Mycorrhiza (2007) 17:527–535
DOI 10.1007/s00572-007-0125-6

ORIGINAL PAPER

Mycorrhizal status of *Eucalyptus* plantations in south China and implications for management

Ying Long Chen · Shuie Liu · Bernard Dell

Received: 27 November 2006 / Accepted: 28 February 2007 / Published online: 4 April 2007

Abstract The aim of this study is to assess the mycorrhizal status of *Eucalyptus* plantations in south China and to determine the need for inoculation. In four provinces in south China, 155 plantations were sampled for sporocarps of ectomycorrhizal (ECM) fungi, spores of arbuscular mycorrhizal (AM) fungi, and mycorrhizas over 2 years. This study revealed a low above-ground diversity of ECM fungi consisting of 15 taxa fruiting beneath *Eucalyptus* plantations. The most common ECM genera were *Scleroderma* and *Pisolithus*, but they were infrequent. A total of 21 AM fungi, mostly *Glomus* species, were recognized from spores collected from eucalypt plantations. Four *Glomus* species were frequently present in soils, but spore density and relative abundance of AM fungi were generally low. Eucalypt roots from all plantation sites were poorly colonized by either ECM fungi or AM fungi. A bioassay with *E. urophylla* as a bait host, using soils collected from 11 eucalypt plantations, confirmed low levels of inoculum of both ECM and AM fungi in field soil. This is the first integrated study on the mycorrhizal status of eucalypt plantations in China. Findings from this research can be used to encourage adoption of mycorrhizal technology by eucalypt nurseries in the region. The potential of using spores of compatible ECM fungi or collections for forest nurseries is discussed.

Keywords Ectomycorrhizal fungi · Arbuscular mycorrhizal fungi · Diversity · *Eucalyptus* plantations · South China · Nursery inoculation

Y. L. Chen · S. Liu · B. Dell
School of Biological Sciences and Biotechnology,
Murdoch University,
Perth, WA 6150, Australia

S. Liu
Research Institute of Tropical Forestry,
Chinese Academy of Forestry,
Guangzhou 510520, China

Present address:
Y. L. Chen (✉)
School of Plant Biology, The University of Western Australia,
Perth, WA 6009, Australia
e-mail: yinglong.chen@uwa.edu.au

Introduction

Eucalyptus plantations have been largely established in south China. These trees predominantly form ectomycorrhizal (ECM) associations in both native forests (Chilvers 2000) and plantations (Brundrett et al. 1996; Giachini et al. 2004). They also have arbuscular mycorrhizal (AM) associations and dual ECM/AM associations (Oliveira et al. 1997; Chen et al. 2000a; Lodge 2000). Preliminary observations in south China on fungal diversity under eucalypt plantations and findings from a few inoculation trials, suggest eucalypt seedlings would benefit from inoculation with compatible symbionts before outplanting on nutrient-poor soils (Malajczuk et al. 1994; Chen et al. 2000b, 2006a; Dell et al. 2002; Brundrett et al. 2005). There is still reluctance by many nursery managers in south China to adopt mycorrhizal technology in eucalypt nurseries. Hence, new eucalypt plantations are still being established using predominantly nonmycorrhizal plants. One of the factors that limits further adoption of inoculation technology in China is the lack of information on the diversity and abundance of mycorrhizal fungi under eucalypt plantations in China. Furthermore, little is known about the symbiotic status of indigenous fungal species with exotic trees. This knowledge is important because of the potentially valuable role of mycorrhizal fungi in the sustainable productivity of eucalypt plantations.

This study examined the frequency of occurrence and the diversity of mycorrhizal fungi in *Eucalyptus* plantations in south China. To determine this, the following components were examined: (1) putative ECM fungi fruiting beneath plantations; (2) the AM fungal community in plantation soil; (3) the extent of mycorrhizal colonization of eucalypts by indigenous symbionts; and (4) the inoculation potential of fungal propagules using a pot bioassay.

Materials and methods

Site location and plantation species involved in fungal surveys

A number of field surveys were carried out to investigate the frequency of occurrence and the diversity of putative mycorrhizal fungi in *Eucalyptus* plantations in south China. A total of 155 plantations at 31 locations (Guangdong [11 locations], Guangxi [eight locations], Hainan [five locations], and Yunnan [seven locations] Provinces) were sampled covering the main regions of commercial eucalypt plantations in south China. In each location, five plantations (generally more than 50 ha in area and at least 5 km apart) were chosen for the survey. Eight eucalypt species, plus a hybrid and a clonal line, were included in the survey, namely, *E. camaldulensis*, *E. citriodora*, *E. exserta*, *E. globulus*, *E. grandis*, *E. grandis* × *E. urophylla*, *E.* Leizhou no.1 (local name—unknown clonal line from Leizhou Forestry Bureau), *E. propinqua*, *E. robusta*, and *E. urophylla*. Each plantation site was visited once in May or June (wet/warm season) over 2 years.

