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Britton & Rose, *Sophora linearifolia* Griseb., *Aa achalensis* Schltr., and *Buddleja cordobensis* Griseb., occur in the Chaco Serrano Woodland; the last two species have been declared as vulnerable and are currently included in the red list of the International Union for Conservation of Nature (Vischi et al. 2004). In revegetation programs of vulnerable or endangered native species, it is important to know the mycorrhizal status of the plants (Gemma et al. 2002; Wubet et al. 2003; Fuchs and Haselwandter 2004). There are few reports of the mycorrhizal status of Argentinian plants. These include one in *Polylepis* woodlands from Córdoba (Menoyo et al. 2007).

The objective of this study was to determine the mycorrhizal status of plant species in a dry native woodland improving our knowledge on the ecology of this severely threatened ecosystem. We included dark septate endophytes (DSE) in our study since they are being increasingly reported in plant surveys (Muthukumar et al. 2006; Weishampel and Bedford 2006) and are receiving more attention as possible mutualistic associations (Jumpponen 2001).

Materials and methods

Study area

Two field sites with topographic differences were selected in the Chaco Serrano Woodland community on the west hillside of the Sierras Chicas, Punilla Department (Córdoba Province). As a result of topographic differences, changes in plant species composition are present and sampling of the two patches provides a most complete view of species richness. The characteristic vegetation is a low and open woodland with a tree layer (8–15 m high), shrubs (1.5–3 m high), herbs and grasses (0–1 m high), many vines, and terrestrial bromeliads. Also, ferns and cacti occur in the region (Cagnolo et al. 2006). The field sites are located at approximately 30° 50' S–64° 30' W (site A) and 30° 51' S–64° 33' W (site B). The annual average air temperature is 16°C (winter=10°C; summer=22°C) and total annual precipitation between 700 and 800 mm, falling mainly in the warm season (November–March). Overall, the region is classified as subtropical.

Site A is located at the base of Uritorco mountain (900 m), which has a steep slope (>40%). The overstorey vegetation is composed of evergreen trees (*Lithrea molleoides* (Vell.) Engl., *Aspidosperma quebracho-blanco* Schltdl., and *Schinus areira* L.) and deciduous trees (*Ruprechtia apetala* Wedd. and *Schinopsis haenkeana* Engl.). The understorey vegetation is dominated by shrubs such as *Acacia caven* Molina, *Acacia atramentaria* Benth., *Flourensia oolepis*, *Cesalpinia gilliessi* (Wall. ex Hook.) D. Dietr., and a wide variety of herbaceous species belonging

to the families Malvaceae, Solanaceae, Asteraceae, and Poaceae. At higher altitudes (>1,100 m), patches of *Heterothalamus alienus* (Spreng.) Kuntze and *Colletia spinosissima* J.F. Gmel. are common. Near the rivers, we also found species of cryptogams and Pteridophytes. This area is well preserved, and anthropogenic fires have not been documented for more than 20 years. Invasion of exotic plant species such as *Ligustrum lucidum* and *Morus alba* is restricted to the lower slope of the area explored.

Site B is at an altitude of 700 m and 4 km from the mountain slope. The plant community is mainly composed of *Schinopsis haenkeana* Engl., *Lithrea molleoides*, *Prosopis* sp., *Senna aphylla* (Cav.) H.S. Irwin et Barneby, *Acacia caven*, and *Buddleja cordobensis*. Patches of *Sophora linearifolia* and *Heterothalamus alienus* are present and the palm *Tritrixax campestris* (Burmeist.) Drude et Griseb. is common. Many cacti species such as *Gymnocalycium monvillei*, *Trichocereus candicans* (Gilles ex Salm-Dyck) Britton et Rose, and *Opuntia sulphurea* Gilles ex Salm-Dyck are common among rocks. Pteridophytes and cryptogams are frequent near temporary water courses.

In both sites (A and B), the soil is lithosolic. The A–AC–C horizons are characterized by having a high percentage of sand and rocks up to 20 cm in diameter, both within the profile and on the surface (Vázquez et al. 1979). Soil physico-chemical characteristics of horizon A are presented in Table 1.

