New Phytol. (1994), **126**, 517–524

The effectiveness of ectomycorrhizal fungi in increasing the growth of *Eucalyptus globulus* Labill. in relation to root colonization and hyphal development in soil

By B. D. THOMSON[1,*], T. S. GROVE[1], N. MALAJCZUK[1] and G. E. St J. HARDY[2,†]

[1] *CSIRO Division of Forestry, Private Bag, P.O. Wembley, W.A. 6014, Australia*
[2] *Bunnings Treefarms, P.O. Box 444, Manjimup, W.A. 6258, Australia*

(*Received 30 August 1993; accepted 8 November 1993*)

SUMMARY

Forty-seven different isolates of ectomycorrhizal fungi, from 16 different genera, were screened for their effectiveness in increasing the growth of *Eucalyptus globulus* Labill. where supply of P is deficient. Plants were grown in a P-deficient sand, in pots, in a temperature-controlled glasshouse. Seedlings were harvested 63 and 87 d after planting, and were assessed for dry matter production and mycorrhizal colonization. Selected treatments were also assessed for P concentrations in the plant and hyphal development in the soil.

Dry weights of inoculated plants ranged from 50 to 350% of the dry weights of uninoculated plants. Growth increases in response to ectomycorrhizal inoculation corresponded with increased P uptake by the plant. 'Early' colonizing fungal species (*Descolea maculata*, *Hebeloma westraliense*, *Laccaria laccata* and *Pisolithus tinctorius*) were generally more effective in increasing plant growth than 'late' colonizing species (*Cortinarius* spp. and *Hysterangium* spp.), although there was also variation in effectiveness among isolates of the same fungal species. Plant dry weights were positively correlated ($r^2 = 0{\cdot}79$–$0{\cdot}84$) with the length of colonized root, indicating that fungi which colonized roots extensively were the most effective in increasing plant growth. For some fungi, however, plant growth responses to inoculation were not related to colonized root length. These responses could not be related to the development of hyphae in soil by the mycorrhizal fungi.

Key words: Ectomycorrhiza, P nutrition, colonization, external hyphae.

INTRODUCTION

There are numerous reports in the literature of increased tree growth following inoculation with ectomycorrhizal fungi (see Harley & Smith, 1983). This effect is generally attributed to increased P uptake by the mycorrhizal trees (Heinrich & Patrick, 1986; Bougher, Grove & Malajczuk, 1990; Jones, Durall & Tinker, 1990). The basis of this increased P uptake is thought to be the ability of the external hyphae of ectomycorrhizal fungi to extend beyond the zones of P depletion which form around plant roots, and to absorb P rapidly from previously unexplored regions of the soil and translocate it to the plant (Tinker, 1975; Nye & Tinker, 1977).

Despite the likely role external hyphae play in the improved P nutrition of ectomycorrhizal plants, there have been relatively few studies which have quantified hyphal development in soil by ectomycorrhizal fungi (Jones *et al.*, 1990; Colpaert, Van Assche & Luijtens, 1992; Wallander & Nylund, 1992). Differences between ectomycorrhizal fungi in their ability to increase plant growth at a deficient supply of P (effectiveness) have been related to differences between fungi in their ability to colonize plant roots (Cline & Reid, 1982; Heinrich & Patrick, 1986), but in other instances there has been a poor correlation

* Present address: Centre for Legumes in Mediterranean Agriculture, The University of Western Australia, Nedlands, W.A. 6009, Australia.
† Present address: School of Biological & Environmental Sciences, Murdoch University, Murdoch, W.A. 6150, Australia.

between plant growth and root colonization (Jones *et al.*, 1990). In the study of Jones *et al.* (1990), the most effective fungus in increasing plant growth developed the greatest length of hyphae in soil. In a similar study with vesicular–arbuscular (VA) mycorrhizal fungi, Graham, Linderman & Menge (1982) related the effectiveness of eight different VA mycorrhizal fungi to the amount of hyphae in soil rather than to the length of colonized root.

