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Arbuscular mycorrhizas in a hot and arid ecosystem in southwest China

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Abstract

The colonization by arbuscular mycorrhizal fungi, arbuscular mycorrhizal fungal spore abundance and community were investigated in a valley-type semi-savanna vegetation of Yuan River in southwest China. Of the 62 plants representing 33 families surveyed, 59 plant species (about 95%) were arbuscular mycorrhizal and 3 species (5%) were possibly arbuscular mycorrhizal. Rhizosphere soils harbored abundant arbuscular mycorrhizal fungal spores in a range of 240–6430 per 100 g soil with an average of 2096, and most spores were small with diameter less than 70 μm (about 78%). The fungi most frequently found were members of the genera *Acaulospora* and *Glomus*. *Acaulospora spinosa*, *A. denticulata*, *A. tuberculata*, *Glomus sinuosa*, *G. clarum*, *G. intraradices* and *G. microaggregatum* were the most common species. These results revealed that arbuscular mycorrhizas are a common and important component in this semi-savanna vegetation; the high spore density and colonization were presumably a selective adaptation toward the hot and arid ecosystem.

Keywords: Hot and arid ecosystem; Arbuscular mycorrhiza; Valley-type semi-savanna vegetation

1. Introduction

Over the past several years there has been a growing appreciation of the importance of plant/fungal interactions, especially arbuscular mycorrhizas (AM), on terrestrial ecosystems (Mukerji and Kapoor, 1986; Koske et al., 1992; Stutz et al., 2000; Zhao et al., 2001; Maremmani et al., 2003; Muthukumar et al., 2003). Mycorrhizas form a critical link between the aboveground plant and the soil by influencing plant nutrient cycling and soil structure (Korb et al., 2003) and make a large direct contribution to soil fertility and quality through soil organic matter (Rillig et al., 2001). Some plant species are arbuscular mycorrhizal dependent in natural ecosystems (Gemma et al., 2002) and in the greenhouse environment (Gemma and Koske, 1997). It has been demonstrated that mycorrhiza was one of the components in natural ecosystems and could potentially affect the aboveground vegetation structure and communities (van der Heijden et al., 1998a,b; Streitwolf-Engel et al., 2001). AMF have

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been used in vegetation establishment in stressful and disturbed ecosystems (Estaún et al., 1997; Smith et al., 1998; Requena et al., 2001; Caravaca et al., 2003). In a comparison of several methods to rehabilitate the fragile degraded lands in La Gran Sabana, Cuenca et al. (1998) concluded that the rehabilitation of tropical land was not possible through the application of chemical fertilizers but also needed AMF.

The Yuan River is the upper part of an international river, which winds through Yunnan province (southwest China) and then enters Vietnam, as Red River. Warm and wet airflow from the Bay of Bengal is blocked by the surrounding mountains (at an altitudinal range of 2500–3000 m). Hot air makes the long and narrow valley, with an altitudinal range of 300–1300 m, of the Yuan River arid. Overgrazing, chopping and burning have accelerated soil degradation and desertification process in this unique arid ecosystem. Yuanjiang county is the most representative site of this ecosystem, which is located at 23°19′N–55′ and 101°39′E–102°22′. Data from Yuanjiang county weather station covering a period of 26 years indicate an annual average temperature of 23.7 °C with a maximum of 42 °C. The mean annual precipitation is 805.1 mm, rainfall between June and October accounts for 81% of all the precipitation, while annual evaporation reaches 2750.9 mm, about 3.4 times of precipitation. Vegetation is dominated by treeless savannas together with scattered forest and scrublands. The most common plant species are *Heteropogon contortus* and *Bothriochloa pertusa* with scattered of *Buchanania latifolia*, *Lannea coromandelia* and *Terminthia paniculata*. The origin, development, evolution, structure and function of this unique valley-type semi-savanna vegetation have been detailed (Zhu, 1990; Jin, 1999, 2002; Jin and Ou, 2000). The role and occurrence of arbuscular mycorrhiza, a functional component and the most significant ecological factor of this unique semi-savanna vegetation in southwest China have been largely ignored. To understand the arbuscular mycorrhizal status of plants growing in this unique ecosystem and the community of AMF in the rhizosphere soils, 62 species of plant (representing 33 families) roots and rhizosphere soils were sampled. AMF colonization and the abundance and communities of AMF were documented.