Field sampling

In both sites, root samples of trees, shrubs, cacti, bromeliads, and herbaceous plant species were collected between September 2006 and January 2007, when the plants were

Table 1 Soil properties of study sites

Parameter	Site A	Site B
Texture	Sandy loam	Sandy loam
pH	6.97± 1.3	6.7± 1.5
Water content at field capacity (%)	38± 3	24± 2
Available P (mg/kg)	13.5± 4.8	32.1± 5
Total N (g/kg)	1.14± 0.3	0.94± 0.4
Total C (g/kg)	13.8± 2.7	11.24± 3.1
Organic matter (%)	27.6± 1	22.48± 1.5
CEC (cmolc/kg)	6.5± 2.8	3.45± 1.4
Ca (cmolc/kg)	9.75± .98	8.45± 1.3
Mg (cmolc/kg)	0.8± 0.1	0.45± 0.1
Na (cmolc/kg)	0.14± 0.08	0.04± 0.01
K (cmolc/kg)	0.3± 0.01	0.55± 0.03
Electric conductivity (dSm/m)	1.33± 0.3	1.23± 0.3

The analyses were as follows: available P, Bray and Kurtz (1945) method; electric conductivity and pH in 1:2.5 suspension of soil in water; cations after equilibrium of soil in 0.1 N NH₄ Cl; percent of water by weight for soil at field capacity
 cmolc centimols of charge, CEC cation exchange capacity

flowering, from an area of approximately 5 ha. At least two individuals per species and site were sampled. Shrub and tree root systems were carefully excavated, taking care that only roots belonging to the root system of the sampled species were collected. Whenever possible, young trees and shrubs plants, at least 1 year old, were uprooted. For herbs, grasses, and ferns, when possible, the whole root system was collected. The samples were placed in polyethylene bags and processed, when possible, the same day.

Mycorrhizal analysis

The roots were carefully washed in running tap water to remove soil debris and observed with a stereo binocular microscope to detect external morphology of ectomycorrhizal type. Roots that were detached from the main root system were assigned to their corresponding species by careful examination of morphological characteristics. Samples were cleared with 10% KOH and stained with trypan blue (Phillips and Hayman 1970). Roots with dark pigments were bleached overnight in alkaline H₂O₂ (30%) at room temperature prior to treatment with KOH. Stained root segments were screened for the presence or absence of the different mycorrhizal types with a compound microscope. The mycorrhizal type of each plant species was determined from 20 subsamples of each individual.

Arbuscular mycorrhiza (AM) colonization type (*Paris*, *Arum*, and intermediate structures) and percent AM colonization were determined in 50 mounted young root pieces (1 cm) of each individual plant. Three different levels of colonization were considered: low, <10% of segments with AM structures; medium, >10% and <30%; and high, >30%. The presence of DSE structures (microsclerotia or melanized hyphae) in at least one root sample was counted as positive for that plant species. Root samples were placed in lactic acid and deposited at the Centro Regional de Investigaciones La Rioja, Anillaco, La Rioja, Argentina.

Results

Four different types of fungal colonization were found in 119 (93%) of the 128 species surveyed (Table 2). The most common associations were AM, colonizing roots of 114 species (89%), orchid mycorrhiza (OR) was found in five species of orchid (100%) and DSE in 96 species (75%) (Fig. 1, 1). We found 12 endemic plants with roots colonized as follows: two of them *Puya spathacea* (Griseb.) Mez and *Amblyopetalum coccineum* (Griseb.) Malme only by AM, *Gomphrena pulchella* only by DSE, eight of them by AM and DSE, and *Aa chalcensis* by OR and DSE (Table 2).

Fifteen out of the 17 samples of pteridophytes were colonized by AM fungi. The five terrestrial native orchids

had colonization characterized by intracellular hyphal pelotons (Fig. 1, 5) and three harbored DSE microsclerotia (Fig. 1, 6). Typical ECM structures were present in one root sample of *Salix humboldtiana* Willd. (0.8%). Nine (7%) of the plant species sampled lacked mycorrhizal structures.

Many plant species harbored more than one type of fungal association, with the co-occurrence of AM and DSE colonization being predominant (Fig. 1, 2). In some families such as Fabaceae, Poaceae, Cactaceae, and Anacardiaceae, all the species sampled showed this mycorrhizal pattern (Table 2). In *Salix humboldtiana*, three types of associations were found (AM, DSE, and ECM) in the root system of an individual plant. On roots of this species, ECM had the typical structures: mantle and Hartig net, and the DSE and AM structures were observed in the same root section. *Ephedra triandra* Tul. emend. J.H. Hunz, the only gymnosperm sampled, had typical AM and DSE hyphae and microsclerotia in fine roots. In 21 plant species of different families, only AM were observed (Fig. 1, 3 and 4).