The primary aim of the present study was to screen a broad range of ectomycorrhizal fungi for their ability to increase the uptake of P and growth of *Eucalyptus globulus* Labill. Plant growth responses to inoculation were also related to root colonization and hyphal development in soil in an attempt to develop a physiological/morphological basis for the screening and selection of inoculant ectomycorrhizal fungi.

MATERIALS AND METHODS

Soil preparation

A yellow sand with a pH of 6·2 in water, a Bray-extractable P of less than 2 mg kg^{-1} sand, and a moderate P-adsorption capacity, was collected from the Spearwood dune system north of Perth, Western Australia. This sand was sieved through a 2 mm stainless steel mesh, steam sterilized at 70 °C for 1·5 h and then oven-dried at 70 °C. Two kilograms of dried soil was added to plastic pots (14 cm diameter) lined with plastic bags. Basal nutrients (minus P) were applied in solution to the surface of each pot at rates sufficient for maximum plant growth (mg kg^{-1} sand): $CaSO_4.2H_2O$, 51·5; K_2SO_4, 111·6; $MgSO_4.7H_2O$, 33·7; $MnSO_4.4H_2O$, 16·9; $CuSO_4.5H_2O$, 8·2; $ZnSO_4.7H_2O$, 9·2; $CoCl_2.6H_2O$, 0·34; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0·46; $Na_2B_4O_7.10H_2O$,1·10. When these solutions had air-dried, 80 mg of $Ca(H_2PO_4)_2.H_2O$ was applied as a powder to each pot [sufficient for 15 % of maximum plant growth (Bougher *et al.*, 1990)] and all nutrients were then thoroughly mixed through the sand. Pots were watered to container capacity (10 % w/w) with deionized water. The sand surface of each pot was covered with aluminium insulation foil to reduce water loss and algal growth, and pots were left to equilibrate for one week before planting.

Seed germination of fungal inoculation

Seeds of *E. globulus* were sieved to uniform size (1·4–1·6 mm diameter) and surface sterilized by shaking them in a solution of 10 % NaOCl with a trace of Tween 80 (1–2 drops l^{-1}) for 15 min. After five rinses in sterile deionized water, seeds were transferred aseptically to plates containing agar with 500 μM $CaSO_4$ and 3 μM H_3BO_3, and incubated in the dark at 25 °C for 5 d. Germinated seeds which were free from contamination were transferred aseptically to polycarbonate jars (65 mm diam, 80 mm high) containing 20 to 30-d-old pure cultures of one of 47 different ectomycorrhizal fungi (Table 1) actively growing on modified Melin–Norkrans (MMN) medium (Marx, 1969). Uninoculated control seedlings were produced in jars without mycorrhizal cultures. The jars were incubated in a growth cabinet (22–23 °C, 16 h photoperiod, 350 μE m^{-2} s^{-1}) until the roots of seedlings were covered with mycelia (10–14 d).

Table 1. *Genus, species (where identified), isolate and state of origin (WA, Western Australia; VIC, Victoria; TAS, Tasmania) of the ectomycorrhizal fungi which were tested for their ability to increase the growth of* Eucalyptus globulus *seedlings*