2. Materials and methods

Roots and their rhizosphere soils were collected to a depth of 5–30 cm in January 2003 (dry season), after ensuring that the roots were connected to plants sampled. Equipment was cleaned between samples. Part of the root system of each plant was fixed in 5 ml formalin, 5 ml acetic acid and 90 ml of 70% alcohol, diluted twice (1/2 FAA), and stored at 4 °C. The remaining roots were air-dried with their rhizosphere soil (about 500 g) for 2 weeks, and then stored in sealed plastic bags at 4 °C for up to 2 months until samples could be processed. Roots were taken from the 1/2 FAA, washed several times in tap water and bleached in 10% (w/v) KOH by heating to approximately 90 °C in a water bath for 2–3 h, the time depending on the size/structure of the roots and their pigmentation. The cooled root samples were washed and cut into 0.5–1 cm segments and stained with 0.5% acid fuchsin according to Berch and Kendrick's method (1982). Fifty 0.5–1 cm root fragments were examined per sample for their AM status under a compound microscope (160–880×). If at least one root segment was found to contain arbuscules or vesicles, then the plant was noted as an AM plant, recorded as "+". If the root cortex was found to be colonized by fungal mycelia, but without arbuscules or vesicles, the corresponding plant was noted as possibly AM, recorded as "±". Plants were recorded as non-mycorrhizal ("−") when neither arbuscules/vesicles nor fungal mycelia were detected in their root cortical cells.

The rhizosphere soil samples were wet-sieved for spores using the method described by An et al. (1990). Twenty grams of soil from each plant rhizosphere were independently suspended in 150 ml water, stirred with a magnetic stirrer for 10 min, sieved using 40, 70, 100 and 150 μm sieves with tap water, filtered onto a filter paper, and then placed in a 9 cm Petri dish for examination under a binocular stereomicroscope (7–45×). The intact, healthy AMF spores with shining appearances were considered to be alive and counted in the four sieved samples. Some spores were tightly grouped in a sporocarp, *Glomus sinuosa* for example. Here it was difficult to count the number of spores in the sporocarp and in these cases a sporocarp was counted as one spore.

Each spore type was mounted sequentially in water, lactophenol, PVA (polyvinyl lactic acid) and Melzer's reagent (Morton, 1988) for identification. Identification was based on spore colour, size, surface ornamentation and wall structure, with reference to the descriptions provided by the International collection of vesicular and arbuscular mycorrhizal fungi (http://invam.caf.wvu.edu) and the original species descriptions.

3. Results

AMF hyphae, hyphal coils, vesicles and arbuscules were stained red while the root cortical cells were unstained or pale red. Of the 62 root samples, 59 plant species (95%) were arbuscular mycorrhizal and the other 3 plant (5%) were possibly arbuscular mycorrhizal (Table 1). AMF colonization patterns varied from single intracellular aseptate hyphae, hyphal coils to vesicles and/or arbuscules. AMF hyphae, hyphal coils, vesicles and arbuscules occurred abundantly in the cortical cells of species, such as *Bothriochloa pertusa*, *Acacia farnesiana*, *Breynia fruticosa*, *Cyanotis cristata*, *Vitex negundo*, *Sida acuta*, *Polyalthia cerasoides*, *Boea hygrometrica*. AMF spores were obtained from all rhizophere soil samples. The rhizosphere soils contained an abundance of AMF spores ranging from 240 to 6430 with an average of 2096 ± 1172 per 100 g soil. Within the AMF spores or/and sporocarps obtained, 78% (20316 spores) were small with diameter less than 70 μm, 17% (4392 spores) were in the 70–100 μm diameter range, with 5% (1281 spores) larger than 100 μm. Some spores were identifiable to species level through their morphological characters (Fig. 1). Among these, *Acaulospora spinosa*, *A. denticulata*, *A. tuberculata*, *Glomus sinuosa*, *G. clarum*, *G. intraradices* and *G. microaggregatum* were the most commonly encountered species. Five genera of AMF were identified, among which *Acaulospora* and *Glomus* were dominant with frequencies of 21% and 69%. The frequencies of AMF from the other three genera were low (Table 2). Some AMF species developed their spores in the host plant tissues (Fig. 1(5)), such as *G. intraradices*, *G. clarum*, *G. microaggregatum* or in a sporocarp with a dense hyphal peridium, such as *G. sinuosa*.