Each plant family had its particular pattern of mycorrhizal status distribution and AM colonization. Only coenocytic hyphae and *Glomus*-type vesicles but not arbuscules or coils were found in *Gomphrena pulchella* (Fig. 1, 4), *Apidosperma quebracho-blanco*, *Jodinia rhombifolia* (Hook ex Arn.) Reisseck, and *Cyperus entrerianus* Boeck roots. We considered these associations as non-functional since mycorrhizal fungi can co-exist as endophytes in roots, without nutrient transfer (Brundrett 2006). The morphology of AM was found to be *Arum* (Fig. 1, 8) or *Paris* type (Fig. 1, 7) and intermediate structures (Table 2). The *Paris* type was found in 23 species belonging to 16 families; all the Pteridophytes had this type of colonization. In contrast, *Arum* type was found in 72 species belonging to 25 families. The type defined as *Arum–Paris* (with *Arum* structure as predominant) was found in 11 species belonging to four families (Anacardiaceae, Fabaceae, Lamiaceae, and Rutaceae), and the intermediate type with no well-defined pattern was presented in 11 species belonging to eight families.

The level of AM colonization was higher in some families like Convolvulaceae and Cactaceae, and lower in Anacardiaceae. In root samples of some species (*Thalictrum decipiens* Bivin., *Achyrocline satureoides* (Lam.) DC, and *Colletia spinosissima*), the level of AM colonization was nearly 100% of the root system (Table 2).

Discussion

Of the 128 species belonging to 53 families examined from the Chaco Serrano Woodlands, the mycorrhizal status of 107 species is reported for the first time. Twelve of these species are endemic to Central Argentina and *Aa achalcensis*

Table 2 Plant species, phenology, types of mycorrhizal association, and AM colonization in two sites (A and B) of the Montane Forest in Córdoba Province, Argentina

Family and species	Field site	Functional type	Phenology	Mycorrhizal type	CT	M/NM	AM colonization	DSE
Acanthaceae								
<i>Stenandrium dulce</i> (Cav.) Nees	B	ah	fl	AM	A	1/2	L	X
<i>Dicliptera squarrosa</i> Nees	A B	h	fl	AM	A	2/0	L	X
Alliaceae								
<i>Nothoscordum gracile</i> (Dryand. ex Aiton) Stearn	A B	h	fl	AM	A	4/0	H	X
Amaranthaceae								
<i>Amaranthus viridis</i> L.	B	h	fr	NM		0/3		
<i>Gomphrena pulchella</i> (Griseb.) Pedersen ^a	B	h	fl	AM	NF	3/0		X
Anacardiaceae								
<i>Schinus fasciculata</i> (Griseb.) IM Johnst.	A B	s	v	AM	P	3/0	L	X
<i>Schinus areira</i> L.	A B	t	v	AM	P	3/0	L	X
<i>Lithraea molleoides</i> (Vell.) Engl.	A B	t	v	AM	I	3/0	L	X
<i>Schinopsis haenkeana</i> Engl.	A B	t	v	AM	A–P	2/0	L	X
Apiaceae								
<i>Eryngium horridum</i> Malme	B	h	fl	AM	A	3/0	L	X
<i>Eryngium paniculatum</i> Cav. et Dombey ex F. Delaroché	B	h	fl	AM	A	2/0	L	X
Apocynaceae								
<i>Aspidosperma quebracho-blanco</i> Schltdl.	A	t	v	AM	NF	3/0		X
<i>Amblyopetalum coccineum</i> (Griseb.) Malme ^a	A	h	fl	AM	P	3/0	H	
Arecaceae								
<i>Trithrinax campestris</i> (Burmeist.) Drude et Griseb.	B	p	v	AM	A	2/0	L	X
Asclepiadaceae								
<i>Asclepias mellodora</i> A. St.-Hill	B	h	fl	AM	A	3/0	L	X
Asteraceae								
<i>Achyrocline satureioides</i> (Lam.) DC	B	s	fl	AM	A	3/0	H	X
<i>Achyrocline tomentosa</i> Rusby	B	s	v	AM	A	2/0	M	X
<i>Baccharis articulata</i> (Lam.) Pers.	B	s	fl	AM	A	3/0	H	
<i>Bidens pilosa</i> L.	A	h	v	AM	A	3/0	L	X
<i>Chaptalia sinuata</i> (Less.) Baker	A B	h	fl	AM	A	3/0	H	X
<i>Eupatorium arnottianum</i> Griseb.	A B	s	fl	AM	A	3/0	M	
<i>Flourensia campestris</i> Cabrera ^a	A	s	v	AM	A	3/0	M	X
<i>Flourensia oolepis</i> Blake ^a	A B	s	v	AM	A	3/0	M	X
<i>Gaillardia megapotamica</i> (Spreng.) Baker	B	h	fl	AM	A	2/0	L	X
<i>Galinsoga parviflora</i> Cav.	B	h	fl	AM	A	3/0	L	X
<i>Grindelia cabreræ</i> Ariza ^a	B	s	fl	AM	A	3/0	L	X
<i>Heterothalamus alienus</i> (Spreng.) Kuntze	A B	s	v	AM	A	5/0	H	X
<i>Parthenium hysterophorus</i> L.	A B	h	fl	AM	A	3/0	M	X
<i>Stevia satureiifolia</i> (Lam.) Sch. Bip.	A B	s	fl	AM	P	3/2	L	X
<i>Tagetes minuta</i> L.	A B	h	fl	AM	A	3/0	H	X
<i>Trichocline reptans</i> (Wedd.) Hieron.	B	h	fl	AM	A	3/0	M	X
<i>Trichocline sinuata</i> (D. Don.) Cabrera	A	h	fl	AM	A	3/1	H	X
<i>Vernonia mollissima</i> D. Don ex Hook. et Arn.	B	s	fl	AM	A	2/1	M	X
<i>Vernonia nudiflora</i> Less.	A B	s	v	AM	A	2/0	M	X
<i>Xanthium cavanillesii</i> Schouw.	B	h	fl	AM	A	2/0	H	X
<i>Zinnia peruviana</i> (L.) L.	A B	h	fl	AM	A	5/0	H	X
Berberidaceae								
<i>Berberis ruscifolia</i> Lam.	A B	s	v	AM	P	3/0	L	
Boraginaceae								
<i>Heliotropium amplexicaule</i> Vahl	B	h	fl	NM		0/3		X
Bromeliaceae								
<i>Deuterocohnia longipetala</i> (Baker) Mez	A B	b	fl	NM		0/3		
<i>Puya spathacea</i> (Griseb.) Mez ^a	A B	b	fl	AM	A	2/1	M	
Buddlejaceae								
<i>Buddleja cordobensis</i> Griseb. ^{a,b}	A B	s	fl	AM	A	3/0	M	X

