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Mycorrhiza formation and growth of *Eucalyptus globulus* seedlings inoculated with spores of various ectomycorrhizal fungi

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Abstract As many eucalypts in commercial plantations are poorly ectomycorrhizal there is a need to develop inoculation programs for forest nurseries. The use of fungal spores as inoculum is a viable proposition for low technology nurseries currently producing eucalypts for outplanting in developing countries. Forty-three collections of ectomycorrhizal fungi from southwestern Australia and two from China, representing 18 genera, were tested for their effectiveness as spore inoculum on *Eucalyptus globulus* Labill. seedlings. Seven-day-old seedlings were inoculated with 25 mg air-dry spores in a water suspension. Ectomycorrhizal development was assessed in soil cores 65 and 110 days after inoculation. By day 65, about 50% of the treatments had formed ectomycorrhizas. By day 110, inoculated seedlings were generally ectomycorrhizal, but in many cases the percentage of roots colonized was low (<10%). Species of *Laccaria, Hydnangium, Descolea, Descomyces, Scleroderma* and *Pisolithus* formed more ectomycorrhizas than the other fungi. Species of *Russula*, *Boletus*, *Lactarius* and *Hysterangium* did not form ectomycorrhizas. The dry weights of inoculated seedlings ranged from 90% to 225% of the uninoculated seedlings by day 110. Although plants with extensively colonized roots generally had increased seedling growth, the overall mycorrhizal colonization levels were poorly correlated to seedling growth. Species of *Laccaria*, *Descolea*, *Scleroderma* and *Pisolithus* are proposed as potential candidate fungi for nursery inoculation programs for eucalypts.

Key words Colonization · Ectomycorrhiza · *Eucalyptus globulus* · Spore inoculation

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Introduction

Inoculation of tree seedlings with specific ectomycorrhizal fungi to improve seedling survival and early growth in forestation programs has been well documented (Trappe 1977). To date, many inoculation programs have used vegetative mycelium as the ectomycorrhizal inoculum (Marx 1991). However, this has often been hampered by factors such as the high cost of vegetative inoculum production, difficulties of isolation and maintenance of pure cultures, and lack of sufficient amounts of inoculum for large scale inoculation programs (Trappe 1977; Marx and Kenney 1982). This is especially the case in developing countries, such as in China and other Asian countries, where the greatest recent development of eucalypt plantations has taken place. In Asia, basidiospores of ectomycorrhizal fungi are relatively cheap inoculum resources and are easier to handle than vegetative mycelium. Large amounts of spores of some ectomycorrhizal fungi can be collected readily in the field. Unlike vegetative inoculum, spores do not require extended culture under aseptic conditions, and they usually remain viable in simple storage from one season to the next (Marx and Kenney 1982). Spores of selected ectomycorrhizal fungi have been effectively used as inoculum to form ectomycorrhizas on pine (Marx 1976; Alvarez and Trappe 1983; Maghembe and Redhead 1984; Martinez-Amores et al. 1991; Torres and Honrubia 1994; Parladé et al. 1996) and other conifer tree species (Castellano et al. 1984; Castellano and Trappe 1985). In the United States, large-scale inoculation of Douglas-fir seedlings with basidiospores of *Rhizopogon* spp. has been applied in commercial nurseries for over a decade (Castellano 1994).

Numerous workers have used vegetative mycelium as ectomycorrhizal inoculum for eucalypts (e.g. Bougher et al. 1990; Burgess et al. 1993; Thomson et al. 1994), but little work has been undertaken on the inoculation of eucalypts with fungal spores. An exception is in the Philippines, where tablets made from a mixture of basidiospores of *Pisolithus tinctorius* and *Scleroderma*

Table 1 Details of fungal spore collections used in this study, giving the code, species, origin and associated vegetation