4. Discussion

The ecosystem surveyed is characterized by high temperature and stress for most of the year. These conditions limit plant establishment and growth. In our study, 95% of plants surveyed were arbuscular mycorrhizal. Obviously no non-mycorrhizal plant species were observed (Table 1). Wubet et al. (2003) reported 11 indigenous trees in the dry Afromontane forests of Ethiopia as typically being arbusucular mycorrhizal. Similar situations have been observed in other drought influenced ecosystems (Mukerji and Kapoor, 1986; Stutz et al., 2000; Li et al., 2004). In contrast, only 56% of plants were arbuscular mycorrhizal in the humid tropical rain forest (Zhao et al., 2001). Tawaraya et al. (2003) found that 77% of tree species grown in peat swamp forests of Central Kalimantan, Indonesia were arbuscular mycorrhizal. Work by Onguene and Kuyper (2001) showed that 79% plants were AM in the rain forest of south Cameroon. Comparison of the AMF colonization rate and intensity of infection in roots from different ecosystems suggested that plants grown in the semiarid (and arid) habitats might be more dependence on AM.

Spore densities are known to vary greatly in different ecosystems. Values range from dozens to 10,000 spores per 100 g soil (Cuenca and Lovera, 1992; Johnson and Wedin, 1997; Picone, 2000; Zhao et al., 2001; Li et al., 2004). Here, the density of AMF spores was relatively high (2096 spores/100 g soil in average). The presence of spores in dead roots provides support for Picone's (2000) view that death or senescence of host plants induced AMF to sporulate. The abundance of AMF spores in the soil of hot and arid valley of the Yuan River suggests that this type of ecosystem may be characterized by a high diversity of AMF.

In the Glomales, the small-spored species mainly fall into the genera *Acaulospora* and *Glomus* (Morton, 1988). Most of the AMF spores (78%) obtained by wet-sieving were small (diameter less than 70 μm). The dominance of small spores may be a selective adaptation to water stress (Brundrett et al., 1999; Boddington and Dodd, 2000). Picone (2000) reported that small spores were more frequent and had a low seasonal variation than larger spores. In addition, small spore species are more common within host

Table 1
AM status of plants and spore densities of AMF in the valley-type savanna soils