Table 2 (continued)

Family and species	Field site	Functional type	Phenology	Mycorrhizal type	CT	M/NM	AM colonization	DSE
Cactaceae								
<i>Opuntia sulphurea</i> Gilles ex Salm-Dyck	B	c	fl	AM	A	2/0	L	X
<i>Trichocereus candicans</i> (Gilles ex Salm-Dyck) Britton et Rose	B	c	fl	AM	A	2/1	L	X
<i>Gymnocalycium monvillei</i> (Lem.) Britton et Rose ^a	B	c	fl	AM	A	1/2	L	X
<i>Acanthocalycium spiniflorum</i> (K. Schum.) Backeb. ^a	B	c	fl	AM	A	2/2	L	X
Cannaceae								
<i>Canna indica</i> L.	B	h	fl	AM	A	2/2	L	X
Celtidaceae								
<i>Celtis tala</i> Gillies ex Planch.	A B	t	v	AM	I	3/0	L	X
<i>Celtis pallida</i> Torrey	B	s	v	AM	P	2/0	L	X
Chenopodiaceae								
<i>Chenopodium ambrosioides</i> L.	B	h	fl	NM		0/2		
Cleomaceae								
<i>Cleome aculeata</i> L.	B	h	fl	NM		0/4		
Commelinaceae								
<i>Commelina erecta</i> L.	A B	h	fl	NM		0/4		X
Convolvulaceae								
<i>Dichondra sericea</i> (O'Donell) Fabris	A B	h	v	AM	A	3/0	H	X
<i>Dichondra microcalyx</i> (Hallier) Fabris	A B	h	v	AM	A	3/0	H	X
<i>Ipomoea purpurea</i> (L.) Roth	A	v	fl	AM	A	3/0	H	
Cruciferaeae								
<i>Lepidium aletes</i> Macbr.	B	h	fr	NM		0/3		
Cyperaceae								
<i>Cyperus entrerianus</i> Boeck.	B	h	fl	AM	NF	2/0		
Ephedraceae								
<i>Ephedra triandra</i> Tul. enmend. J.H. Hunz	A B	h	v	AM	I	2/0	L	X
Equisetaceae								
<i>Equisetum giganteum</i> L.	A	f	v	AM	P	2/1	L	
Euphorbiaceae								
<i>Croton sarcopetalus</i> Mull. Arg.	A	s	fl	AM	A	2/0	L	X
<i>Acalypha communis</i> Mull. Arg.	A B	h	fl	AM	A	3/0	L	
<i>Euphorbia portulacoides</i> L.	A B	h	fl	AM	A	2/0	M	
<i>Euphorbia serpens</i> Kunth.	B	h	v	AM	A	3/0	M	
Fabaceae								
<i>Acacia atramentaria</i> Benth.	A B	s	fr	AM	A	3/0	L	X
<i>Acacia caven</i> Molina	A B	s	fr	AM	A	3/0	L	X
<i>Acacia praecox</i> Griseb.	B	t	v	AM	A	2/0	L	X
<i>Acacia visco</i> Griseb.	A	t	v	AM	A	2/0	L	X
<i>Caesalpinia gilliessi</i> (Wall. ex Hook.) D. Dietr.	A B	s	fl	AM	A	3/0	M	X
<i>Collaea argentina</i> Griseb.	B	s	fl	AM	A	3/0	L	X
<i>Geoffroea decorticans</i> (Gill. ex Hook. et Arn.) Burkart	A B	t	fl	AM	I	2/0	M	X
<i>Prosopis alba</i> Griseb.	B	t	v	AM	A	2/0	L	X
<i>Prosopis nigra</i> (Griseb.) Hieron	B	t	fr	AM	A	2/0	L	X
<i>Senna aphylla</i> (Cav.) H.S. Irwin et Barneby	B	s	fl	AM	A–P	2/0	L	X
<i>Sophora linearifolia</i> Griseb. ^a	B	s	v	AM	A	4/0	M	X
Iridaceae								
<i>Sisyrinchium chilense</i> Hook.	A B	h	fl	AM	A	3/0	L	X
Lamiaceae								
<i>Hedeoma multiflora</i> Benth.	B	h	fr	AM	A–P	2/0	L	X
Lythraceae								
<i>Heimia salicifolia</i> Link	B	s	fl	AM	P	2/0	L	X
<i>Cuphea glutinosa</i> Cham. et Schldl.	A B	h	fl	AM	A	3/0	M	X
Malpighiaceae								
<i>Janusia guaranitica</i> (St. Hill) Juss.	B	v	fl	AM	A	2/0	H	X