Observations of ECM fungal sporocarps

An area of approximately 100 m^2 was sampled from inside each plantation, and the presence of sporocarps of putative ECM fungi was recorded for each taxon. Fungal abundance was recorded as: +++, very frequent (four to five plantations); ++, frequent (two to three plantations); +, infrequent or rare (one plantation); and 0, absent. This ranking referred to each location (other than overall plantations) to reveal the variation of fungal abundance between locations. Species frequency by plantation (SF_p) was calculated as: $SF_p = (S_p/S_{tp}) \times 100$, where S_p=number of plantations in which the fungus was observed, and S_{tp}=total plantations, i.e., 155. SF by location (SF_l) was calculated as: $SF_l = (S_l/S_{tl}) \times 100$, where S_l=number of locations in which the fungus was observed, and S_{tl}=total locations (i.e., 31). Fungal species richness (SR) was defined as the number of putative ECM fungal taxa present in each location over the total taxa. Notes on the morphological characteristics of sporocarps and site information were taken during the field survey, and sporocarp collections were made for further identification in the laboratory based on the morphological characters. Fresh specimens were compared to color illustrations in field guides, and simple keys and descriptions were used to recognize fungal genera and species (such as Mao 2000; Fuhrer 2005).

Soil sampling procedure

Two sets of soil samples were taken. The first set of soil (S_a) was collected from all 155 plantations to examine mycorrhizal colonization on roots ("Root processing to measure mycorrhizal colonization") and the AM fungal community in soil ("AM fungus spore quantification and identification"). A bulk sample of soil was obtained for each plantation by combining five samples (ca. 500 g each) taken randomly from 10–30 cm below the surface horizon. Both fine roots of eucalypts and rhizosphere soils were included. When possible, soil was sampled from sites that were free of weeds and understorey to maximize the presence of eucalypt roots in the samples. A second set of soil (S_b) was collected from 55 plantations of 11 locations in Guangdong Province to examine inoculation potential within the soil profile using a eucalypt seedling bioassay (Brundrett and Abbott 1995; Chen et al. 1999; "Bioassay experiment"). Approximately 3 kg of bulk soil (S_b) from three replicates of each plantation was taken from the surface horizon (1–30 cm). There were five replicate samples for each location.

Root processing to measure mycorrhizal colonization

Fresh fine roots of eucalypts were picked out from each soil sample (S_a) to examine mycorrhizal colonization. Although the roots of weeds or other plants were minimized during field sampling, care was taken to distinguish eucalypt roots from the roots of other plants. Fresh roots were washed free of soil. Morphological descriptions of each ECM morphotype were made using terminology based on Ingleby et al. (1990) and images in Brundrett et al. (1996). For colonization measurement, roots were cleared in 10% (w/v) KOH in a 90°C water bath for 60–90 min depending on root age. The cooled root samples were then washed with water and stained with 0.5% (w/v) acid fuchsin (Biermann and Linderman 1981). The ECM root tips were counted under a dissecting microscope, whereas the percentages of root length colonized by AM fungal structures were measured using the magnified line-intersect method under a compound microscope (Brundrett et al. 1996). Colonization of roots by AM fungi was determined by the presence of hyphae, arbuscules, or vesicles at an intersection. Three replicates, a minimum of 50 root segments per replicate, were counted for each sample.

AM fungus spore quantification and identification

AM spores and sporocarps were extracted from 40 g of air-dried subsamples of each soil sample (S_a) in triplicate by wet sieving followed by flotation centrifugation in 50% sucrose (Dalpe 1993). Spores were collected on a grid patterned (4× 4 mm) filter paper, washed three times with distilled water to spread them evenly over the entire grid, and counted using a dissecting microscope. The number of spores was expressed as the mean of three replicates. Relative abundance (RA) and SF were expressed as the number of spores of a species/total spores × 100 and the number of plantations in which the species was observed/total plantations × 100, respectively. Spores were mounted on glass slides in polyvinyl alcohol lactoglycerol for identification according to Schenck and Perez (1988), Morton and Redecker (2001), and information published by the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (INVAM, http://www.invam.caf.wvu.edu) and the AM fungal phylogeny (http://www.amf-phylogeny.com).

Bioassay experiment

Soils (S_b) collected from 11 plantation locations in Guangdong Province were used for a bioassay experiment to analyze soil inoculum potential using roots of bait plants (*E. urophylla*). A complete randomized design consisting of 12 soil treatments (11 field soils, one potting mix autoclaved at 121°C for 20 min) with five replicates was used. Air-dried soils were placed in plastic pots (8-cm top diameter, 10-cm deep) lined with clean plastic bags. Soils were watered to field capacity (about 12%). Seeds of *E. urophylla* (seedlot no. 19393, Pantar Island, Indonesia) were surface sterilized by shaking in a 1.2% solution of NaOCl for 5 min. After four rinses in sterile water, seeds were transferred to a tray filled with autoclaved river sand (121°C, 30 min). Seeds were incubated in darkness at room temperature for 10 days and in the light for 3 days before pricking out for planting. Two uniform seedlings were transplanted into each pot. There were five replicate pots for each soil treatment. The pots were placed in a glasshouse in Guangzhou. Water was added by hand as required, and no mineral nutrients were applied. All seedlings were harvested at 12 weeks. Roots were cleared and stained to assess mycorrhizal colonization ("Root processing to measure mycorrhizal colonization").

Data analysis

The colonization data collected from field samples and the bioassay experiment were subjected to one-way ANOVA using SPSS software version 11.0. The differences in root colonization were separated by Duncan's multiple range test (Gomez and Gomez 1984). Percentage data were transformed to Arc sin before performing statistical analysis.

Results

Diversity and abundance of putative ECM fungi

Based on observations on the presence of sporocarps, 15 putative ECM fungi belonging to six genera were recorded under 155 *Eucalyptus* plantations in south China (Table 1). However, SF (SF_p) was low and ranged from 1.5 (*Russula puellaris*) to 22.6% (*Scleroderma cepa*) across plantations. Only a few species were frequently observed across 31 geographic locations. *S. cepa* and *Pisolithus* sp2 had the highest frequency (61 and 58%, respectively). Other fungi were present in a small number of plantations. There were six *Scleroderma* species (Table 1).