Plant species	M	ASD	Plant species	M	ASD
Acanthaceae			Labiatae		
Barleria cristata	+	705	*Ocimum basilicum var. pilosum*	+	675
Woodfordia fruticosa	+	1595	Malvaceae		
Agavaceae			*Sida acuta*	+	3215
Agave americana	±	240	*Sida cordifolia*	+	1835
Amaranthaceae			*Sida szechuensis*	+	3365
Achyranthes aspera	+	1145	Meliaceae		
Anacardiaceae			*Cipadessa cinerascens*	+	4095
Buchanania latifolia	+	1305	Mimosaceae		
Lannea coromandelica	+	1565	*Albizzia kalkora*	+	1190
Terminthia paniculate	+	1450	*Leucaena leucocephala*	+	1805
Annonaceae			Moraceae		
Polyalthia cerasoides	+	1845	*Ficus cyrtophylla*	+	2530
Asclepiadaceae			Nyctaginaceae		
Calotropis gigantea	+	2140	*Boerhavia diffusa*	+	2235
Dregea volubilis	+	2240	Papilionaceae		
Bauhinia			*Atylosia mollis*	+	1970
Bauhinia aurea	+	5230	*Cassia occidentalis*	+	2250
Cactaceae			*Desmodium zonatum*	+	2730
Opuntia dillenii	+	1085	*Indigofera* sp.	+	1730
Caesalpiniaceae			*Indigofera cinerascens*	+	2180
Acacia farnesiana	+	3225	*Indigofera linifolia*	+	1705
Cassia siamea	+	1095	*Lespedeza juncea*	+	4065
Commelinaceae			Rhamnaceae		
Cyanotis cristata	+	2860	*Ziziphus mauritiana*	+	4735
Compositae			Rutaceae		
Bidens bipinnata	+	3680	*Clausena lenis*	+	3680
Eupatorium odoratum	+	1580	Sapindaceae		
Laggera pterodonta	+	1895	*Dodonaea viscose*	+	2265
Sonchus arvenisis	+	2400	Selagineuaceae		
Tridax procumbens	+	2400	*Selaginella moellendorfii*	+	3015
Convolvulaceae			Sinopteridaceae		
Evolvulus alsinoides var. decumbens	+	3610	*Aleuritopteris rufa*	+	1795
Porana discifera	+	705	Solanaceae		
Euphorbiaceae			*Physalis alkekengi*	+	2190
Breynia fruticosa	+	900	*Solanum indicum*	+	1230
Euphorbia antiquorum	+	960	Symplocaeae		
Euphorbia hirta	+	1195	*Symplocos racemosa*	+	2370
Phyllanthus emblica	+	6430	Tiliaceae		
Phyllanthus urinaria	+	1180	*Grewia abutilifolia*	+	1430
Gesneriaceae			*Triumfetta pilosa*	±	1525
Boea hygrometrica	+	1110	Ulmaceae		
Gramineae			*Trema tomentosa*	+	2175
Bothriochloa pertusa	+	1435	Verbenaceae		
Capillipedium assimile	+	1435	*Vitex negundo*	+	1395
Heteropogon contortus	+	1900	Zygophyllaceae		
Neyraudia neyraudiana	+	1675	*Tribulus cistoides*	±	540
Paspalum orbiculare	+	1810			

Notes: M: AM status of plants, + mycorrhizal, ± possibly mycorrhizal; ASD: average AMF spore density (number of AMF spores in 100 g soil from the corresponding plant rhizosphere).