Table 2 (continued)

Family and species	Field site	Functional type	Phenology	Mycorrhizal type	CT	M/NM	AM colonization	DSE
Malvaceae								
<i>Abutilon grandifolium</i> (Willd.) Sweet	A B	s	fl	AM	I	3/0	L	
<i>Gaya parviflora</i> (Phil.) Krapov.	A B	h	fl	AM	A	2/0	H	X
<i>Mediolastrum gilliesii</i> (Steud.) Krapov.	B	h	fl	AM	I	3/0	H	X
<i>Sphaeralcea cordobensis</i> Krapov.	A B	s	fl	AM	P	4/0	M	X
Orquidaceae								
<i>Aa achalensis</i> Schltr. ^{a,b}	A	h	fl	OR		3/0		X
<i>Sacoila lanceolata</i> (Aubl.) Garay	A B	h	fl	OR		3/0		X
<i>Pelexia bonaerensis</i> (Lindl.) Schltr.	A	h	fl	OR		2/0		X
<i>Cyclopogon elatus</i> (Sw.) Schltr.	A	h	v	OR		3/0		
<i>Habenaria hexaptera</i> Lindl.	A B	h	fl	OR		4/0		
Onagraceae								
<i>Oenothera affinis</i> Cambess.	A B	h	fl	AM	A	2/1	L	X
Oxalidaceae								
<i>Oxalis conorrhiza</i> Jacq.	A B	h	fl	AM	I	2/1	M	X
Papaveraceae								
<i>Argemone subfusiformis</i> G. Ownbey	B	h	fl	NM		0/3		
Passifloraceae								
<i>Passiflora caerulea</i> L.	A B	v	fl	AM	A	2/0	M	X
Plantaginaceae								
<i>Plantago tomentosa</i> Lam.	B	h	fl	AM	I	3/0	H	X
<i>Plantago australis</i> Lam	B	h	fl	AM	A	3/0	H	X
Poaceae								
<i>Chloris</i> sp.	B	g	fl	AM	P	2/0	L	X
<i>Bromus</i> sp.	B	g	fl	AM	A	2/0	L	X
<i>Cortaderia selloana</i> (Schultes) Asch. & Graeb.	A B	g	fl	AM	A	2/0	L	X
<i>Cenchrus incertus</i> Curtis	A B	g	fr	AM	A	3/0	H	X
Polygonaceae								
<i>Ruprechtia apetala</i> Wedd.	A	t	v	AM	P	2/0	L	X
Portulacaceae								
<i>Portulaca grandiflora</i> Hook.	A	h	v	NM		0/3		
Pteridaceae								
<i>Argyrochosma nivea</i> (Poir.) Prantl	A	f	s	AM	P	2/1	M	
<i>Adiantum raddianum</i> Presl.	A B	f	s	AM	P	3/0	M	
<i>Adiantum</i> sp.	A	f	s	AM	P	2/0	L	
Ranunculaceae								
<i>Clematis montevidensis</i> Speg.	A B	v	fl	AM	P	3/0	M	X
<i>Thalictrum decipiens</i> Boivin.	A	h	v	AM	P	3/0	H	X
Rhamnaceae								
<i>Colletia spinosissima</i> J.F. Gmel.	A B	s	v	AM	A	3/0	H	X
Rosaceae								
<i>Acaena myriophylla</i> Lindl.	A	h	fl	AM	A	2/0	M	X
<i>Kageneckia lanceolata</i> Ruiz ex Pavon.	A B	t	v	AM	A	2/0	L	X
Rubiaceae								
<i>Galium latoramosum</i> Clos	A	h	v	AM	P	3/0	L	X
<i>Borreria densiflora</i> DC	A B	h	fl	AM	P	3/1	M	
Rutaceae								
<i>Fagara coco</i> (Gilles) Engl.	A B	t	v	AM	A–P	3/0	H	X
Salicaceae								
<i>Salix humboldtiana</i> Willd.	A B	t	v	AM+ECM	A	3/0	M	X
Santalaceae								
<i>Jodinia rhombifolia</i> (Hook. ex Arn.) Reisseck	A B	t	v	AM	NF	2/0		
Schizaeaceae								
<i>Anemia tomentosa</i> Mickel	A	f	s	AM	P	3/0	M	