Code	Species	Origin	Vegetation
AMA1	*Amanita preisii* Fries	Perth	*Eucalyptus* spp.
AMA2	*Amanita umbrinella* Gilbert & Cleland	Perth	*Eucalyptus* spp.
AMA3	*Amanita umbrinella* Gilbert & Cleland	Manjimup	*E. globulus* Labill.
AMA4	*Amanita xanthocephala* (Berkeley) Reid & Hilton	Pemberton	*E. regnans* Muell.
AMA5	*Amanita* sp.	Perth	*E. marginata* Donn ex Smith *E. calophylla* Brown ex Lindl.
AMA6	*Amanita* sp. 2	Perth	*E. marginata* Donn ex Smith *E. calophylla* Brown ex Lindl.
AUS	*Austrogautieria manjimupana* Trappe & Stewart	Dwellingup	*E. microcorys* Muell.
BOL1	*Boletus* sp. 1	Watermans	
BOL2	*Boletus* sp. 1	Kellerberrin	*E. wandoo* Blakely
CORT1	*Cortinarius basirubescence* Cleland & Harris	Augusta	*E. globulus* Labill.
CORT2	*Cortinarius radicatus* Cleland	Dwellingup	*E. marginata* Donn ex Smith
CORT3	*Cortinarius* sp. 1	Perth	*Melaleuca* spp.
CORT4	*Cortinarius* sp. 2	Perth	*Melaleuca* spp.
DESL1	*Descolea maculata* Bougher & Malajczuk	Perth	*Melaleuca* spp.
DESL2	*Descolea maculata* Bougher & Malajczuk	Augusta	*E. globulus* Labill.
DESM	*Descomyces albellus* (Massee & Rodway) Bougher & Castellano	Northcliff	*E. globulus* Labill.
HYDG1	*Hydnangium carneum* Wallroth	Northcliff	*E. globulus* Labill.
HYDG2	*Hydnangium sublamellatum* Bougher, Tommerup & Malajczuk	Perth	*Melaleuca* spp.
HYNO	*Hydnotrya* sp.	Pemberton	*E. marginata* Donn ex Smith *E. calophylla* Brown ex Lindl.
HYNU	*Hydnum repandum* L. ex Fr.	Northcliff	*E. globulus* Labill.
HYST	*Hystanerium* sp.	Dwellingup	*E. microcorys* Muell.
LAC1	*Laccaria lateritia* Malencon	Manjimup	*E. globulus* Labill.
LAC2	*Laccaria lateritia* Malencon	Bridgetown	*E. globulus* Labill.
LAC3	*Laccaria laccata* (Scop. ex. Fr.) Berk.	Yunnan, CHINA	*E. globulus* Labill.
LAC4	*Laccaria* sp.	Manjimup	*E. globulus* Labill.
LACT	*Lactarius eucalypti* Miller & Hilton	Northcliff	*E. globulus* Labill.
LEUC	*Leucopaxillus lilacinus* Bougher	Dwellingup	*E. marginata* Donn ex Smith
MESO1	*Mesophellia* sp. 1	Margaret River	*E. diversicolor* F. Muell.
MESO2	*Mesophellia* sp. 2	Margaret River	*E. diversicolor* F. Muell.
PAX1	*Paxillus muelleri* (Berkeley) Saccardo	Pemberton	*E. regnans* Muell
PAX2	*Paxillus* sp.	Pemberton	*E. regnans* Muell
PIS1	*Pisolithus albus* (Cooke & Massee) Priest	Perth	*E. camaldulensis* Dehnh.
PIS2	*Pisolithus microcarpus* Cooke & Massee	Perth	*E. rudis* Endl.
PIS3	*Pisolithus tinctorius* (Pers.) Coker & Couch	Yunnan, CHINA	*E. globulus* Labill.
PIS4	*Pisolithus* sp.	Northcliff	*E. globulus* Labill.
PIS5	*Pisolithus* sp.	Augusta	*E. marginata* Donn ex Smith
RUS1	*Russula* sp. 1	Northcliff	*E. globulus* Labill.
RUS2	*Russula* sp. 2	Northcliff	*E. globulus* Labill
RUS3	*Russula* sp. 3	Manjimup	*E. globulus* Labill.
SCL1	*Scleroderma cepa* Persoon	Perth	*E. camaldulensis* Dehnh.
SCL2	*Scleroderma cepa* Persoon	Manjimup	E. marginata Donn ex Smith
SCL3	*Scleroderma cepa* Persoon	Bridgetown	*E. globulus* Labill.
SCL4	*Scleroderma* sp. 1	Bridgetown	*E globulus* Labill.
SCL5	*Scleroderma* sp. 2 (Hypogeous)	Manjimup	*E. globulus* Labill.
TRIC	*Tricholoma* sp.	Augusta	*E. globulus* Labill.

cepa have proven to be effective on several eucalypt species (de la Cruz 1990). The primary aim of the present study was to test a broad range of ectomycorrhizal fungi for their potential to form ectomycorrhizas with *Eucalyptus globulus* seedlings when applied as a spore inoculum. Seedling growth responses to inoculation were also assessed. *E. globulus* was chosen because of its importance in afforestation and land rehabilitation in many temperate regions of the world.

