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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*

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Abstract Plantation forestry with exotic trees in south China needs compatible symbionts to improve the growth of seedlings in nurseries and to enhance establishment and growth in the field. *Scleroderma*, a potentially suitable symbiont for inoculation, is not being used in containerized nurseries in the region due to poor knowledge of its host range. The ability of 15 collections of *Scleroderma*, nine from Australia and six from Asia, to colonize and promote growth of four important exotic plantation trees (*Eucalyptus globulus* Labill., *Eucalyptus urophylla* ST Blake, *Pinus elliottii* Engl., and *Pinus radiata* D. Don) was examined in a nursery potting mix. There was generally low host specificity of *Scleroderma* between tree genera. At 12 weeks after inoculation, 13 to 14 of the 15 spore collections formed ectomycorrhizas on seedlings of eucalypts or pines. The extent of colonization differed between spore treatments with two or four collections forming abundant mycorrhizas (>50% fine roots colonized) on *E. globulus* or *E. urophylla*, respectively, and three or five on *P. radiata* or *P. elliottii*, respectively. Three collections from Australia strongly colonized all hosts resulting in 26 to 100% of short roots being colonized. Chinese *Scleroderma* collections resulted in fewer mycorrhizas on eucalypts than on pines. Inoculation stimulated the growth (shoot height and dry weight) of eucalypt and pine seedlings by up to 105% where *Scleroderma* mycorrhizas developed. The results suggest that there is a need to source *Scleroderma* from outside China for inoculating eucalypts in Chinese nurseries whereas Chinese collections of *Scleroderma* could be used in pine nurseries. Further screening of Australian and Chinese *Scleroderma* should be performed in Chinese nurseries and in the field before final commercial decisions are made.

Keywords Ectomycorrhiza (ECM) · *Scleroderma* · Host range · *Eucalyptus* · *Pinus* · China

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Introduction

Exotic eucalypts and pines are important plantation species in south China, where the climate is conducive to high growth rates. Due to a large domestic demand for fuelwood, paper pulp, poles, and sawn timber in China, the plantation area of these fast-growing species is continuing to expand. *Eucalyptus* species were first introduced to south China for large-scale plantations in the 1950s (Qi 2002). Widely planted species in the region include *Eucalyptus citriodora*, *Eucalyptus camaldulensis*, *Eucalyptus globulus*, *Eucaplytus grandis*, *Eucaplytus maidenii*, *Eucaplytus robusta*, *Eucaplytus tereticornis* and *Eucaplytus urophylla*. Among them, *E. globulus*, *E. urophylla*, and a hybrid known as *Eucalyptus* leizhou No. 1 are now the main plantation eucalypts in China, and the plantation area is over 1.5 million ha. In addition to eucalypts, some commercial pine species have been introduced to south China, such as *Pinus elliottii*, *Pinus caribaea*, and *Pinus taeda*. It is estimated that there are over 150,000 ha of exotic pine plantations in south China (Jiang and Lu 2000). However, productivity of exotic eucalypts and pines in south China is relatively low compared to plantation productivity outside China and is variable depending on site and silvicultural inputs (Jiang and Lu 2000; Xu et al. 2000). Impediments to productivity include abiotic (e.g., infertile soil and alkaline soil) and biotic (e.g., pathogenic bacteria and fungi) factors (Xu et al. 2000; Hardy et al. 2003).

Inoculating *Eucalyptus* and *Pinus* with compatible ectomycorrhizal (ECM) fungi has been shown to be beneficial in many parts of the world (Marx et al. 1985; Thomson et al. 1996; Duñabeitia et al. 2004). It has been suggested that ECM fungi can help improve productivity of eucalypt plantations in China (Chen et al. 2000c; Dell et al. 2002). An early introduction of three exotic pine species in Guangdong Province failed in 1974, and subsequent success in re-establishing plantations with mycorrhizal seedlings suggests that the failure was due to lack of compatible ECM fungi in the soil (Ge and Bi 1989). A number of ECM fungi, collected from under *Eucalyptus* in Australia, have been introduced in research trials into eucalypt plantations in south China to screen compatible fungi with exotic eucalypt species (Dell and Malajczuk 1997; Chen et al. 2000c; Brundrett et al. 2005). Two genera favored for introduction are *Pisolithus* and *Scleroderma* because they readily colonize eucalypt roots in disturbed habitats (Lu et al. 1998). At present, spores of *Pisolithus* collected from the field in south China are being used to inoculate clonal eucalypts in a commercial nursery. It is also easy to collect spores of *Scleroderma* from species that form large epigeous basidiocarps and then to produce spore inoculum for nursery inoculation programs. However, *Scleroderma* spores are not being used commercially in China because of a lack of knowledge regarding its host range and compatibility with the main plantation species. This fungal genus has potential for application in commercial plantation forests in the region, as some beneficial isolates can aggressively compete with other ECM fungi in the field (Garbaye et al. 1988; Hall et al. 1995; Thomson et al. 1996; Cairney and Chambers 1999; Dell et al. 2002; Martin et al. 2003). In plantations with exotic eucalypts and pines, these fungi are desirable as inoculum if they are compatible with the host tree and are effective in promoting survival and growth in the field.

Previously, we determined optimum spore densities and spore storage conditions for mycorrhization of eucalypts with a few collections of *Scleroderma* (Chen et al. 2006). In this study, we investigate the capacity of *Scleroderma* to form associations with seedlings of *Eucalyptus* and *Pinus* under glasshouse conditions. This work addresses whether there is a need to source *Scleroderma* fungi from outside China to inoculate these exotic plantation trees and to match inocula with host genera.