Table 2 (continued)

Family and species	Field site	Functional type	Phenology	Mycorrhizal type	CT	M/NM	AM colonization	DSE
Selaginellaceae								
<i>Selaginella</i> sp.	B	f	v	AM	P	4/0	H	
Solanaceae								
<i>Cestrum parqui</i> Lhér	A B	s	fl	AM	I	3/0	M	X
<i>Nicotiana glauca</i> Graham	B	s	v	AM	I	2/0	M	X
<i>Nicotiana longiflora</i> Cav.	A	h	fl	AM	P	3/0	M	X
<i>Nierembergia linariaefolia</i> Gaham ^a	B	h	fl	AM	P	2/0	L	X
<i>Petunia axillaria</i> (Lam.) Britton, Stern et Poggenb.	A B	h	fl	AM	A	2/0	M	
<i>Salpichroa origanifolia</i> (Lam.) Baillon	A B	h	fr	AM	A	3/0	M	
<i>Solanum palinacanthum</i> Dunal	B	h	fl	AM	I	3/0	M	
<i>Solanum sisymbriifolium</i> Lam.	B	h	fl	AM	P	3/0	H	X
Turneraceae								
<i>Turnera sidoides</i> L.	A B	h	fl	AM	A	3/1	L	X
Verbenaceae								
<i>Aloysia gratissima</i> (Gillies ex Hook.) Tronc.	A B	s	v	AM	A	2/0	M	X
<i>Glandularia dissecta</i> (Willd. ex Spreng.) Schnack & Covas	A B	h	fl	AM	A	3/0	H	
<i>Glandularia peruviana</i> (L.) Small	A B	h	fl	AM	A	4/0	H	
<i>Lippia turbinata</i> Griseb.	A B	s	fl	AM	A	2/0	L	X
<i>Verbena litoralis</i> Kunth	B	h	fl	AM	A	2/0	H	X

Plant identification following Zuloaga et al. (1994) and Zuloaga and Morrone (1996, 1999a, b); functional type after Cagnolo et al. (2006). Field sites: A 30° 50' S–64° 30' W, B 30° 51' S–64° 33' W. Functional type: *b* bromelid, *f* fern, *g* grass, *h* herb, *p* palm, *s* shrub, *t* tree, *v* vine. Phenology: *fl* flowering, *fr* fruiting, *v* vegetative, *s* sporangia present. Type of mycorrhiza: *AM* arbuscular mycorrhizas, *ECM* ectomycorrhizas, *OR* orchid mycorrhizas. AM colonization type (CT): *P* Paris, *A* Arum, *A–P* Arum–Paris, *I* intermediate AM structures, *NF* non-functional. *M/NM*: the ratio of the number of individuals examined that had mycorrhiza (*M*) versus the number lacking mycorrhiza. AM Colonization: *L* low, *M* medium, *H* high, *DSE* dark septate endophytes

^a Endemic species

^b Endangered species

and *Buddleja cordobensis* have been declared endangered species (Vischi et al. 2004) (Table 2).