Species of *Scleroderma*, especially *S. cepa* and *S. polyrhizum*, *Pisolithus*, and *Laccaria* were the dominant ECM fungi fruiting above ground. These fungi were present in a wide range of soils including granitic loam, red clay, and doleritic soils. Furthermore, these taxa were observed equally under young (1–3 years) and older (4–7 years) eucalypt stands with no apparent host specificity between eucalypt species. In contrast, *Amanita griseoverrucosa*, *Boletus griseus*, and *Russula puellaris* usually occurred in newly established plantations on land that had been cleared of native secondary forests containing members of Pinaceae or Fagaceae particularly in highland Yunnan Province.

Species richness and abundance of putative ECM fungi varied between geographic locations (Table 2). Thirteen species were recorded from highland Yunnan Province, ten from Guangdong, nine from Guangxi, and six from Hainan Island. The higher species richness was obtained from Chuxiong (0.47), Jingdong (0.40) and Baoshan (0.40) in Yunnan, Liuzhou (0.33) and Pingxiang (0.33) in Guangxi, and Qingyuan (0.33) in Guangdong. Plantations in the Hainan Province had lower species richness (0.13 or less). It was noted that eucalypt plantations in Hainan were mostly established on barren areas or semiagricultural land where there were few indigenous ECM trees in the landscape.

AM fungal diversity

A total of 21 AM fungi in four genera were recorded in soils collected from under eucalypt plantations in south China (Table 3). There were 16 *Glomus* species and five other species distributed across three genera as follows:

Table 1 Putative ECM fungi fruiting under *Eucalyptus* plantations in south China

Fungus	Code	No. of locations (n=31)	No. of plantations (n=155)	SF_p^a	SF_l^a	Tree[b]
Amanita gemmata (Fr.) Gill	A1	5	5	5.2	16.1	EGL, EUR
Amanita griseoverrucosa Yang	A2	3	3	1.9	9.7	ECA, EGL
Boletus griseus Forst	B1	3	3	1.9	9.7	EGL, EPR
Laccaria amethystea (Bull. ex Gray.) Murr.	L1	7	7	4.5	22.6	EEX, EPR, ERO, EUR,
Laccaria laccata (Scop.:Fr.) Berk et Br.	L2	8	9	6.8	25.8	EGL, EGU, ERO
Pisolithus sp1	P1	4	4	2.6	12.9	EGL, EPR
Pisolithus sp2	P2	18	32	20.6	58.1	EGL, EPR, ERO, EUR
Russula aeruginea Lindb.:Fr.	R1	8	8	6.2	25.8	EEX, EGL
Russula puellaris Borszcz.	R2	2	2	1.5	6.5	ECA, EEX
Scleroderma areolatum Ehrenb.	S1	2	2	1.5	6.5	EGL
Scleroderma bovista Fr.	S2	1	2	1.5	3.2	EGL
Scleroderma cepa Pers.	S3	19	35	22.6	61.3	ECA, ECL, EEX, ERO, EUR
Scleroderma citrinum Pers.	S4	11	20	12.9	35.5	EGU, EGR, EGL, EUR,
Scleroderma paradoxum Beaton	S5	3	3	1.9	9.7	ELE, EGL
Scleroderma polyrhizum Pers.	S6	15	19	12.5	48.4	ELE, EGL, EUR

Species frequency (SF) by plantation and location are given.

[a] Species frequency by plantation (SF_p) or by location (SF_l): $SF_p = \left(S_p/S_{tp}\right) \times 100$, $SF_l = (S_l/S_{tl}) \times 100$, where S_p (or S_l)=the number of plantations (or locations) in which the fungus was observed, and S_{tp} (or S_{tl})=total plantations (or locations); names of locations are given in Table 2.

[b] *Eucalyptus* species: *ECA E. camaldulensis*, *ECI E. citriodora*, *EEX E. exserta*, *EGL E. globulus*, *EGR E. grandis*, *EGU E. grandis* × *E. urophylla*, *ELE E.* Leizhou no. 1, *EPR E. propinqua*, *ERO E. robusta*, *EUR E. urophylla*

Acaulospora (3), *Gigaspora* (1), and *Scutellospora* (1). Spore density and relative abundance of each AM fungal taxon was generally low. *G. mosseae* had a higher relative abundance of spores (22.5) followed by *G. geosporum* (15.4). SF ranged from 2.6 (*A. scrobiculate*) to 67% (*G. mosseae*). Four *Glomus* species, i.e., *G. mosseae*, *G. formosanum*, *G. versiforme*, and *G. geosporum*, were frequently observed in more than one third of the plantation sites.

Mycorrhizal colonization in field samples

Examination of mycorrhizal colonization of roots collected from under eucalypt plantations in south China showed that mycorrhizal colonization rates were low (Table 4). The average colonization rate for each location ranged from 0 to 15.2% (short roots colonized) for ECM and from 0 to 16.3% (root length) for AM. Samples from Gaoyao, Xinhui, Yangxi, and Yulin had 10–15% roots colonized by ECM fungi, whereas those from seven locations did not form ECM, or fewer than 1% roots were colonized. About 45% of root samples (14 locations) were colonized by AM fungi with colonization rates of 5% or more. Roots were usually colonized by one type of mycorrhizal association (either ECM or AM) or were not mycorrhizal, although dual ECM/AM associations were occasionally observed in a few samples.