Genus	Species	Isolate	Origin
Protubera	sp.	A	WA
Rhizopogon-like	sp.	A	VIC
Chondrogaster	sp.	A	TAS
Cortinarius	sp.	A	TAS
Cortinarius	sp.	B	TAS
Cortinarius	sp.	C	VIC
Cortinarius	sp.	D	TAS
Cortinarius	sp.	E	TAS
Cortinarius	sp.	F	TAS
Cortinarius	sp.	G	TAS
Hysterangium	sp.	A	WA
Hysterangium	sp.	B	WA
Hysterangium	sp.	C	TAS
Hysterangium	sp.	D	TAS
Amanita	*xanthocephla*	A	WA
Amanita	*umbrenella*	B	TAS
Amanita	*xanthocephla*	C	WA
Amanita	*xanthocephla*	D	WA
Hydnangium	*carneum*	A	TAS
Hydnangium	*carneum*	B	TAS
Hydnangium	*archeri*	C	TAS
Zelleromyces	sp.	A	TAS
Zelleromyces	sp.	B	TAS
Zelleromyces	sp.	C	TAS
Hymenogaster	*viscidus*	A	TAS
Hymenogaster	*albus*	B	TAS
Hymenogaster	*zeylanicus*	C	WA
Hymenogaster	*zeylanicus*	D	WA
Thaxterogaster	sp.	A	TAS
Thaxterogaster	sp.	B	TAS
Scleroderma	*verrucosum*	A	TAS
Scleroderma	*verrucosum*	B	TAS
Scleroderma	*paradoxum*	C	TAS
Scleroderma	*paradoxum*	D	WA
Scleroderma	*verrucosum*	E	TAS
Setchelliogaster	sp.	A	TAS
Pisolithus	*tinctorius*	A	WA
Pisolithus	*tinctorius*	B	WA
Pisolithus	*tinctorius*	C	WA
Pisolithus	*tinctorius*	D	WA
Laccaria	*laccata*	A	TAS
Laccaria	*laccata*	B	TAS
Laccaria	*laccata*	C	VIC
Laccaria	*laccata*	D	TAS
Hebeloma	*westraliense*	A	WA
Descolea	*maculata*	A	WA
Descolea	*maculata*	B	WA