Four fungal colonization types were found: AM, ECM, OR, and DSE, similar to that observed in other semi-arid ecosystems (McGee 1986; Maremmani et al. 2003). AM was the most widespread, present in 89% of species. All sampled species belonging to the families Fabaceae, Asteraceae, and Anacardeaceae had AM. The prevalence of AM might suggest the importance of this symbiosis in the Chaco Serrano Woodlands ecosystem. The extent of this symbiosis is similar to or higher than that reported in other plant communities (Dhillon et al. 1995; Fontela et al. 2001; Camargo-Ricalde et al. 2003).

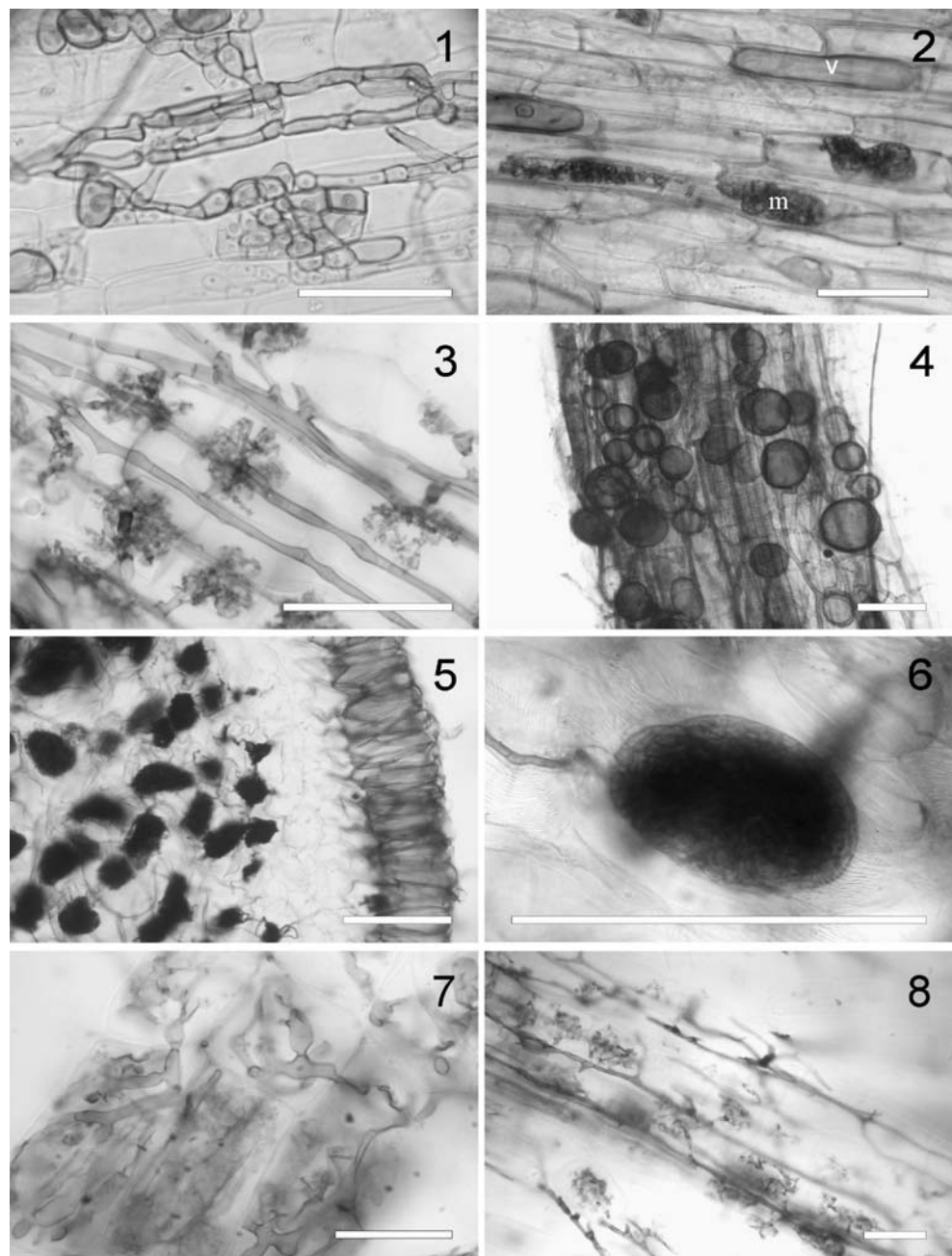
In this study, we observed a dominance of *Arum*-type AM colonization among herbs, shrubs, and grasses. Several studies showed that many trees and forest herbs form *Paris*-type intracellular hyphae, coils, and arbuscular coils in natural ecosystems (Brundrett and Kendrick 1990). However, it is important to emphasize that, although AM morphology depends on plant species and AM fungi, environmental factors such as light and temperature, which affect plant growth and soil factors like aeration, water content, pH, and nutrient supply, may also contribute to

morphological variability (Dickson 2004; Dickson et al. 2007).