Materials and methods

Inoculum preparation

Sporocarps were collected from June to October 1995 from under *E. globulus* plantations and native forests of several other eucalypt species in southwestern Australia. In addition, two collections from *E. globulus* plantations in Yunnan Province, China were included (Table 1). Freshly collected mature sporocarps were gently brushed free of soil or humus, the stipes removed

from the caps and discarded (gilled fungi), and air-dried with a commercial electric drying unit at 35 °C for 2–7 days (depending on the size and texture of sporocarps). After drying, sporocarps of puffballs and truffle-like fungi (such as *Pisolithus* and *Mesophellia*) were crushed by hand and sieved through a 211-μm metal sieve within a fume cupboard. For gilled fungi (like *Laccaria*), the dried fungal caps were crushed by hand and screened through a series of metal sieves with a final mesh diameter of 211 μm. Those sporocarps which hardened with drying (such as *Boletus* and hypogeous *Scleroderma*) were broken up using a light hammer and sieved as for gilled fungi. After each usage, the sieves were autoclaved at 121 °C for 15 min to prevent cross-contamination. The screened spores or spore masses were stored in sealed plastic bags in the dark at 6 °C for 2–6 months before use.

Seedling preparation

Eucalyptus globulus seeds were sieved to uniform size (1.4–1.6 mm diameter) and surface sterilized by shaking in 10% $NaClO_3$ for 15 min. After 5 rinses with sterile distilled water, seeds were aseptically planted on water agar medium containing 500 μM $CaSO_4$, 3 μM H_3BO_3 and 0.2% glucose, and incubated in the dark at 25 °C for 4 days. Following germination, the plates were exposed to day light at 25 °C. This procedure was used to ensure that seedlings were free of fungal spores at planting.

Soil preparation

A P-deficient (P less than 2 mg kg^{-1}, pH 5.5) yellow sand, collected from the Spearwood dune system north of Perth, Western Australia, was steam pasteurized twice at 70 °C for 1 h, oven dried at 70 °C and sieved through a 2-mm metal mesh. Plastic pots (140 mm top diameter), presterilized with $NaClO_3$ (5%), were lined inside with plastic bags and 2500 g dry sand was added to each pot. Basal nutrients were applied in solution to the surface of each pot at the following rates (mg kg^{-1}): 50 K, 29 Ca, 18 N, 4.2 Mn, 3.3 Mg, 2.1 Zn, 2.1 Cu, 0.25 Mo, 0.12 B, and 0.08 Co. Soils were then left to air dry for 1 week. Phosphorus was added as $Ca(H_2PO_4)_2 .H_2O$ (6 mg P kg^{-1} soil, Burgess et al. 1993) and all nutrients were thoroughly mixed through the soil. Pots were watered to 12% soil moisture content (v/v) with deionized water. The surface of each pot was covered with aluminium insulation foil to reduce water loss and algal growth, and pots were left to equilibrate for 1 week before planting.

Planting and inoculation

Seven-day-old seedlings were transplanted into pots in an evaporatively cooled glasshouse in December 1995. Four seedlings were initially planted and then thinned to 2 per pot 3 weeks after planting. There were three replicate pots for each treatment. Pots were randomly arranged on glasshouse benches and the benches were rotated weekly. Soil moisture was maintained at 12% by regular watering to weight. Nitrogen was applied in solution as NH_4NO_3 at intervals of 3 weeks (18 mg N kg^{-1} soil).

The inoculum was prepared by weighing approximately 500 mg of the dry sieved spore mass (spores or spores and gill fragments previously stored at 6 °C for 2–6 months) into a plastic vial containing 20 ml sterile distilled water and shaking vigorously on a Microid flask shaker for 15–20 min until an even spore suspension was obtained with a final concentration of 25 mg spores ml^{-1}. The final spore densities were within the range of 10^6 (*Laccaria*, *Descolea*) to 10^7 spores ml^{-1} (*Pisolithus*). The spore water suspensions were prepared about 1 week before inoculation. At the time of planting, 1 ml of spore suspension was aseptically injected into the planting hole (approximately 2 cm deep) and a seedling was placed on top. Control seedlings were not inoculated.