Materials and methods

Experimental design

A complete randomized design consisting of 64 host–fungus combinations (four hosts, 15 fungi, and one non-fungus control) was used to compare fungal ability to colonize roots and promote plant growth under glasshouse conditions. There were eight replicate plants for each treatment.

Fungal inoculum

Details of the 15 collections of *Scleroderma* from eight species used in this trial are given in Table 1. Fungi were identified to putative taxa using morphological features, and voucher specimens are housed at the Research Institute of Tropical Forestry and Murdoch University. Fungal sporocarps were mostly collected from *E.globulus* plantations in temperate Western Australia, while some that were collected from south China, Indonesia, and Thailand were also used to compare their compatibility. *Scleroderma* species associated with eucalypts in subtropical or tropical parts of Australia were not included, as there was no difference in the relative success of temperate and tropical

Table 1 Details of fungi used in the host range trial

Fungus	Code	Collection	Place of origin[a]	Country of origin	Tree of origin
S. albidum	SAL-1	CY-2019	Albany, WA	Australia	*Eucalyptus globulus* plantation
S. albidum	SAL-2	CY-2080	Albany, WA	Australia	*Eucalyptus globulus* plantation
S. areolatum	SAR-1	CY-2021	Albany, WA	Australia	*Eucalyptus globulus* plantation
S. areolatum	SAR-2	CY-2033	Deep Creek, WA	Australia	*Eucalyptus globulus* plantation
S. areolatum	SAR-3	CY-2064	Albany, WA	Australia	*Eucalyptus globulus* plantation
S. cepa	SCE-1	CY-2013	Perth, WA	Australia	*Eucalyptus camaldulensis* plantation
S. cepa	SCE-2	CY-2004	Albany, WA	Australia	*Eucalyptus globulus* plantation
S. cepa	SCE-3	CY-1029	Conghua, GD	China	*Pinus massoniana* secondary forest
S. citrinum	SCI-1	CY-1035	Guangzhou, GD	China	*Eucalyptus* and *Acacia* nursery bed
S. citrinum	SCI-2	CY-2024	Lockhart, WA	Australia	*Eucalyptus globulus* plantation
S. flavidum	SFL-1	CY-1032	Conghua, GD	China	*Pinus massoniana* forest
S. flavidum	SFL-2	CY-3002	Lodge Care, MHS	Thailand	*Pinus*, *Shorea*, *Castanopsis* forest
S. paradoxum	SPA-1	CY-1037	Lingao, HN	China	*Acacia crassicarpa* plantation
S. sp.7	SSP-7	CY-3001	Habisaran, NS	Indonesia	*Pinus tecumansi* plantation
S. verrucosum	SVE-1	CY-2023	Albany, WA	Australia	*Eucalyptus globulus* plantation

Nine collections were from Australia, four from China, and one each from Indonesia and Thailand
[a]*GD* Guangdong Province, *HN* Hainan Province, *MHS* Mae Hong Song, *NS* North Sumatra, *WA* Western Australia

fungal isolates in nursery trials in China in an earlier study (Brundrett et al. 2005). The spore mass was dried at 30°C for 48 h and crushed by hand and sieved (400 μm) to produce spore powders. Spores were stored dry at 4°C for 3–18 months before use. The fungal spore mass was blended in distilled deionized (DI) water (1:10, vol.) for 5 s on low speed in a food blender. A drop of Tween 80 was added to assist suspension. The initial spore concentration was measured with a hematocytometer, and the bulk spore suspension was serially diluted to obtain a concentration of 10^5 spores/ml.

Host species and seed germination

Four exotic plantation tree species from two genera were used: *E. globulus* Labill., *E.urophylla* ST Blake (Myrtaceae); *P. elliottii* Engl. and *P. radiata* D. Don (Pinaceae). Seeds of eucalypts (*E. globulus* seedlot no. 18398, from Triabunna, Tasmania; *E. urophylla* seedlot no. 19393, from Pantar Island, Indonesia) were surface-sterilized by shaking in a 1.2% solution of NaOCl for 5 min (*E. urophylla*) or 10 min (*E. globulus*). After four rinses in sterile DI water, 20 seeds were transferred to each 90-mM Petri dish with 20 ml of germination medium (0.7% water agar amended with 500 μM $CaSO_4$ and 3 μM H_3BO_3). Plates were incubated in darkness at 25°C for 8 days and in the light at 25°C for 2 days before planting (Chen et al. 2000a).

Seeds of *P. elliottii* (seedlot no. 11578, from CSO7E, Woodford) and *P. radiata* (seedlot no. K109S; from Grimwade, WA) were soaked in 1% H_2O_2 solution at 4°C for 5 days. Seeds were rinsed in sterile DI water and then surface-sterilized in 30% H_2O_2 for 25 min. After washing four times with sterile DI water, seeds were placed in 90-mM Petri dishes with germination medium (0.7% water agar amended with 500 μM $CaSO_4$ and 3 μM H_3BO_3). Plates were incubated in darkness at 25°C for 6 days and in the light at 25°C for 3 days before transplanting.

Seedling growth medium

A nursery potting mix containing expanded coarse perlite, peat moss (natural sphagnum), and river sand (4:2:1 v/v) was used. The mix was moistened and steam-pasteurized twice at 60°C for 2 h. Plastic pots (70×70 mm top diameter, 200 mm height) were surface-sterilized with $KClO_3$ (2–5%) and allocated 500 g each of air-dried soil mix.