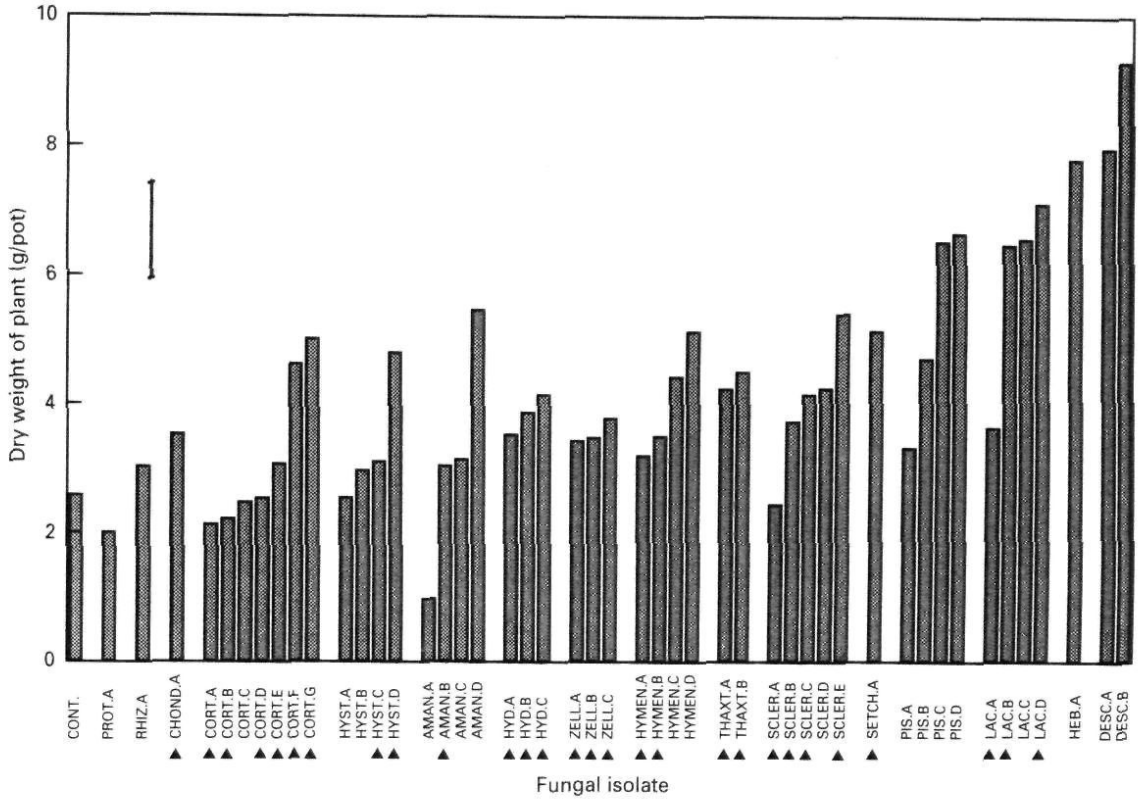

Figure 1. Dry weights at 87 d of *Eucalyptus globulus* seedlings inoculated with selected ectomycorrhizal fungi (PROT., *Protubera*; RHIZ., *Rhizopogon*-like; CHOND., *Chondrogaster*; CORT., *Cortinarius*; HYST., *Hysterangium*; AMAN., *Amanita*; HYD., *Hydnangium*; ZELL., *Zelleromyces*; HYMEN., *Hymenogaster*; THAXT., *Thaxterogaster*; SCLER., *Scleroderma*; SETCH., *Setchelliogaster*; PIS., *Pisolithus*; LAC., *Laccaria*; HEB., *Hebeloma*; DESC., *Descolea*). Tasmanian isolates are indicated with a triangle. Control seedlings (CONT.) were uninoculated. Vertical bar represents 2 × SEM.

Transplanting and maintenance

There were three replicates of each fungal treatment and two harvests, requiring a total of six pots per fungus. Four seedlings were transplanted into each pot through holes in the aluminium foil. A small plug of inoculum (approximately 0·2 g agar and fungal colony) was also placed with the seedling roots at planting to ensure maximum success of the inoculation procedure. Uninoculated control seedlings received an equivalent quantity of agar without mycorrhizal fungi. Pots were placed on benches in an air-cooled glasshouse (air temperatures ranged from 10 °C to 30 °C) and soil moisture maintained by watering to weight. Following establishment, seedlings were thinned to two per pot. Nitrogen (18·0 mg kg^{-1} sand) was added fortnightly as NH_4NO_3 in solution so that a total of 126·0 mg N kg^{-1} sand was applied to each pot.

Plant harvest

Seedlings were harvested 63 and 87 d after transplanting. At each harvest, shoots were cut at the sand surface, oven-dried at 70 °C and weighed. For selected treatments (see Table 4), three soil cores (13 mm diam, 110 mm deep) were taken at random from the pot, and these soil cores bulked and stored in a freezer at −15 °C until they could be assessed for length of external hyphae (see below). The roots in all pots were washed free of sand and divided into coarse and fine root (less than 0·2 mm diam) fractions. The fine root fraction was cut into 10 mm segments and a subsample taken for assessing mycorrhizal colonization. This subsample was stained with lactic glycerol Trypan blue for at least 7 d, after which fine root length and mycorrhizal fine root length was assessed under a binocular microscope using the line intercept method of Newman (1966). Mycorrhizas appeared as short, thickened, dark blue roots. The remaining fine root and the coarse root fractions were dried in an oven at 70 °C and weighed. For those treatments which were assessed for external hyphae, the dried fine roots, coarse roots and shoots were ground, digested in H_2SO_4/H_2O_2 and analyzed for P according to the method of Salt (1968).

External hyphae

The length of external hyphae in bulked soil samples was determined after the hyphae had been extracted

using a modification of a membrane filter technique (Abbott, Robson & De Boer, 1984). A 2 g subsample was taken from each soil sample and blended with 500 ml of deionized water for 20 s in a kitchen blender. This solution was washed through a 500 μm mesh sieve into a 1 l beaker and left to stand for 60 s. The washings were decanted into another 1 l beaker and gently stirred while two 25 ml aliquots were removed. These aliquots were filtered under suction through a 1·2 μm millipore filter. The filter was cut

Table 2. *Partitioning of dry matter between roots and shoots and between fine roots and coarse roots, length of fine root, and length of mycorrhizal fine root (expressed as m/pot and as a percentage) of* Eucalyptus globulus *seedlings inoculated with selected ectomycorrhizal fungi. Control seedlings were uninoculated. Plants were harvested 87 d after planting*