Plant measurements and harvest

Seedling height was measured at 10-day intervals from 3 weeks after planting. Pots were checked periodically for signs of fungal fruiting as well as mycelium growth on the soil surface. In order to measure early mycorrhizal development, one soil core (about 50 mg soil) was taken from each pot using a sterilized cylindrical stainless steel corer (20 mm diameter) at day 65. The cores were replaced with newly steamed sand so that the weight of pots remained unchanged. At day 110, seedlings were harvested for final assessment. Plant shoots were cut at 10 cm from the sand surface and a further soil core was taken from each pot. The pots were left in the glasshouse for a further period to study the fruiting habits of mycorrhizal fungi. Plant shoots were oven-dried at 70 °C for 1 week and dry weights were recorded. Sample roots from each soil core were washed free of sand and a small amount of fresh root material was removed for characterization of ectomycorrhizas. A subsample of fine roots (0.2 g fresh weight) was cut into 2- to 3-mm segments and fixed in 50% ethanol for root colonization assessment. This subsample was cleared with 10% KOH by autoclaving at 121 °C for 15 min and stained with 0.05% lactic glycerol Trypan blue at 25 °C for 24 h. Ectomycorrhizal root colonization was quantified by a line intercept method (Brundrett et al. 1996). Fine roots with a fungal mantle and Hartig net were scored as ectomycorrhizal. Ectomycorrhizas initiated by different fungal symbionts were characterized by the method of Agerer (1991) and Ingleby et al. (1990). Descriptions of relevant eucalypt ectomycorrhizas were available for comparison (Bougher and Malajczuk 1985; Dell et al. 1990; Brundrett et al. 1996).

Data were subjected to one-way analysis of variance (ANOVA) and treatment means were compared using the Least Significant Difference test (LSD, $P < 0.05$).

Results

Ectomycorrhiza colonization

Morphological features enabled the main groups of eucalypt ectomycorrhizas to be easily distinguished. For example, ectomycorrhizas formed by *Laccaria* species were creamy white, simple bifurcate or pyramidal with a dense mantle. *Scleroderma* ectomycorrhizas were easily identified by the shiny, pure white mycelial mats formed on the root surface and the distinctive white rhizomorphs radiating through the soil. *Pisolithus* ectomycorrhizas were yellowish-brown with numerous emanating yellow hyphae, forming mycelial mats and rhizomorphs. Ectomycorrhizas formed by species of *Descolea* and *Descomyces* were characterized by a mantle composed of two distinctive types of hyphae (hyaline, thin-walled and dark, thick-walled) and the hyaline, thin-walled capitate cystidia formed on the mantle surface. Seedlings inoculated with *Mesophellia* spp. developed mycorrhizas of the superficial type with a diffuse mantle and shallow Hartig net.

At day 65, 18 of the 45 fungal treatments had formed ectomycorrhizas (data not presented), but in only the four *Laccaria* treatments, including one collected from China, was there abundant colonization of fine roots (about 20%). Treatments with less colonization (<10%) included *Scleroderma* (5 treatments), *Pisolithus* (4), *Hydnangium* (2), *Descolea* (1), *Descomyces* (1) and *Cortinarius* (1). The fine roots of the uninoculated seedlings were not colonized by fungal hyphae.

Table 2 Height, dry weight of shoots and percent of mycorrhizal feeder roots of *Eucalyptus globulus* seedlings inoculated with 45 fungal spore collections. Control seedlings were uninoculated. Seedlings were sampled at day 110. Data are the average of 3 (ectomycorrhiza) or 6 (seedling height, shoot dry weight) replicates. Values within columns followed by the same letter are not significantly different ($P<0.05$, LSD)