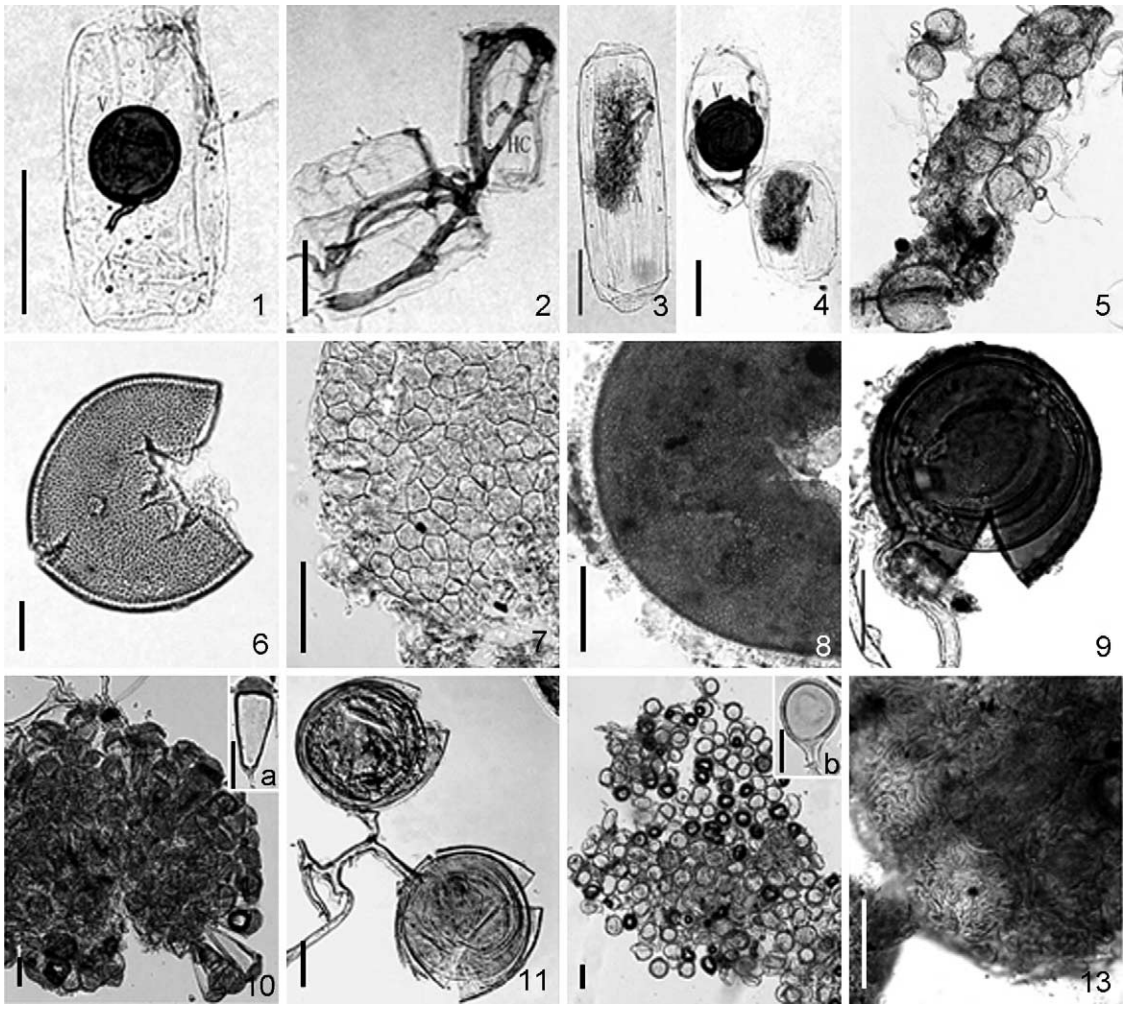

Fig. 1. Arbuscular mycorrhizal colonization in the roots and some identified AMF: (1) a vesicle (V) in a root cell; (2) aseptate hyphal coils (HC) in root tissue cells; (3) a typical arbuscule (A) in a cortical cell; (4) A vesicle (V) and an arbuscule (A) in root tissue; (5) one cluster of AMF spores (S) in root tissue; (6) *Acaulospora scrobiculata*; (7) *A. bireticulata*; (8) *Entrophospora infrequens*; (9) *Glomus claroideum*; (10) *G. clavispora*; (11) *G. intraradices*; (12) *G. microaggregatum*; (13) *G. sinuosa*. 1–13: bar = 40 μm; a and b: bar = 20 μm.

Table 2
Identified AMF genera and their frequencies of occurrence in rhizosphere soils

AMF genus	OT	*F* (%)
Acaulospora	342	21
Entrophospora	15	1
Gigaspora	68	4
Glomus	1122	69
Scutellospora	85	5
Total	1632	100

Notes: OT: the occurrence times of the corresponding AMF identified; *F*: frequency of occurrence.

plant roots, as was found for *G. intraradices*, *G. clarum*, *G. microaggregatum*. These AMF species appeared the most adapted to the hot and arid environment.

The results of the current work showed that AM are a common and important component in the hot and arid environment, AMF with small spores may be more adaptive to this ecosystem. This suggested that AM may take an important role in the developing and sustaining of vegetation in this area. AM cannot be ignored in the reestablishment of this ecosystem and

small spore AMF might have a potential application in the practice.

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