Genus	Isolate	Root/shoot ratio	Fine root/ coarse root ratio	Fine root length (m/pot)	Fine root length colonized (m/pot)	Fine root length colonized (%)
	Control	$0{\cdot}41^{b-e}$	$0{\cdot}37^{a}$	$55{\cdot}5^{a-c}$	$4{\cdot}5^{ab}$	9^{a-c}
Protubera	A	$0{\cdot}34^{a-d}$	$0{\cdot}50^{ab}$	$44{\cdot}4^{ab}$	$2{\cdot}3^{a}$	4^{ab}
Rhizopogon-like	A	$0{\cdot}28^{a}$	$0{\cdot}38^{a}$	$45{\cdot}3^{ab}$	$2{\cdot}9^{a}$	9^{a-c}
Chondrogaster	A	$0{\cdot}42^{c-e}$	$0{\cdot}96^{b-d}$	$86{\cdot}3^{b-g}$	$12{\cdot}1^{ab}$	18^{a-e}
Cortinarius	A	$0{\cdot}37^{a-e}$	$0{\cdot}52^{ab}$	$53{\cdot}5^{a-c}$	$5{\cdot}0^{ab}$	11^{a-e}
	B	$0{\cdot}37^{a-e}$	$0{\cdot}51^{ab}$	$57{\cdot}2^{a-c}$	$5{\cdot}0^{ab}$	8^{a-c}
	C	$0{\cdot}31^{a-c}$	$0{\cdot}54^{ab}$	$49{\cdot}7^{ab}$	$4{\cdot}4^{ab}$	11^{a-e}
	D	$0{\cdot}33^{a-d}$	$0{\cdot}56^{a-c}$	$66{\cdot}8^{a-e}$	$6{\cdot}2^{ab}$	9^{a-c}
	E	$0{\cdot}37^{a-e}$	$0{\cdot}51^{ab}$	$71{\cdot}7^{a-e}$	$7{\cdot}8^{ab}$	13^{a-e}
	F	$0{\cdot}32^{a-c}$	$0{\cdot}50^{ab}$	$108{\cdot}9^{c-j}$	$11{\cdot}7^{ab}$	10^{a-d}
	G	$0{\cdot}32^{a-c}$	$0{\cdot}34^{a}$	$82{\cdot}1^{b-f}$	$3{\cdot}5^{ab}$	6^{a-c}
Hysterangium	A	$0{\cdot}32^{a-c}$	$0{\cdot}55^{a-c}$	$68{\cdot}8^{a-e}$	$5{\cdot}7^{ab}$	7^{a-c}
	B	$0{\cdot}38^{a-e}$	$0{\cdot}48^{ab}$	$52{\cdot}4^{a-c}$	$3{\cdot}1^{ab}$	6^{a-c}
	C	$0{\cdot}33^{a-d}$	$0{\cdot}56^{a-c}$	$65{\cdot}3^{a-e}$	$3{\cdot}3^{ab}$	6^{a-c}
	D	$0{\cdot}35^{a-d}$	$0{\cdot}49^{ab}$	$94{\cdot}7^{b-h}$	$10{\cdot}5^{ab}$	10^{a-d}
Amanita	A	$0{\cdot}41^{b-e}$	$0{\cdot}67^{a-c}$	$18{\cdot}9^{a}$	$0{\cdot}6^{a}$	2^{a}
	B	$0{\cdot}33^{a-d}$	$0{\cdot}51^{ab}$	$88{\cdot}9^{b-h}$	$2{\cdot}6^{a}$	3^{ab}
	C	$0{\cdot}38^{a-e}$	$0{\cdot}43^{ab}$	$67{\cdot}7^{a-e}$	$4{\cdot}9^{ab}$	7^{a-c}
	D	$0{\cdot}33^{a-d}$	$0{\cdot}47^{ab}$	$123{\cdot}7^{e-k}$	$12{\cdot}3^{ab}$	10^{a-d}