Code	Species	Height (cm)	Shoot dry weight (g/seedling)	Ectomycorrhizal roots (%)
CONT	Control	43.3$^{a–g}$	3.5$^{k–m}$	0^{k}
AMA1	*Amanita preissi*	42.2$^{a–h}$	5.0$^{c–m}$	8.8$^{f–k}$
AMA2	*Amanita umbrinella*	42.2$^{a–h}$	6.8$^{a–d}$	*0^{k}
AMA3	*Amanita umbrinella*	43.2$^{a–g}$	6.5$^{a–f}$	*0^{k}
AMA4	*Amanita xanthocephala*	43.0$^{a–g}$	5.0$^{c–m}$	10.6$^{d–k}$
AMA5	*Amanita* sp. 1	42.0$^{a–h}$	4.7$^{d–m}$	2.3$^{l–k}$
AMA6	*Amanita* sp. 2	39.2$^{d–i}$	4.5$^{f–m}$	1.7$^{l–k}$
AUS	*Austrogautieria manjimupana*	40.7$^{c–i}$	4.9$^{d–m}$	3.4$^{h–k}$
BOL1	*Boletus* sp. 1	38.2$^{d–i}$	4.3$^{g–m}$	4.7$^{h–k}$
BOL2	*Boletus* sp. 2	36.3$^{g–i}$	3.5$^{k–m}$	0^{k}
CORT1	*Cortinarius basirubescence*	44.2$^{a–f}$	6.6$^{a–e}$	19.3$^{a–f}$
CORT2	*Cortinarius radicatus*	42.8$^{a–g}$	5.9$^{a–i}$	3.5$^{h–k}$
CORT3	*Cortinarius* sp. 1	37.2$^{f–i}$	5.4$^{b–k}$	3.9$^{h–k}$
CORT4	*Cortinarius* sp. 2	43.8$^{a–f}$	6.4$^{a–g}$	5.5$^{h–k}$
DESL1	*Descolea maculata*	44.5$^{a–e}$	5.9$^{a–i}$	5.7$^{h–k}$
DESL2	*Descolea maculata*	47.0$^{a–c}$	6.8$^{a–d}$	14.8$^{c–h}$
DESM	*Descomyces albellus*	49.2^{a}	6.5$^{a–f}$	12.6$^{c–i}$
HYDG1	*Hydnangium carneum*	40.3$^{c–i}$	5.5$^{b–k}$	21.6$^{a–e}$
HYDG2	*Hydnangium sublamellatum*	42.2$^{a–h}$	5.7$^{b–j}$	18.6$^{b–g}$
HNYO	*Hydnotrya* sp.	41.5$^{b–h}$	5.9$^{a–i}$	4.3$^{h–k}$
HYNU	*Hydnum repandum*	42.0$^{a–h}$	7.9^{a}	5.4$^{h–k}$
HYST	*Hysterangium* sp.	40.8$^{c–i}$	5.6$^{b–j}$	0^{k}
LAC1	*Laccaria lateritia*	43.2$^{a–g}$	5.3$^{b–l}$	28.4^{ab}
LAC2	*Laccaria lateritia*	43.3$^{a–g}$	6.2$^{a–g}$	31.0^{a}
LAC3	*Laccaria laccata*	43.0$^{a–g}$	6.0$^{a–h}$	24.2$^{a–c}$
LAC4	*Laccaria* sp.	44.2$^{a–f}$	6.2$^{a–g}$	24.0$^{a–c}$
LACT	*Lactarius eucalypti*	34.0^{i}	3.9$^{l–m}$	0^{k}
LEUC	*Leucopaxillus lilacinus*	42.3$^{a–h}$	6.1$^{a–h}$	7.9$^{f–k}$
MESO1	*Mesophellia* sp. 1	37.5$^{e–i}$	3.2^{m}	3.5$^{h–k}$
MESO2	*Mesophellia* sp. 2	42.3$^{a–h}$	5.5$^{b–k}$	6.3$^{h–k}$
PAX1	*Paxillus muelleri*	42.7$^{a–h}$	5.0$^{c–m}$	3.0$^{h–k}$
PAX2	*Paxillus* sp.	40.7$^{c–i}$	5.0$^{c–m}$	4.0$^{h–k}$
PIS1	*Pisolithus albus*	41.0$^{c–i}$	4.9$^{c–m}$	12.2$^{c–j}$
PIS2	*Pisolithus microcarpus*	41.8$^{b–h}$	5.4$^{b–k}$	6.6$^{g–k}$
PIS3	*Pisolithus tinctorius*	35.5^{hi}	3.5$^{k–m}$	0.5^{jk}
PIS4	*Pisolithus* sp.	43.0$^{a–g}$	5.9$^{a–i}$	2.1$^{l–k}$
PIS5	*Pisolithus* sp.	42.0$^{a–h}$	4.1$^{h–m}$	3.3$^{h–k}$
RUS1	*Russula* sp. 1	39.5$^{d–i}$	7.3^{ab}	*0^{k}
RUS2	*Russula* sp. 2	48.5^{ab}	6.3$^{a–g}$	*0^{k}
RUS3	*Russula* sp. 3	45.2$^{a–d}$	7$^{a–c}$	*0^{k}
SCL1	*Scleroderma cepa*	42.2$^{a–h}$	4.8$^{d–m}$	9.6$^{e–k}$
SCL2	*Scleroderma cepa*	44.0$^{a–f}$	4.6$^{e–m}$	22.0$^{a–d}$
SCL3	*Scleroderma cepa*	38.7$^{d–i}$	5.2$^{c–m}$	13.2$^{c–i}$
SCL4	*Scleroderma* sp. 1	38.3$^{d–i}$	3.6$^{j–m}$	10.8$^{d–k}$
SCL5	*Scleroderma* sp. 2 (Hypogeous)	38.2$^{d–i}$	3.3^{lm}	7.4$^{f–k}$
TRIC	*Tricholoma* sp.	44.0$^{a–f}$	5.2$^{c–m}$	2.7$^{h–k}$