Planting, inoculating, and maintenance

Two uniform healthy seedlings were transplanted into each free-draining pot, and seedlings were thinned to one after 2 weeks. Seedlings in each treatment were given 10 ml spore suspension, at a rate of 10^6 spores/seedling, 1 week after transplanting. The spore slurry was added in a 2-cm deep hole near the plant using a 5-ml pipette. All pots were put on to holding trays and randomly placed on benches in a glasshouse. The glasshouse was evaporatively cooled, and the maximum temperature ranged from 30–42°C and the minimum temperature ranged from 13–16°C during the period of the experiments. The position of each tray was randomly changed biweekly. The potting mix was watered to field capacity (ca. 10% w/w) before planting. Soil moisture levels were then maintained by an automatic overhead watering system (twice a day, 5 min each). A complete liquid fertilizer was applied biweekly to the surface of the containers based on previous experiments for eucalypt mycorrhization (Dell and Malajczuk 1995). The following were supplied at each application: macronutrients (milligrams/plant): N 1.5, P 0.7, K 0.9, Ca 0.5, S 0.5, and Mg 0.2; micronutrients (micrograms/plant): B 1.5, Cu 1.2, Fe 2.9, Mn 2.4, and Zn 0.9.

Data collection

Seedlings were harvested 12 weeks after planting. The shoot of each seedling was removed and its height measured. Mycorrhizal morphological characters of each fungus–host pair were visually assessed. Putative ECM root tips were further examined by transverse section or longitudinal section under light or scanning electronic microscopy and confirmed if both mantle and Hartig net were present. Root and shoot dry weights (60°C, 48 h) were determined. For each fungal treatment, individual root tips were selected to make fresh microscopic preparations. A randomly selected 2 g subsample of roots was preserved in 50% ethanol for mycorrhizal assessment. The samples were cleared in 10% KOH for 30 min at 90°C and stained with Trypan blue. Percentage of ECM colonization was under a dissecting microscope using the gridline intersect method (Brundrett et al. 1996). Colonization data were expressed as relative mycorrhizal abundance in three categories: +, 1–25% root-colonized; ++, 26–50% colonized; and +++, 50–100% colonized.

Statistical treatment of the data

Two separate analyses were performed. Two-way ANOVA was used to test the effect of both tree and fungus on the mean mycorrhizal colonization, shoot height, root dry weight, and shoot dry weight and total dry weight for each host. These variables measured on each seedling were dependent, so the initial significance test used the multivariate Wilk's Lamba statistic, followed by univariate *F* test where the initial multivariate analysis was significant. One-way ANOVA was used for possible differences in the above variables. For percentage mycorrhizal colonization,

data were transformed by Arc sin $\sqrt{\times}$ before performing statistic analysis. The significance of mean values was determined using Duncan's Multiple Range Test ($P<0.05$) (Gomez and Gomez 1984).

Results

Colonization and specificity

After 12 weeks, *Scleroderma* ECMs were present on all four hosts; however, the number of effective spore collections and percentage of mycorrhizal seedlings differed between tree genera and between tree species. There were significant differences in mycorrhizal colonization within tree species and between fungus collections ($P<0.001$). Interactions between tree and fungus were also observed. The majority of spore collections formed characteristic ECMs on pines and eucalypts (Table 2). Fourteen of the 15 fungus collections formed ECMs on pine seedlings and 13 on eucalypt seedlings. Over 93% of the inoculated seedlings of pines and 86% of eucalypts were ECMs. ECMs were extensive on some treatments of pine or eucalypt seedlings. Three spore collections colonized 50–100% of fine roots on *P. elliottii* seedlings, and two inoculation treatments of *P. radiata* reached similar levels of colonization. As in eucalypt seedlings, three spore collections resulted in abundant ECMs on *E. urophylla*, while two collections produced 50–100% ECMs on *E. globulus*.

Collections of *Scleroderma* from under eucalypt plantations in Western Australia were capable of colonizing eucalypts and pines tested in this trial. Among them, three collections (SAR-1, SCE-2, and SVE-1) resulted in well-developed ECMs on seedlings across the four hosts. However, there were differences within taxa from Australia. For example, ECMs formed extensively on both eucalypts and pines (50–100% of short roots) with SAR-1 (*Scleroderma areolatum*), while SAR-2 from the same taxon failed to colonize seedlings of *P. elliottii* and colonized less than 25% of the short roots of the other three hosts. Two collections of *Scleroderma cepa* (SCE-1 and SCE-2) formed ECMs on all hosts, while SCE-3 only colonized roots of pines. All four Chinese collections were compatible with *P. elliottii*; however, only one produced abundant mycorrhizas on the seedlings of other hosts. The two Chinese *Scleroderma* fungi collected from under secondary pine forests colonized larger component of short roots with pines than with eucalypts. The *Scleroderma* collected from the nursery bed formed ECMs with both eucalypts and pines, while a collection from under acacias in the field (SPA-1) only colonized roots of *P. elliottii*. Collection SAL-1 was an effective colonizer (26–50% of short roots colonizeed) for eucalypts only and SCE-1 and SCI-2 for pines. Seedlings of both eucalypts inoculated with SCE-3 and SPA-1 did not form ECMs. SAR-2 and SPA-1 did not form mycorrhizal associations on *P. elliottii* and *P. radiata*, respectively. These results suggest that there were some differences between collections in the ability to form ECMs on each host.