There were seven morphotypes of ECM on roots of eucalypts (Table 5). ECMs formed by some fungal groups had distinct characteristics and were easily distinguished under a microscope. These included *Laccaria* (white, thin, and long tips), *Scleroderma* (distinctly white and glabrous), and *Cenococcum* (black with firm external hyphae). *Scleroderma* ECMs were the dominant type in some root samples, whereas others were present occasionally in one or more samples only. ECM status of some fungi was confirmed by tracking hyphae from sporocarps to roots. AM associations were largely formed by *Glomus* species, whereas other types were also observed. AM morphotypes at the genus level were determined by the characters of mycorrhizal structures (arbuscules, vesicles, and hyphae) and spores if present in roots as described in mycorrhizal manuals (such as Brundrett et al. 1996).

Bioassay assessment

Roots harvested from 12-week-old container-grown seedlings in a bioassay experiment were poorly colonized (5% or less roots colonized) in all soil treatments collected from 11 eucalypt locations in Guangdong Province. There were no significant differences in ECM (P=0.07) nor AM (P=0.14) colonization across soils tested. Distinct white ECMs of *Scleroderma* were observed in soils collected from Leizhou and Guangzhou. A few light brown ECM tips

Table 2 Abundance and species richness of putative ECM fungi under *Eucalyptus* plantations in south China

Location	Ectomycorrhizal fungi															
	A1[a]	A2	B1	L1	L2	P1	P2	R1	R2	S1	S2	S3	S4	S5	S6	SR[b]
Guangdong Province																
Gaoyao	0[c]	0	0	+	0	0	++	0	0	0	0	++	++	0	+	0.33
Guangzhou	+	0	0	+	0	0	0	0	0	0	0	+	+++	0	0	0.27
Kaiping	0	0	0	+	0	0	++	0	0	0	0	++	0	0	+	0.27
Leizhou	0	0	0	0	0	0	+++	0	0	0	0	+++	0	0	++	0.20
Qingyuan	0	+	0	0	+	0	+	+	0	0	0	0	+	0	0	0.33
Shaoguan	+	0	0	0	0	0	0	+	0	0	0	+	0	0	+	0.20
Xinhui	0	0	0	0	+	0	+	0	0	0	0	++	0	0	++	0.27
Xuwen	0	0	0	+	0	0	+	0	0	0	0	+++	0	0	+	0.27
Yangjiang	0	0	0	0	0	+	+	0	0	0	0	+	0	0	0	0.20
Yangxi	0	0	0	+	0	0	0	0	0	0	0	++	0	0	+	0.20
Zhanjiang	0	0	0	0	+	0	++	0	0	0	0	+	+	0	0	0.27
Guangxi Province																
Baise	0	0	0	0	0	0	+	0	+	0	0	0	0	0	+	0.20
Fusui	0	0	0	0	0	0	++	0	0	0	0	+	0	0	0	0.13
Liuzhou	0	0	0	+	+	0	0	+	0	0	0	+	0	0	+	0.33
Nanning	0	0	0	0	0	0	+	0	0	0	0	+	0	0	0	0.13
Pingxiang	0	0	+[d]	0	0	0	+	0	+	0	0	0	+	0	+	0.33
Qinzhou	0	0	0	0	0	0	+	0	0	0	0	+	0	0	0	0.13
Wuzhou	0	0	0	0	+	0	0	+[d]	0	0	0	0	0	0	0	0.13
Yulin	0	0	0	0	0	0	0	+	0	0	0	0	+	0	+	0.20
Hainan Province																
Anding	0	0	0	0	0	0	+	0	0	0	0	0	+	0	0	0.13
Chengmai	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0.07
Danzhou	0	0	0	+	0	0	0	0	0	+	0	0	0	+	0	0.13
Ledong	0	0	0	0	0	0	+	0	0	0	0	0	++	0	0	0.13
Lingao	0	0	0	0	0	0	++	0	0	0	0	+	0	0	0	0.13
Yunnan Province																
Anning	0	+	0	0	0	0	0	0	0	+	0	0	0	0	++	0.27
Baoshan	0	0	+[d]	0	0	+	+	0	0	0	0	+	+	0	+	0.40
Chuxiong	+	+	0	0	++	0	+++	+	0	0	0	++	0	+[d]	0	0.47
Dali	+	0	0	0	+	0	0	0	0	0	+[d]	0	++	0	0	0.27
Gejiu	0	0	0	0	0	+	0	0	0	0	0	+[d]	0	0	0	0.20
Jingdong	+	0	0	0	+	0	0	+	0	0	0	0	+	+	+	0.40
Simao	0	0	+	0	0	+	0	+	0	0	0	0	0	0	+	0.27

[a] Codes of fungi are given in Table 1.
[b] *SR* Species richness (the number of ECM fungal taxa present in each location over the total taxa)
[c] Abundance levels are given in "Observations of ECM fungal sporocarps." Five plantations were sampled for each location.
[d] Probably associated with remnant pine forests

were present in a Yangxi soil, which were similar to those collected from the field as described in Table 5.