* Mycorrhizas formed by contaminating fungus occurred in these treatments but are not included here

By the final sampling at day 110, 37 of the 45 fungal treatments had formed ectomycorrhizas and colonization rates varied greatly (Table 2). Seedlings inoculated with all collections of *Laccaria*, *Hydnangium* and *Descomyces*, and some collections of *Scleroderma* (SCL2, SCL3), *Descolea* (DESL2), *Pisolithus* (PIS1) and *Cortinarius* (CORT1), formed abundant ectomycorrhizas. The four collections of *Laccaria*, three local and one exotic, had the highest ectomycorrhizal colonization rates in this study. Eight fungal treatments, including *Amanita* (2), *Russula* (3), *Boletus* (1), *Lactarius* (1) and *Hysterangium* (1), did not form ectomycorrhizas. Control seedlings remained uncolonized at the final sampling.

Contamination occurred in pots of several treatments (Table 2). There were two types of contamination. The first was contamination of *Laccaria* which fruited in AMA3, a non-*Laccaria* treatment and was identified as *L. lateritia*, a fungus used in this study. Since, under our glasshouse conditions, we have not previously observed contamination with *Laccaria,* it is likely that limited cross-contamination occurred in this experiment. The second was caused by a *Cenococcum*-like fungus which formed distinct black mycorrhizas

(AMA2, RUS1, RUS2, RUS3), a common contaminant in our glasshouses.

There was considerable variation between spore collections of *Pisolithus* in their ability to colonize roots of *E. globulus* seedlings with only one (PIS1) of the five collections forming numerous ectomycorrhizas. The two exotic fungal species collected from under *E. globulus* plantations in China differed in their performance: *L. laccata* (LAC3) formed abundant ectomycorrhizas, whereas *P. tinctorius* (PIS3) did not form any mycorrhiza. Basidiomes of *Laccaria*, the only fungal genus to fruit, first appeared at day 70 (Chinese *L. laccata* collection) and was followed by the three Australian *Laccaria* collections.

Seedling growth

Uninoculated control seedlings had generally poor growth with thinner stems than most mycorrhizal seedlings in the glasshouse. Inoculation with ectomycorrhizal fungal spores generally had little effect on the height growth of *E. globulus* seedlings (Tab. 2). The mean heights of inoculated seedlings measured at the final sampling time did not differ from that of the uninoculated control seedlings, except for two cases where inoculation depressed seedling height growth.

By day 110, dry weights of shoots of inoculated seedlings ranged from 90% to 225% of the dry weights of uninoculated control seedling shoots, depending on the fungal treatment (Table 2). Generally, those treatments where roots were extensively colonized increased seedling growth. However, there was no positive relationship between seedling growth and the rate of root colonization ($r^2 = 0.03$). This indicates that plant growth was not related to mycorrhizal colonization levels in the present study. For example, the two *Hydnangium* collections, which colonized roots extensively, failed to increase seedling growth significantly. Treatments inoculated with the Chinese *L. laccata* increased seedling growth, whereas the Chinese *P. tinctorius* failed to do so. Two *Amanita* (AMA2, AMA3) and three *Russula* treatments, where contamination occurred, also had increased seedling growth.

Discussion

A wide range of ectomycorrhizal fungi is associated with *E. globulus* plantations in Western Australia (X. Lu, N. Malajczuk and B. Bell, unpublished work). We tested 45 collections of ectomycorrhizal fungi, 20 of them from *E. globulus* plantations, for the effectiveness of spore inoculum to colonize the root system of *E. globulus* seedlings. This is the first report of *E. globulus* seedlings inoculated with spores of a wide range of eucalypt-associated ectomycorrhizal fungi. Most of the tested fungal spore collections formed ectomycorrhizas at final sampling, although colonization rates were low in some cases. Spore collections of *Laccaria*, *Hydnangium* and *Descomyces*, and some collections of *Descolea*, *Scleroderma*, *Pisolithus* and *Cortinarius*, were the most effective.