Scleroderma ECMs of pines and eucalypts had shiny, white extramatrical mycelia and distinctive white rhizomorphs. Three typical morphological features of *Scleroderma* ECMs are the dichotomous, multiple dichotomous,

Table 2 Relative mycorrhizal abundance on four plantation tree species inoculated with eight *Scleroderma* species from 15 collections

Fungus	Code	*E. globulus*	*E. urophylla*	*P. elliottii*	*P. radiata*
S. albidum	SAL-1	+ +[a]	+ +	+	+
S. albidum	SAL-2	+	+ +	+ +	+
S. areolatum	SAR-1	+ + +	+ + +	+ + +	+ + +
S. areolatum	SAR-2	+ +	+	–	+
S. areolatum	SAR-3	+ + +	+	+ +	+
S. cepa	SCE-1	+	+	+	+ +
S. cepa	SCE-2	+ +	+ +	+ +	+ + +
S. cepa	SCE-3	–	–	+	+ +
S. citrinum	SCI-1	+ +	+ + +	+ +	+
S. citrinum	SCI-2	+	+ +	+ +	+ +
S. flavidum	SFL-1	+	++	+ + +	+ +
S. flavidum	SFL-2	+	+	+ +	+
S. paradoxum	SPA-1	0	–	+ +	–
S. sp.7	SSP-7	+	++	+ + +	+ +
S. verrucosum	SVE-1	+ +	+ + +	+ +	+ +
Control	CONT	–	–	–	+[b]

Data are the averages of four replicate seedlings

[a] – = no ECM, 0 = roots colonized by hyphae but Hartig net absent or weakly developed, + to +++ = ECM with mantle and Hartig net: + = 1–25% root colonized, ++ = 26–50% colonized, +++ = 50–100% colonized

[b] ECMs formed by contaminating species

or pyramidal branching patterns. Examination of transverse sections of putative ECM roots confirmed that the Hartig net hyphae were present around both epidermal and cortical cells of pine roots but they were present around epidermal cells only of eucalypt roots.

Growth of the hosts

There were significant differences in the height, shoot dry weight, root dry weight, and the total dry weight between hosts, fungus treatment, and their interactions, respectively, when a two-way ANOVA was performed ($P<0.001$). For each host, effects of fungus treatments were significant ($P<0.001$) on the above variables, except for root dry weight of *E. globulus* (one-way ANOVAs). Inoculated seedlings of pines or eucalypts were generally larger than uninoculated controls of each tree (Figs. 1 and 2). Inoculation significantly stimulated the height and total biomass of *E. globulus* by up to 45 and 60%, and by 70 and 105% for *E. urophylla*. SAL-1, SCE-2, SCI-2, SFL-2, SPA-1, SSP-7, and SVE-1 extremely promoted the growth of *E. urophylla*, and SAR-3, SFL-2, SPA-1 and SSP-7 were the better growth promoters for *E. globulus* (Fig. 1). In terms of the effects on the growth of *P. elliottii*, seedlings of four fungus treatments (SAL-1, SAL-2, SCI-2, and SFL-1) were significantly taller than the other treatments, and nine of 15 fungus collections increased seedling total dry weight (Fig. 2). All fungus collections, except SCI-1, greatly enhanced the height and total biomass of *P. radiata*.

Discussion

Differences between the collections of *Scleroderma*, in their ability to form ECMs and promote growth, were apparent on seedlings of the four plantation hosts under glasshouse conditions. Fully developed ECM symbioses were present on the roots of 14 (pines) or 13 (eucalypts) of the 15 fungal inoculant treatments, although the proportion of mycorrhizal roots varied between tree species and collections. The results suggest that there is low host specificity of *Scleroderma* between host genera. However, the collections of *Scleroderma* from under pines were less aggressive on eucalypts than were some of the *Scleroderma* collections from under eucalypts in plantations in