Discussion

Both field observations and the bioassay experiment revealed that there was a low diversity of ECM fungi in eucalypt plantations in south China. Species richness and abundance varied between geographic locations. Factors that may have contributed to these differences include climatic and topological characteristics, soil properties, plantation age, and land history. According to Chilvers (2000), more than 140 named ECM fungi are known to be associated with eucalypts in the field around the world. Among them, 104 have been reported from Australia and 37 from outside Australia. By contrast with south China, a large diversity of ECM fungi has been reported for Australia where native eucalypt forests or plantations have been established (Bougher 1995; Lu et al. 1999; Chilvers 2000). In Brazil, Giachini et al. (2000) claimed a higher diversity of ECM fungi including some sequestrate species

Table 3 Diversity of AM fungal spores extracted from soil samples collected from *Eucalyptus* plantations in south China

AM fungus	No. of plantations	SF	RA
Acaulospora foveata Trappe and Janos	17	11.0	0.8
Acaulospora myriocarpa Spain, Sieverding and Schenck	8	5.2	2.2
Acaulospora scrobiculate Trappe	4	2.6	3.4
Gigaspora margarita Becker and Hall	16	10.3	1.2
Glomus aggregatum Schenck and Smith	9	5.8	2.5
Glomus claroideum Schenck and Smith	15	9.7	4.5
Glomus constrictum Trappe	23	14.8	0.8
Glomus coremioides (Berk. and Broome) Redecker and Morton	10	6.5	0.7
Glomus dolichosporum Zhang et. Wang	36	23.2	1.6
Glomus formosanum Wu and Chen	75	48.4	10.0
Glomus geosporum (Nicol. and Gerd.) Walker	47	30.3	15.4
Glomus intraradices Schenck and Smith	8	5.2	3.3
Glomus macrocarpum Tul. and Tul.	3	1.9	4.6
Glomus microaggregatum Koske, Gemma and Olexia	37	23.9	7.4
Glomus microcarpum Tul. and Tul.	12	7.7	5.5
Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe	104	67.1	22.5
Glomus rubiformis (Gerd. and Trappe) Almeida and Schenck	21	13.5	0.6
Glomus sinuose (Gerd. and Bakshi) Almeida and Schenck	7	4.5	2.4
Glomus taiwanense (Wu and Chen) Almeida and Schenck	16	10.3	3.3
Glomus versiforme (Karst.) Berch	58	37.4	6.5
Scutellospora castanea Walker	24	15.5	0.5

SF Species frequency, the number of plantations in which the species was observed/total plantations × 100; *RA* relative abundance, the number of spores of a species/total spores × 100

in plantations of eucalypts and pines than previously reported. More recently, 23 ECM fungi were recorded under *E. dunnii* plantations in southern Brazil (Giachini et al. 2004).

In Australia, a high diversity of ECM fungi often occurs in soils of similar low fertility. Sporocarps of *Laccaria*, *Descolea*, *Pisolithus*, and *Scleroderma* were dominant in young plantations of *E. globulus* in Western Australia, and these were considered to be early-stage fungi (Lu et al. 1999; Gardner and Malajczuk 1988). Early-stage and late-stage ECM fungi were also reported for New Zealand (Chu-Chou and Grace 1982) and Brazil (Bellei et al. 1992). It is possible, however, that the same fungus could act as an 'early-stage fungus' as well as a 'late-stage fungus' depending on host species and habitat. It has been suggested that older plantations (≥10 years) have a higher fungal diversity than young plantations (Lu et al. 1999). In China, eucalypt plantations are managed as short-term crops (3–7 years) largely for the pulp and paper industries. Further, some ECM fungi, such as members of the Ascomycota, Corticiaceae, and Tuberaceae, produce small or cryptic fruiting bodies or are hypogeous; thus, they may be overlooked during field surveys concentrating on above-ground sporocarps. Therefore, results from this study may not reflect the below-ground ECM fungal communities as some epigeous fungi may not have been fruiting, and many may fruit below ground. Nevertheless, there were a limited number of ECM morphotypes in the plantations that were sampled. Molecular tools, such as gene sequencing of rDNA ITS and LSU regions, were used for *Scleroderma* collections (unpublished data), whereas molecular work was not engaged for other taxa in this study. Such tools will be useful to explore the below ground diversity of mycorrhizal fungi under eucalypt plantations in China in the future.

Although some fungi present in the young eucalypt stands were able to complete their life cycles, as evidenced by sporocarps with spores, they were mostly weak colonizers of roots that were collected from near sporocarps such as *Pisolithus*. These plantations often had some isolated plants of native members of the Pinaceae or Fagaceae emanating from remnant patches of vegetation. As most eucalypt plantations in China were established on precleared farm land or coniferous plantations, or were surrounded by native forests, there is opportunity for indigenous ECM fungi to present in newly established plantations. These fungi could be partly dependent on non eucalypt trees or are using eucalypts in the absence of indigenous hosts, assuming the ECM fungi are native (Chen et al. 2006c). Spores of some ECM fungi could be blown from remnant nearby vegetation into plantations. Further, the presence of ECM fungi in plantations may not mean that they were beneficial to the plantations.

Bioassays have been previously used to evaluate the inoculum potential of infective propagules of mycorrhizal fungi in soil profiles in Western Australia (Brundrett and

Table 4 Mycorrhizal colonization of roots collected from *Eucalyptus* plantations in south China