Spores of the same fungus, yet collected from different locations, differed in their ability to colonize roots of *E. globulus* seedlings. Most previous studies have used only a single spore collection for each tested fungus (e.g. Marx 1976; Torres and Honrubia 1994; Parladé et al. 1996). Our results demonstrated that there is a need to select the appropriate spore collection for inoculation of tree species, as shown previously for the selection of fungal isolates for vegetative inoculum (Thomson et al. 1994). This is because large genetic variation can be expected when spores collected from different locations and tree hosts are combined into a single collection (Marx and Kenney 1982).

Ectomycorrhizal colonization was not consistently related to growth of *E. globulus* seedlings. This inconsistency has been previously reported on spore inoculation studies of conifer tree species (Martinez-Amores et al. 1991; Parladé et al. 1996). In fact, growth stimulation is not essential for eucalypts in the nursery. Rather, hardy seedlings that are well colonized are preferred for outplanting.

From a practical point of view, a fungus selected for spore inoculation of seedlings must satisfy at least two criteria: firstly, it must possess the ability to colonize the host plant rapidly and extensively and, secondly, large amounts of spores must be available in the field. Species of *Laccaria*, *Descolea*, *Scleroderma* and *Pisolithus* either fruit abundantly (the former three genera) or produce large sporocarps (the latter two genera) in the field and it would thus be feasible to use spores of these fungi to inoculate seedlings on a large scale. Species of *Descomyces*, *Hydnangium* and *Cortinarius*, however, are not suitable for large-scale inoculation programs because of low spore availability, even though they formed abundant ectomycorrhizas in this study. Of special interest is the genus *Laccaria*. Species of this genus, such as *L. laccata* and *L. bicolor*, have been widely used in mycelium inoculation programs, particularly for conifer tree species (Le Tacon et al. 1988). Since species of *Laccaria* readily form basidiomes under glasshouse (this study) and nursery (Brundrett et al. 1996) conditions, it should be possible to cultivate basidiome lawns for the production of spore inoculum.

Our results demonstrate that the use of spore suspensions is effective for delivering spores of ectomycorrhizal fungi to eucalypt seedlings. It provides a simple method for controlled ectomycorrhizal inoculation of *Eucalyptus* seedlings, as well as for assessing the viability of spore inoculum. Spore suspensions have been widely used to inoculate conifer tree species where plants remain in the nursery for a long period of time (Castellano et al. 1985; Castellano and Trappe 1985; Torres and Honrubia 1994; Parladé et al. 1996). A number of alternative methods have been used to deliver

spores to seedlings. These include encapsulating or coating seeds (Theodorou and Bowen 1973; Marx et al. 1984) and mixing with physical carriers (Marx 1976; Maghembe and Redhead 1984; de la Cruz 1990; Martinez-Amores et al. 1991).

We used a uniform spore inoculation density, because of the large amount of fungal spore collections employed in this study. The optimal spore density will need to be determined for those fungi chosen for commercial use. It is not possible to generalize, as several studies have shown that different tree-fungus associations require different inoculation densities for the maximum production of ectomycorrhizas (Marx 1976; Castellano et al. 1984; Parladé et al. 1996).

Further work is required for those inoculation candidates, species of *Laccaria*, *Descolea*, *Scleroderma* and *Pisolithus*, with potential for commercial use. Studies are currently in progress to establish the optimal inoculation density for each fungus under nursery conditions, to define storage conditions for spore survival, and to establish the effectiveness of these fungi to produce ectomycorrhizal seedlings in eucalypt nurseries in Australia and Asian countries.