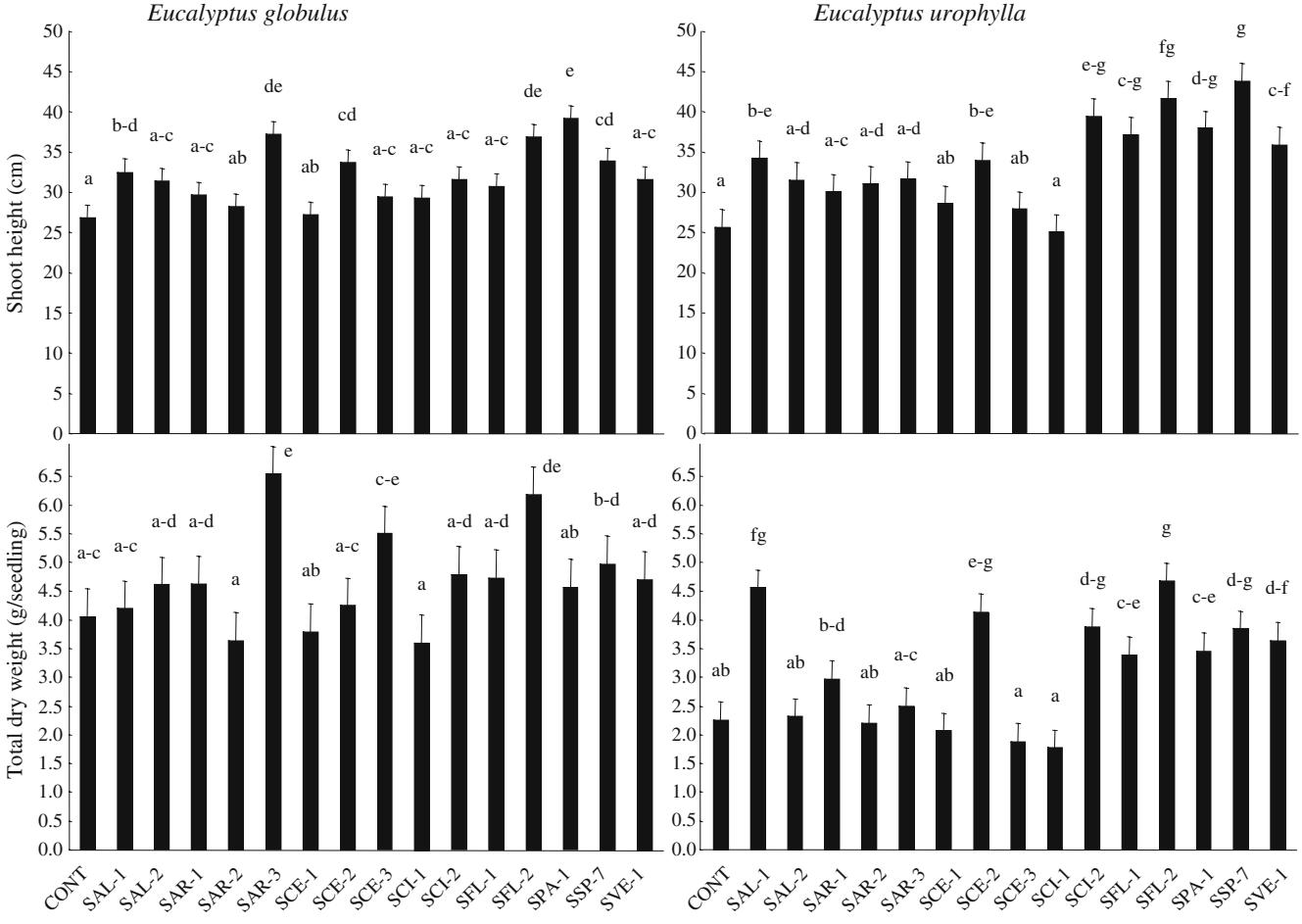

Fig. 1 Effects of *Scleroderma* inoculation on shoot height and total dry weight of *Eucalyptus globulus* (*left*) and *E. urophylla* (*right*). Standard errors are given. For each host, bars with same letters in each parameter are not significantly different by Duncan's Multiple Comparison ($P<0.05$). *CONT* uninoculated control; other codes are given in Table 1

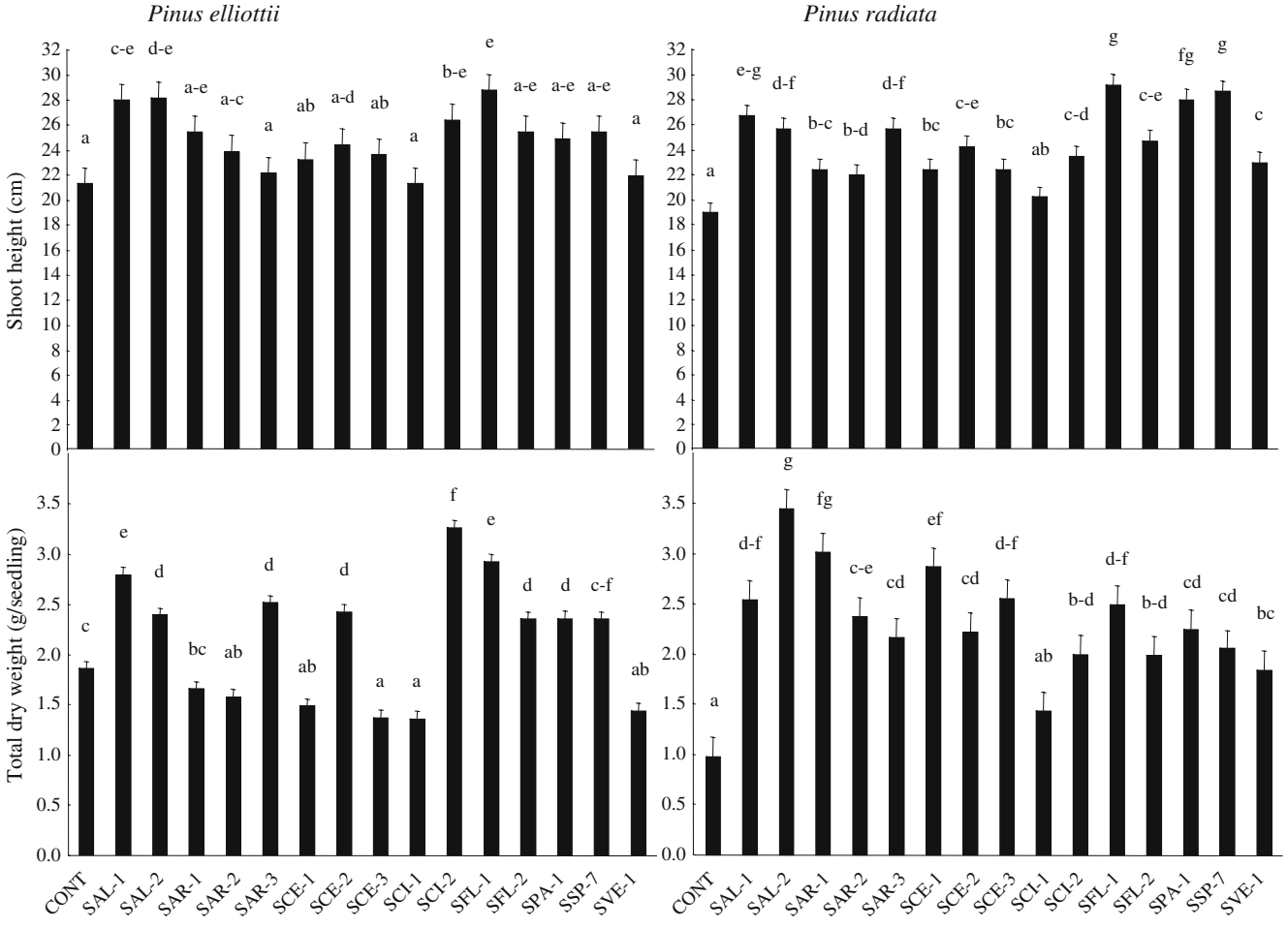

Fig. 2 Effects of *Scleroderma* inoculation on shoot height and total dry weight of *Pinus elliottii* (*left*) and *P. radiata* (*right*). Standard errors are given. For each host, bars with same letters in each parameter are not significantly different by Duncan's Multiple Comparison ($P<0.05$). *CONT* unicoculated control; other codes are given in Table 1