Location	ECM (%)[a]	AM (%)[a]	ECM/AM[b]
Guangdong Province			
Gaoyao	12.7 d[c]	8.5 d	−
Guangzhou	2.9 b	12 e	−
Kaiping	4.6 bc	3.7 c	−
Leizhou	9.5 cd	5.1 cd	+
Qingyuan	0 a	7.4 d	−
Shaoguan	6.4 c	0 a	−
Xinhui	11.5 cd	2.6 ab	+
Xuwen	4.5 bc	2.2 ab	−
Yangjiang	0 a	4.3 c	−
Yangxi	15.2 d	7.4 d	+
Zhanjiang	6.6 c	0 a	−
Guangxi Province			
Baise	0 a	3.5 c	−
Fusui	2.5 b	8.2 d	−
Liuzhou	0 a	2.7 ab	−
Nanning	8.0 c	5.3 cd	−
Pingxiang	3.8 b	16.3 f	+/−
Qinzhou	7.5 c	10.2 e	+
Wuzhou	8.4 c	7.0 d	−
Yulin	10.3 cd	4.3 c	−
Hainan Province			
Anding	4.2 bc	0 a	−
Chengmai	2.6 b	2.5 ab	−
Danzhou	0.6 a	0 a	−
Ledong	0 a	1.5 a	−
Lingao	3.1 b	2.9 ab	−
Yunnan Province			
Anning	3.7 b	8.4 d	−
Baoshan	4.8 bc	12.9 e	+
Chuxiong	5.6 c	6.8 d	+/−
Dali	4.5 bc	0 a	−
Gejiu	8.9 cd	0 a	−
Jingdong	0 a	3.6 c	−
Simao	2.5 b	7.4 d	−

[a] ECM, percentage of short roots colonized; AM, percentage of root length colonized

[b] Dual ECM/AM on the same root system present (+), rarely present (+/−), or absent (−)

[c] Data are means of five plantation sites; means with the same letters in each column are not significantly different by Duncan's multiple range test ($P \leq 0.05$)

Table 5 Seven typical ECM morphotypes present on roots of *Eucalyptus* collected from 155 plantations in south China

Type	Genus	Brief description
A	*Cenococcum*	Black; usually monopodial; tips short, straight, or bulbous; 0.5–2 mm long, 0.4–1 mm diameter; with firm black external hyphae arising from the outer layer of the mantle; mantle thick; Hartig net present. Very rare type in root samples.
B	*Laccaria*	Creamy white to light vinaceous grey when older; monopodial to pyramidal; long, straight, and spindly; 1–6 mm long, 0.5–2 mm diameter; smooth; mantle thin except near the rootcap; white velvety hyphae around or near the roots; Hartig net present. Rare in root samples.
C	*Pisolithus*-like	Sulphur yellow to yellowish brown; dichotomous, bifurcate to irregular; straight; 1–5 mm long, 0.7–1.2 mm diameter; velvety with some external hyphae; mantle thin to nearly lacking showing the epidermal cells underneath, especially near rootcaps; Hartig net usually absent. Common in some root samples.
D	*Scleroderma*	Distinctly white and glabrous; monopodal to monopodal pyramidal, rarely dichotomous; tips usually straight or tortuous; 0.5–6 mm long, 0.2–0.4 mm diameter; woolly, with white mantle mycelia and extramatrical mycelia; white rhizomorphs commonly present; mantle thick covering root tips; abundant clamp connections in mantle; Hartig net present. Dominant ECM type in some root samples collected.
E	Unknown 1	Brown to vinaceous brown; monopodial; tips long, straight; 2–5 mm long, 1–2 mm diameter; smooth; external hyphae rare; mantle thick; Hartig net seldom present. Very rare in root samples.
F	Unknown 2	Green-grey to dark brown or black; dichotomous; 0.3–2 mm long, 0.2–1 mm diameter; felty; mantle thick; firm white hyphae sparsely present outside the root; Hartig net absent. Very rare in root samples.
G	Unknown 3	Light brown to cinnamon; monopodial; straight; 0.5–4 mm long, 0.2–0.8 mm diameter; cottony; mantle thin and host epidermal cells visible; ECMs not well developed; Hartig net absent. Common in some root samples.

Abbott 1995; Chen et al. 1999), Canada (Plenchette et al. 1989; Massicotte et al. 1999), and Senegal (Duponnois et al. 2005). Results from the bioassay experiment, using soils collected from under eucalypt plantations, confirmed the low diversity of mycorrhizal fungi and poor inoculum levels in soils for eucalypt plantations in south China. In addition to this, we have tested the inoculum levels in four typical soils on which eucalypt plantations are extensively established in south China and effects of soil on inoculation with *Scleroderma* spores in a nursery (Chen et al. 2006a). It is presumed that increasing the fungal diversity could benefit the health of eucalypts through enhanced growth of trees, increased tolerance of trees to adverse soil or environmental conditions, and improved nutrient cycling, which may increase the sustainability of plantations. The apparent low diversity of ECM fungi and their weak colonization suggest that the introduction of more compatible

eucalypt symbionts could improve the establishment of eucalypt plantations and their productivity in south China.

Examination of the diversity and abundance of AM fungi showed that there were relatively more species in soils than ECM fungi from eucalypt plantations in south China. However, the intensity of colonization on roots was generally low. It is presumed that some AM fungi might associate with remnant vegetation, such as grasses and shrubs as most eucalypt plantations were young and land history and vegetations may vary between locations and plantation sites. This study confirmed early findings of occasional multiple colonization by ECM and AM fungi on eucalypt roots (Lapeyrie and Chilvers 1985; Brundrett et al. 1996; Oliveira et al. 1997; Chen et al. 2000a; Lodge 2000). However, the ECM associations have been considered to be more important than AM fungi in studies of eucalypt root structure, physiology, and for healthy eucalypt plantations (Hilbert and Martin 1988; Chen et al. 2000a).