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References

Agerer R (1991) Characterization of ectomycorrhiza. In: Noris JR, Read DJ, Varma AK (eds) Methods in microbiology, vol 23. Techniques for the study of mycorrhiza. Academic, London, pp 25–73

Alvarez IF, Trappe JM (1983) Effects of application rates and cold soaking pretreatment of *Pisolithus* spores on effectiveness as nursery inoculum on western conifers. Can J For Res 13:533–537

Bougher NL, Malajczuk N (1985) A new species of *Descolea* (Agaricales) from Western Australia, and aspects of its ectomycorrhizal status. Aust J Bot 33:619–627

Bougher NL, Grove TS, Malajczuk N (1990) Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. New Phytol 114:77–85

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

Burgess TI, Malajczuk N, Grove TS (1993) The ability of 16 ectomycorrhizal fungi to increase growth and phosphorus uptake of *Eucalyptus globulus* Labill. and *E. diversicolor* F. Muell. Plant Soil 153:155–164

Castellano MA (1994) Current status of outplanting studies using ectomycorrhiza-inoculated forest trees. In: Pfleger RF, Linderman RG (eds) Mycorrhizae and plant health. APS, St Paul, Minn, pp 261–281

Castellano MA, Trappe JM (1985) Ectomycorrhizal formation and plantation performance of Douglas-fir nursery stock inoculated with *Rhizopogon* spores. Can J For Res 15:613–617

Castellano MA, Trappe JM, Molina R (1985) Inoculation of container-grown Douglas-fir seedlings with basidiospores of *Rhizopogon vinicolor* and *R. colossus*: effects of fertility and spore application rate. Can J For Res 15:10–13

de la Cruz RE (1990) Current status of nursery and field applications of ectomycorrhizas in the Philippines In: Proceedings of the 8th North American Conference on Mycorrhizas, Jackson, Wyoming, p 75

Dell B, Malajczuk N, Grove TS, Thomson GB (1990) Ectomycorrhiza formation in *Eucalyptus*. IV. Ectomycorrhizas in the sporocarps of the hypogeous fungi M*esophellia* and *Castorium* in eucalypt forests of Western Australia. New Phytol 114:449–456

Le Tacon F, Garbaye J, Bouchard D, Chevalier G, Olivier JM, Guimberteau J, Poitou N, Frochot H (1988) Field results from ectomycorrhizal inoculation in France. In: Lalonde M, Piché Y (eds) Proceedings of Canadian Workshop on Mycorrhizae in Forestry, Quebec, Canada, pp 51–74

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

Maghembe JA, Redhead JF (1984) Growth and ectomycorrhizal development of *Pinus caribaea* seedlings inoculated with basidiospores of *Scleroderma dictyosporum* in fertilized nursery soil in Tanzania. For Ecol Manag 8:211–228

Martinez-Amores E, Valdes M, Quintos M (1991) Seedling growth and ectomycorrhizal colonization of *Pinus patula* and *P. radiata* inoculated with spores of *Helvella lacunosa*, *Russula brevipes* or *Lycoperdon perlatum*. New For 4:237–245

Marx DH (1976) Synthesis of ectomycorrhizae on loblolly pine seedlings with basidiospores of *Pisolithus tinctorius*. For Sci 22:13–20

Marx DH (1991) The practical significance of ectomycorrhizae in forest establishment. In: Ecophysiology of ectomycorrhizae of forest trees. The Marcus Wallenberg Foundation Symposia Proceedings 7, Sweden, pp 54–90

Marx DH, Kenney DS (1982) Production of ectomycorrhizal fungus inoculum. In: Schenck NC (ed) Methods and principles of mycorrhizal research. The American Phytopathological Society, St Paul, Minn, pp 131–146

Marx DH, Jarl K, Ruehle JL, Bell W (1984) Development of *Pisolithus tinctorius* ectomycorrhizae on pine seedlings using basidiospore-encapsulated seeds. For Sci 30:897–907

Parladé J, Pera J, Alvarez IF (1996) Inoculation of containerized *Pseudotsuga menziesii* and *Pinus pinaster* seedlings with spores of five species of ectomycorrhizal fungi. Mycorrhiza 6:237–245

Theodorou C, Bowen GD (1973) Inoculation of seeds and soil with basidiospores of mycorrhizal fungi. Soil Biol Biochem 5:765–771

Thomson BD, Grove TS, Malajczuk N, Hardy GE StJ (1994) The effectiveness of ectomycorrhizal fungi in increasing the growth of *Eucalyptus globulus* Labill. in relation to root colonization and hyphal development in soil. New Phytol 126:517–524

Torres P, Honrubia M (1994) Inoculation of containerized *Pinus halepensis* (Miller) seedlings with basidiospores of *Pisolithus arhizus* (Pers) Rauschert, *Rhizopogon roseolus* (Corda) Th M Fr and *Suillus collinitus* (Fr) O Kuntze. Ann Sci For 51:521–528

Trappe JM (1977) Selection of fungi for ectomycorrhizal inoculation in nurseries. Annu Rev Phytopathol l 15:203–222