The DSE association was present in 75% of species examined. In contrast with other authors, we observed that DSE associations were not more prevalent in herbs than in trees (Ahlich and Sieber 1996; Muthukumar et al. 2006). Nearly all (>90%) of shrubs and trees had typical DSE structures which were present in cortical cells, but not within the xylem as reported in some studies (Yu et al. 2001; Barrow 2003). In the terrestrial bromelid *D. longipetala* and the Papaveraceae *A. subfusiformis*, profuse melanized hyphae were present only in the rhizoplane; these species were categorized as non-mycorrhizal plants.

Co-occurrence of AM and DSE was observed in 88 (68.7%) of species examined. The prevalence of this simultaneous colonization seems to be common in semi-arid regions suggesting an eventual role in such ecosystems (Mandyam and Jumpponen 2005; Muthukumar et al. 2006). The three different fungal root associations reported in *Salix commutata* Bebb and *S. planifolia* Pursh, ECM, AM, and DSE (Trowbridge and Jumpponen 2004), were also observed in *Salix humboldtiana*. Multiple colonization has been reported in *Eucalyptus*, *Populus*, and *Alnus* species

Fig. 1 Patterns of mycorrhizal fungi and dark septate endophytes roots colonization of native plants in the Chaco Serrano Woodland from central Argentina. **1** Melanized hyphae present in *Apidosperma quebracho-blanco* (Apocynaceae). **2** Microsclerotium and AMF vesicles in *Heterothalamus alienus* (Asteraceae) (*v* vesicle, *m* microsclerotium). **3** Arbuscules in *Tagetes minuta* (Asteraceae). **4** Intra- and inter-cellular vesicles and mycelia of AM fungi in *Gomphrena pulchella* (Amaranthaceae). **5** Hyphal coils forming pelotons in *Aa achalensis* (Orchidiaceae). **6** DSE microsclerotium within root cortical cell of *Sacoila lanceolata* (Orchidiaceae). **7** Paris-type AM in *Borreria densiflora* (Rubiaceae). **8** Arum-type AM in *Trichocline sinuata* (Asteraceae). Scale bars: 1, 2, 3, 7, 8=25 μ m; 4, 5, 6=100 μ m



(Chilvers et al. 1987; Maremmani et al. 2003). Although the tree *Fagara coco* (Gilles) Engl. has been reported to associate with the edible fungus *Phlebopus bruchii* (Speg.) Heineman & Rammeloo (Deschamps and Moreno 1999), we did not find typical ECM structures in the roots of this species. However, as in *S. humboldtiana*, AM showing a dual colonization of *Arum* and *Paris* morphologies and DSE structures were abundant in fine roots.

Characteristic OR colonization was observed in the five terrestrial orchid species. The roots of *Sacoila lanceolata* (Aubl.) Garay, *Aa achalensis*, and *Pelexia bonaerensis* (Lindl.) Schltr. showed also DSE colonization in the first layers of cortical cells, similar to that reported for another

terrestrial orchid *Calypso bulbosa* (L.) Oakes (Currah et al. 1988). The species *Gomphrena pulchella* (Amaranthaceae) and *Cyperus entrerianus* (Cyperaceae), belonging to families considered non-mycorrhizal (Miller et al. 1999), were devoid of any mycorrhizal symbiosis although intra-radical aseptate hyphae and/or vesicles were present in some roots.

As we considered the DSE association as endophytic and mutualistic, the proportion of non-colonized plant species found in the Chaco Serrano Woodland was low (7%) as compared with that reported for other vegetation communities (Brundrett and Abbott 1991; Zhao et al. 2001). When only mycorrhizal colonization is considered, the value for non-colonized plants (10.1%) is similar to that found in other

studies (Maremmani et al. 2003; Muthukumar et al. 2006). The low incidence of non-mycotrophic species may reflect an inability of such plants to compete successfully with the mycotrophic species in this ecosystem (Peat and Fitter 1993).

If the observed rates of deforestation of the Chaco Serrano Woodlands continue, the forest cover is expected to be reduced by the year 2020 to 20% of the area occupied in 1970 (Gavier and Bucher 2004). This situation indicates the need for management and conservation programs in this severely threatened ecosystem. Considering the predominance of AM and DSE associations and the various potential benefits that these associations may bring to plant establishment (Brundrett 2006, Schulz 2006), they should receive more attention in the conservation and reforestation of these woodlands

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