Knowledge on the functional diversity of mycorrhizal fungi provides opportunities to select fungi adapted to specific combinations of host/soil conditions to assist establishment of exotic plantations and to optimize tree performance in the field. This is the first intensive study on the mycorrhizal status of eucalypt plantations in south China, although some early observations on the presence of sporocarps in the region were noted (Gong et al. 1997; Chen et al. 1998; Dell et al. 2002; Brundrett at al. 2005). This work suggests that very few, if any, indigenous symbionts are compatible with exotic eucalypt trees in the region. It was concluded that inoculation with compatible fungi in eucalypt nurseries may be advantageous in the production of high-quality stock for outplanting and for performance of trees in the field. However, knowledge of which species to use and how inoculum should be managed to meet local nursery requirements remains poor. This study showed that several *Scleroderma* species were dominant under eucalypt plantations in south China. These fungi usually produce large sporocarps, which are good sources for spore inoculum. So far, effort has been placed on identifying eucalypt-compatible *Scleroderma* species and optimizing conditions for the formation of *Scleroderma* mycorrhizas in nursery containers (Chen et al. 2006a–c). Previous field studies showed that inoculation with *Scleroderma* spores from Australia increased the production of eucalypts in plantations by up to 26% in south China (Chen et al. 2000b; Xu et al. 2001). Therefore, it is recommended that *Scleroderma* be harvested from outside China to inoculate eucalypts until the time basidiomes are sufficiently abundant in Chinese eucalypt plantations to enable the local collection of spores. Existing spores of *Scleroderma* in south China can be used if the introduction of suitable resource from the outside is not feasible because of certain circumstances, such as the unavailability of large amount of spores and quarantine restrictions on importation.

Pisolithus sp2 sporocarps were also abundant in some eucalypt stands in south China, such as the Leizhou Peninsula. As discussed early, however, this fungus is believed to be unsuitable for inoculating exotic eucalypt plantations because of its poor ability to fully develop mycorrhizas on eucalypt roots, as reported previously (Malajczuk et al. 1990; Lei et al. 1990; Dell et al. 2002). Spores of *Laccaria* spp. have been shown to be an effective inoculum for containerized eucalypt seedlings (Lu et al. 1998; Chen et al. 2000a; Brundrett et al. 2005). The use of these fungi in eucalypt nurseries needs to be explored. Other suitable ECM fungi should be sourced to provide a larger diversity of inoculum before wider applications are performed.

Exotic ECM fungi that can aggressively invade native forests and compete with indigenous organisms reducing their diversity (Fuhrer 2005) should be excluded from inoculation programs. Host-specific species or collections are considered to be important for introduction. Together with our recent work (Chen et al. 2006a–c), findings from these studies should assist the commercialization of *Scleroderma* spores for eucalypt plantations in south China. Hopefully, by using spores that are relatively easy to collect, store, and apply, the percentage of mycorrhizal plants being produced for reforestation will increase quickly. Some follow-up studies should be undertaken to evaluate the following: (1) changing in ECM fungal diversity and abundance with plantation rotation; (2) the persistence of inoculum in nurseries and in the field; and (3) the effect of colonization by ECM fungi and plantation yield.

Acknowledgment This study was supported by the Chinese Academy of Forestry, Murdoch University and the International Foundation for Science. Li Hua Kang, Zheng Mei Zhen, Ye Gen Jiang, and Hong Li of CAF assisted in the field investigations and sampling.

References

Bellei MM, Garbaye J, Gil M (1992) Mycorrhizal succession in young *Eucalyptus viminalis* plantations in Santa Catarina (south Brazil). For Ecol Manag 54:205–213

Biermann B, Linderman RG (1981) Quantifying vesicular-arbuscular mycorrhizae: a proposed method towards standardization. New Phytol 87:63–67

Bougher NL (1995) Diversity of ectomycorrhizal fungi associated with eucalypts in Australia. In: Brundrett M, Dell B, Malajczuk N, Gong MQ (eds) Mycorrhizas for plantation forestry in Asia—ACIAR proceedings no. 62. ACIAR, Canberra, pp. 28–15

Brundrett MC, Abbott LK (1995) Mycorrhizal fungus propagules in the jarrah forest. II. Spatial variability in inoculum levels. New Phytol 131:461–469

Brundrett MC, Bougher N, Dell B, Grove G, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. ACIAR, Canberra

Brundrett MC, Malajczuk N, Gong M, Xu D, Snelling S, Dell B (2005) Nursery inoculation of *Eucalyptus* seedlings in Western Australia and southern China using spores and mycelial inoculum of diverse ectomycorrhizal fungi from different climatic regions. For Ecol Manag 209:193–205

Chen YL, Gong MQ, Wang FZ, Chen Y, Brundrett M, Dell B (1998) Diversity of VA mycorrhizal and ectomycorrhizal fungi in *Eucalyptus* plantations in southern China. In: Program and abstracts of 2nd international conference on mycorrhiza. Uppsala, Sweden, July 5–10 1998

Chen YL, Mark BC, Dell B, Gong MQ, Malajczuk N (1999) Bioassay measurements of mycorrhizal inoculum in soils from eucalypt plantations of varying ages in Western Australia. For Stud in Chin 1:26–32

Chen YL, Brundrett MC, Dell B (2000a) Effects of ectomycorrhizas and vesicular-arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. New Phytol 146:545–556

Chen YL, Gong MQ, Xu DP, Zhong CL, Wang FZ, Chen Y (2000b) Screening and inoculant efficacy of Australian ectomycorrhizal fungi on *Eucalyptus urophylla* in field. For Res 13:569–576