Australia. Furthermore, there were differences within taxa, even from same collecting region. For example, one collection of Australian *S. areolatum* (SAR-1) formed extensive ECMs on both eucalypts and pines (over 50% of short roots), while SAR-2 (same storage as SAR-1) failed to colonize seedlings of *P. elliottii* and colonized less than 25% of short roots of *P. radiata* and *E. urophylla* by week 12. By contrast, *Scleroderma citrinum* and *Scleroderma flavidum* collected from either south China, Western Australia, or northern Thailand showed no or little host specificity on all hosts in this trial. *S. citrinum* has been found to be commonly associated with various tree species in nurseries, plantations, and forests (Schramm 1966; Garido 1984; Ingleby et al. 1985; Duñabeitia et al. 2004), and collections of this fungus have been tested to be compatible with many pine species (Takacs 1961; Trappe 1962; Schramm 1966; Azevedo 1982; Parladé et al. 1996; Duñabeitia et al. 2004). The *S. areolatum* collections used in this trial may be polymorphic, and molecular tools are required to define species boundaries.

Scleroderma fungi, such as *Scleroderma bovista, S. citrinum*, and *S. verrucosum*, have been observed to be present in coniferous or eucalypt forests and plantations (Pryor 1956; Takacs 1961; Bakshi 1966; Birch 1973; Guzmán and Varela 1978; Dunstan et al. 1998; Cairney and Chambers 1999; Chen et al. 2000b, 2004). *S. bovista, S. cepa*, *S. citrinum*, *Scleroderma meridionale*, and *Scleroderma verrucosum* have been previously used to inoculate *Eucalyptus* and *Pinus* species (Trappe 1962; Marx 1969; Chu-Chou 1979; Richter and Bruhn 1989; Dell et al. 1994; Parladé et al. 1996; Lu et al. 1998; Rincón et al. 2001; Chen et al. 2000b). While most studies tested the comparability of *Scleroderma* in one host genus only (Richter and Bruhn 1987; Duponnois et al. 2005), Dell at al. (1994) and Sanon et al. (1997) compared across a few tree genera from Australia or the Northern hemisphere, respectively. *Scleroderma* has been considered to be incompatible with *Casuarina* (Dell at al. 1994) or *Alnus* (Molina 1981). Reddy and Satyanarayana (1998) reported that isolates of *S. cepa* and *S. flavidum* failed to form ECMs on *E. tereticornis* in vitro. Apart from these studies, the literature suggests that *Scleroderma* generally colonizes a wide range of host trees.

Results on colonization ability of 15 collections of *Scleroderma* on four plantation trees showed that variable degrees of compatibility of fungal collection–host species

influenced successful colonization and development of mycorrhizas. These are consistent with previous findings on eucalypt mycorrhization (Malajczuk et al. 1990; Dell et al. 1994; Parladé et al. 1996). Previous studies conclude that many ECM fungi show specificity at the host genus level rather than at the host species level (Chilvers 1973; Malajczuk et al. 1982). A few broad-host-range fungal symbionts, such as *Amanita muscaria* and *Hebeloma crustuliniforme*, are capable of forming ECMs in common on eucalypts and pines (Malajczuk et al. 1982). In this experiment, *Scleroderma* collected from under eucalypt plantations in Australia formed ECMs on both *P.elliottii* and *P. radiata*. By contrast, *Scleroderma* associated with *Pinus massoniana* in China was a poor colonizer of eucalypts than pines.

Inoculation with some *Scleroderma* species generally enhanced the growth of all host plants involved in this trial confirming the potential usefulness of this genus for slow-growing nursery stock. Growth promotion at the nursery stage is not necessarily important for fast-growing species, such as *E. urophylla*, but is advantageous for most pines. The priority of inoculating containerized-grown seedlings is the extent of mycorrhizal success and the subsequent improvement in survival and growth in the field. Nevertheless, previous work has shown that *Scleroderma* can enhance the growth of eucalypts (Burgess and Malajczuk 1990; Reddell and Milnes 1992; Dell et al. 1994; Reddy and Satyanarayana 1998), pines (*Pinus caribaea*, *P. contorta*, and *P. kesiya*) (Rangarajan et al. 1990; Rao et al. 1996; Fay et al. 1997), *Castanopsis hystrix* (Chen et al. 2001), and some dipterocarps (Santoso 1991; Omon 1996). The finding that *Scleroderma* species promoted plant growth confirms four previous studies where *Scleroderma* was inoculated onto *Afzelia africana* (Bâ et al. 1999), *E. globulus* (Lu et al. 1998), *Pinus sylvestris* (Colpaert et al. 1992), or *Quercus ilexballota* (Seva et al. 1996). The experimental conditions applied in our trial have been previously tested to grow mycorrhizal eucalypts with inoculation of *Scleroderma* spores (Chen et al. 2006). Results from inoculation of eucalypts at three nurseries in Western Australia (Mediterranean) and south China (subtropical and temperate) with 18 collections of *Scleroderma* revealed that there were no significant differences in the performance of congeneric fungal collections from different climatic regions (Brundrett et al. 2005). This suggests that fungi of this genus may be particularly suitable for inoculating eucalypts in south China, where climate ranges from lowland subtropical/tropical to highland temperate.

Results on the efficiency in colonizing eucalypts between Australian and Chinese collections of *Scleroderma* expand previous findings of a field inoculation trial on *E. urophylla* in south China (Chen et al. 2000c), where 10 of the 11 collections of *Scleroderma* from Australia were effective in improving tree survival and productivity while there was no response inoculation by a single Chinese collection. While Chinese *Scleroderma* collected from under secondary pine forests or pine plantations could be used for inoculating seedlings in pine nurseries, there may be a risk to using these fungi for inoculating eucalypts in Chinese nurseries. Hence, it is recommended that *Scleroderma* be sourced from outside China to inoculate eucalypts until the time basidiomes are sufficiently abundant in eucalypt plantations to enable the local collection of spores. Due to the ease of application and the availability of large quantities of spores from a few sporocarps for nursery inoculation needs, spore inoculum is preferable for application on an operational scale in China.

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References

Azevedo N (1982) Ectomycorrhizae del *Pinus pinaster* Sol. ex Ait. Bol Estac Cent Ecol (ICONA) 11:37–42

Bâ AM, Sanon KB, Duponnois R, Dexheimer J (1999) Growth response of *Afzelia africana* Sm. seedlings to ectomycorrhizal inoculation in a nutrient-deficient soil. Mycorrhiza 9:91–95

Bakshi BK (1966) Mycorrhiza in eucalypts in India. Indian For 92:19–20

Birch TTC (1973) A synopsis of forest fungi of significance in New Zealand. NZ J For 6:109–125

Brundrett M, Bougher N, Dell B, Grove G, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. ACIAR, Canberra, pp 174–182

Brundrett M, 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

Burgess T, Malajczuk N (1990) The effect of ectomycorrhizal fungi on reducing the variation of seedling growth of *Eucalyptus globulus*. Agric Ecosyst Environ 28:41–46

Cairney JWG, Chambers SM (1999) Ectomycorrhizal fungi: key genera in profile. Springer-Verlag Berlin Heidelberg, New York, pp 369

Chen YL, Brundrett MC, Dell B (200a) 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, Wang FZ, Zhang MQ, Chen Y (200b) Diversity and colonization of putative mycorrhizal fungi in *Eucalyptus* plantations in Southern China. For Stud China 2:34–42

Chen YL, Gong MQ, Xu DP, Zhong CL, Wang FZ, Chen Y (200c) Screening and inoculant efficacy of Australian ectomycorrhizal fungi on *Eucalyptus urophylla* in field. For Res 13:569–576

Chen YL, Gong MQ, Wang FZ, Chen Y (2001) Effects of inoculation with 11 ectomycorrhizal fungal isolates on growth and photosynthesis of *Castanopsis hystrix* saplings. For Res 14:515–522

Chen YL, Kang LH, Dell B (2004) Some useful macrofungi from Liuxihe National Forest Park, Guangzhou, China. China For Sci Tech 3:35–42

Chen YL, Dell B, Malajczuk N (2006) Effect of *Scleroderma* spore density and age on mycorrhiza formation and growth of containerized *Eucalyptus globulus* and *E. urophylla* seedlings. New For (in press)

Chilvers GA (1973) Host range of some eucalypt mycorrhizal fungi. Aust Plant J Bot 21:103–111

Chu-Chou M (1979) Mycorrhizal fungi of *Pinus radiata* in New Zealand. Soil Biol Biochem 11:557–562

Colpaert JV, Assche JA van, Luijtens K, Van Assche JA (1992) The growth of the extramatrical mycelium of ectomycorrhizal fungi and the growth response of *Pinus sylvestris* L. New Phytol 120:127–135

Dell B, Malajczuk N (1995) Fertiliser requirements for ectomycorrhizal eucalypts in forest nurseries and field plantings in Southern China. In: Brundrett M, Dell D, Malajczuk N, Gong M (eds) Mycorrhizas for Plantation Forestry in Asia. ACIAR Monograph 62. ACIAR, Canberra, pp 96–100

Dell B, Malajczuk N (1997) L'inoculation des *Eucalyptus* introduits en Asie avec des champignons ectomycorhiziens australiens en vue d'augmenter la productivite des plantations. In: Le Tacon F (ed) Champignons et Mycorhizes en Foret. Revue Forestière Française, Numéro spécial 1997, pp 174–184

Dell B, Malajczuk N, Bougher NL, Thomson G (1994) Development and function of *Pisolithus* and *Scleroderma* ectomycorrhizas formed in vivo with *Allocasuarina*, *Casuarina* and *Eucalyptus*. Mycorrhiza 5:129–138

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

Duñabeitia MK, Hormilla S, Garcia-Plazaola JI, Txarterina K, Arteche U, Becerril JM (2004) Differential responses of three fungal species to environmental factors and their role in the mycorrhization of *Pinus radiata* D. Don. Mycorrhiza 14:11–18

Dunstan W, Dell B, Malajczuk N (1998) The diversity of ectomycorrhizal fungi associated with introduced *Pinus* spp. in the Southern Hemisphere, with particular reference to Western Australia. Mycorrhiza 8:71–79

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

Fay DA, Mitchell DT, Parkes MA (1997) A preliminary study of the mycorrhizal associations of tree seedlings growing on mine spoil at Avoca, Co. Wicklow. Special issue. The ecology of old mine sites. Proceedings of a workshop organised by the Mining Heritage Society of Ireland at the Geological Survey of Ireland, 18–19 October 1997

Garbaye J, Delwaulle JC, Diangana D (1988) Growth response of eucalypts in the Congo to ectomycorrhizal inoculation. For Ecol Manag 24:151–157

Garido N (1984) Notes on *Scleroderma citrinum* Pers. (Mycota-Gasteromycetes) in *Pinus radiata* forests in Chile. Nova Hedwigia Kryptogamenkd 40:511–516

Ge XZ, Bi GC (1989) Forest mycorrhiza and application. China Forestry, Beijing, pp 1–305

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, 2nd edn. Wiley, New York, pp 1–680