Chen YL, Kang LH, Dell B (2006a) Inoculation of *Eucalyptus urophylla* with spores of *Scleroderma* in a nursery in south China: comparison of field soil and potting mix. For Ecol Manag 222:439–449

Chen YL, Dell B, Malajczuk N (2006b) Effect of *Scleroderma* spore density and age on mycorrhiza formation and growth of containerized *Eucalyptus globulus* and *E. urophylla* seedlings. New For 31:453–467

Chen YL, Kang LH, Malajczuk N, Dell B (2006c) Selecting ectomycorrhizal fungi for inoculating plantations in south China: effect of *Scleroderma* on colonization and growth of exotic *Eucalyptus globulus*, *E. urophylla*, *Pinus elliottii* and *P. radiata*. Mycorrhiza 16:251–259

Chilvers GA (2000) Mycorrhizas of eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN (eds) Diseases and pathogens of eucalypts. CSIRO, Melbourne

Chu-Chou M, Grace LJ (1982) Mycorrhizal fungi of *Eucalyptus* in the north island of New Zealand. Soil Biol Biochem 14:133–137

Dalpe Y (1993) Vesicular-arbuscular mycorrhiza. In: Carter MR (ed) Soil sampling and methods of analysis. Lewis, Boca Raton, FL

Dell B, Malajczuk N, Dunstan WA (2002) Persistence of some Australian *Pisolithus* species introduced into eucalypt plantations in China. For Ecol Manag 169:271–281

Duponnois R, Founoune H, Masse D, Pontainer R (2005) Inoculation of *Acacia holosericea* with ectomycorrhizal fungi on a semiarid site in Senegal: growth response and influences on the mycorrhizal soil infectivity after 2 years plantation. For Ecol Manag 207:351–362

Fuhrer B (2005) A field guide to Australian fungi. Bloomings Books, Melbourne, Australia

Gardner JH, Malajczuk N (1988) Recolonisation of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. For Ecol Manag 24:27–42

Giachini AJ, Oliveira VL, Castellano MA, Trappe JM (2000) Ectomycorrhizal fungi in *Eucalyptus* and *Pinus* plantations in southern Brazil. Mycologia 92:1166–1177

Giachini AJ, Souza LAB, Oliveira VL (2004) Species richness and seasonal abundance of ectomycorrhizal fungi in plantations of *Eucalyptus dunnii* and *Pinus taeda* in southern Brazil. Mycorrhiza 14:375–381

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research (2nd edn). Wiley, New York

Gong MQ, Chen YL, Zhong CL (1997) Mycorrhizal research and application. China Forestry Publishing House, Beijing

Hilbert JL, Martin F (1988) Regulation of gene expression in ectomycorrhizas. I. Protein changes and the presence of ectomycorrhiza-specific polypeptides in the *Pisolithus-Eucalyptus* symbiosis. New Phytol 110:39–346

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of Ectomycorrhizas. HMSO, London

Lapeyrie FF, Chilvers GA (1985) An endomycorrhizal-ectomycorrhiza succession associated with enhanced growth by *Eucalyptus dumosa* seedlings planted in a calcareous soil. New Phytol 100:93–104

Lei J, Lapeyrie F, Malajczuk N, Dexheimer J (1990) Infectivity of pine and eucalypt isolates of *Pisolithus tinctorius* on roots of *Eucalyptus urophylla* in vitro. II. Ultrastructural and biochemical changes at the early stage of mycorrhiza formation. New Phytol 116:115–122

Lodge DJ (2000) Ecto- or arbuscular mycorrhizas, which are best? New Phytol 146:353–354

Lu XH, Malajczuk N, Dell B (1998) Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi. Mycorrhiza 8:81–86

Lu XH, Malajczuk N, Brundrett M, Dell B (1999) Fruiting of putative ectomycorrhizal fungi under blue gum (*Eucalyptus globulus*) plantations of different ages in Western Australia. Mycorrhiza 8:255–261

Malajczuk N, Lapeyrie F, Garbaye J (1990) Infectivity of pine and eucalypt isolates of *Pisolithus tinctorius* on roots of *Eucalyptus urophylla* in vitro. I. Mycorrhiza formation in model systems. New Phytol 114:627–631

Malajczuk N, Grove TS, Bougher NL, Dell B, Gong MQ (1994) Ectomycorrhizas and nutrients—their importance to eucalypts in China. In: Brown AG (ed) Australian tree species research in China. ACIAR Proceedings 48, Canberra

Mao XL (2000) The Macrofungi of China. Henan Science and Technology, Zhengzhou

Massicotte HB, Molina R, Tackaberry LE, Smith JE, Amaranthus MP (1999) Diversity and host specificity of ectomycorrhizal fungi retrieved from three adjacent forest sites by five host species. Can J Bot 77:1053–1076

Morton JB, Redecker D (2001) Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. Mycologia 93:181–195

Oliveira VL, Schmidt VDB, Bellei MM (1997) Patterns of arbuscular- and ecto-mycorrhizal colonisation of *Eucalyptus dunnii* in southern Brazil. Ann Sci For 54:473–481

Plenchette C, Perrin R, Duvert P (1989) The concept of soil infectivity and a method for its determination as applied to endomycorrhizas. Can J Bot 67:112–115

Schenck NC, Perez Y (1988) Manual for identification of VA mycorrhizal fungi (2nd edn). INVAM, Gainesville, FL

Xu DP, Dell B, Malajczuk N, Gong MQ (2001) Effects of P fertilisation and ectomycorrhizal fungal inoculation on early growth of eucalypt plantations in Southern China. Plant Soil 233:47–57