Guzmán G, Varela L (1978) Los hongos de Colombia III. Obsercaciones sobre los hongos, liquenes y mixomicetos de Colombia. Caldesia 12:309–338

Hall IR, Brown GD, Byars J (1995) The black truffle—its history, uses and cultivation. New Zealand Crop and Food Research, Christchurch, pp 1–105

Hardy GJ, Burgess T, Dell B (2003) Potential threats of plant pathogens to eucalypts plantations in China. In: Wei R, Xu D (eds) *Eucalyptus* plantations, research, management and development. Proceedings of the international symposium of eucalypts, Guangzhou, China. World Scientific, Singapore, pp 358–366

Ingleby K, Last FT, Mason PA (1985) Vertical distribution and temperature relations of sheathing mycorrhizas of *Betula* spp. growing on coal spoil. For Ecol Manag 12:279–285

Jiang JM, Lu BS (2000) High-efficiency intensive culture of industrial timber forest of exotic pines. J Zhejiang For Sci Tech 20:76–79

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

Malajczuk N, Molina R, Trappe JM (1982) Ectomycorrhiza formation in *Eucalyptus* I. Pure culture synthesis, host specificity and mycorrhizal compatibility with *Pinus radiata*. New Phytol 91:467–482

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

Martin TP, Harris JR, Eaton GK, Miller OK (2003) The efficacy of ectomycorrhizal colonization of pin and scarlet oak in nursery production. J Environ Hortic 21:45–50

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Marx DH, Hedin A, Toe IV, SFP (1985) Field performance of *Pinus caribaea* var. *hondurensis* seedlings with specific ectomycorrhizae and fertilizer after three years on a savanna site in Liberia. For Ecol Manag 13:1–25

Molina R (1981) Ectomycorrhizal specificity in the genus *Alnus*. Can J Bot 59:325–334

Omon RM (1996) The effect of some mycorrhizal fungi and media on the growth of *Shorea leprosula* Miq. cuttings. Bul Penelitian Hutan 603:27–36

Parladé J, Alvarez IF, Pera J (1996) Ability of native ectomycorrhizal fungi from northern Spain to colonize Douglas-fir and other introduced conifers. Mycorrhiza 6:51–55

Pryor LD (1956) Ectotrophic mycorrhizae in renantherous species of *Eucalyptus*. Nature 177:587–588

Qi SX (2002) Brief introduction of eucalypt cultivation and utilization in China. In: Wei RP, Xu D (eds) *Eucalyptus* plantations, research, management and development. Proceedings of the international symposium of euclaypts, Guangzhou, China. World Scientific, Singapore, pp 37–41

Rangarajan M, Narayanan R, Kandasamy D, Oblisami G (1990) Studies on the growth of certain ectomycorrhizal fungi in culture media and in the host under axenic conditions. In: Jalali BL, Chand H (ed) Trends in mycorrhizal research. Proceedings of the national conference on mycorrhiza, held at Haryana Agricultural University, Hisar, India, 14–16 Feb, 1990, pp 126–127

Rao CS, Sharma GD, Shukla AK (1996) Ectomycorrhizal efficiency of various mycobionts with *Pinus kesiya* seedlings in forest and degraded soils. Proc Indian Natl Sci Acad, B Biol Sci 62:427–434

Reddy SM, Satyanarayana T (1998) Inoculation of micropropagated plantlets of *Eucalyptus tereticornis* with ectomycorrhizal fungi. New For 16:273–279

Reddell P, Milnes AR (1992) Mycorrhizas and other specialized nutrient-acquisition strategies—their occurrence in woodland plants from Kakadu and their role in rehabilitation of waste rock dumps at a local uranium-mine. Aust J Bot 40:223–242

Richter DL, Bruhn JN (1987) *Scleroderma* spp. ectomycorrhizae for use in greenhouse and nursery to increase *Pinus resinosa* outplanting success. Mycol Soc Am Newsl 38:45

Richter DL, Bruhn JN (1989) *Pinus resinosa* ectomycorrhizae: seven host–fungus combinations synthesized in pure culture. Symbiosis 7:211–228

Rincón A, Alvarez IF, Pera J (2001) Inoculation of containerized *Pinus pinea* L. seedlings with seven ectomycorrhizal fungi. Mycorrhiza 11:265–271

Sanon KB, Bâ AM, Dexheimer J (1997) Mycorrhizal status of some fungi fruiting bebeath indigenous trees in Burkina Faso. For Ecol Manag 98:61–69

Santoso E (1991) Effect of mycorrhizal fungi on nutrient uptake of five dipterocarp seedlings. Bul Penelitian Hutan 532:11–18

Schramm JM (1966) Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans Am Philos Soc 56:1–194

Seva JP, Vilagrosa A, Valdecantos A, Cortina J, Vallejo VR, Bellot J (1996) Mycorrhization and application of urban compost for the improvement of survival and growth of *Quercus ilex* subsp. *ballota* seedlings in an arid zone. Cah Options Mediterr 20:105–121

Takacs EA (1961) Algunas especies de hongos formadores de mycorrhizas en árboles forestales en la Argentina. Rev For Argent 5:80–82

Thomson BD, Hardy GES, Malajczuk N, Grove TS (1996) The survival and development of inoculant ectomycorrhizal fungi on roots of outplanted *Eucalyptus globulus* Labill. Plant Soil 178:247–253

Trappe JM (1962) Fungus associates of ectotrophic mycorrhizae. Bot Rev 28:538–606

Xu DP, Bai JY, Dell B (2000) Overcoming the constraints to productivity of plantation eucalypts in Southern China. In: Gong MQ, Xu DP, Zhong CL, Chen YL, Dell B, Brundrett M (eds) Proceedings of ACIAR Workshop on Mycorrhiza, Guangzhou, China