Hydnangium	A	$0{\cdot}40^{b-e}$	$0{\cdot}81^{a-c}$	$90{\cdot}7^{b-h}$	$28{\cdot}4^{a-c}$	30^{d-g}
	B	$0{\cdot}37^{a-e}$	$0{\cdot}71^{a-c}$	$95{\cdot}1^{b-h}$	$34{\cdot}9^{b-d}$	37^{f-h}
	C	$0{\cdot}38^{a-e}$	$0{\cdot}37^{a}$	$97{\cdot}4^{b-h}$	$5{\cdot}7^{ab}$	4^{ab}
Zelleromyces	A	$0{\cdot}30^{ab}$	$0{\cdot}45^{ab}$	$64{\cdot}1^{a-e}$	$0{\cdot}6^{a}$	1^{a}
	B	$0{\cdot}31^{a-c}$	$0{\cdot}57^{a-c}$	$93{\cdot}7^{b-h}$	$3{\cdot}3^{ab}$	6^{a-c}
	C	$0{\cdot}31^{a-c}$	$0{\cdot}52^{ab}$	$93{\cdot}0^{b-h}$	$10{\cdot}6^{ab}$	13^{a-e}
Hymenogaster	A	$0{\cdot}32^{a-c}$	$0{\cdot}62^{a-c}$	$86{\cdot}2^{b-g}$	$11{\cdot}2^{ab}$	13^{a-e}
	B	$0{\cdot}36^{a-e}$	$0{\cdot}61^{a-c}$	$97{\cdot}1^{b-h}$	$17{\cdot}1^{ab}$	17^{a-e}
	C	$0{\cdot}44^{de}$	$0{\cdot}91^{a-c}$	$112{\cdot}3^{c-j}$	$57{\cdot}9^{de}$	51^{h-j}
	D	$0{\cdot}47^{e}$	$1{\cdot}42^{d}$	$170{\cdot}7^{k}$	$97{\cdot}0^{f}$	55^{h-k}
Thaxterogaster	A	$0{\cdot}35^{a-d}$	$0{\cdot}46^{ab}$	$98{\cdot}6^{b-i}$	$6{\cdot}8^{ab}$	7^{a-c}
	B	$0{\cdot}31^{a-c}$	$0{\cdot}37^{a}$	$83{\cdot}1^{b-f}$	$4{\cdot}0^{ab}$	4^{ab}
Scleroderma	A	$0{\cdot}33^{a-d}$	$0{\cdot}67^{a-c}$	$62{\cdot}2^{a-d}$	$9{\cdot}1^{ab}$	14^{a-e}
	B	$0{\cdot}33^{a-d}$	$0{\cdot}51^{ab}$	$87{\cdot}9^{b-h}$	$6{\cdot}8^{ab}$	7^{a-c}
	C	$0{\cdot}35^{a-d}$	$0{\cdot}71^{a-c}$	$100{\cdot}8^{b-j}$	$23{\cdot}5^{a-c}$	24^{c-f}
	D	$0{\cdot}36^{a-e}$	$0{\cdot}63^{a-c}$	$109{\cdot}6^{c-j}$	$23{\cdot}4^{a-c}$	23^{b-f}
	E	$0{\cdot}44^{de}$	$1{\cdot}12^{cd}$	$144{\cdot}4^{g-k}$	$74{\cdot}9^{ef}$	51^{h-j}
Setchelliogaster	A	$0{\cdot}39^{a-e}$	$0{\cdot}65^{a-c}$	$119{\cdot}9^{d-k}$	$57{\cdot}2^{de}$	46^{g-j}
Pisolithus	A	$0{\cdot}31^{a-c}$	$0{\cdot}39^{ab}$	$62{\cdot}7^{a-d}$	$3{\cdot}6^{ab}$	8^{a-c}
	B	$0{\cdot}38^{a-e}$	$0{\cdot}80^{a-c}$	$96{\cdot}5^{b-h}$	$34{\cdot}8^{b-d}$	37^{f-h}
	C	$0{\cdot}36^{a-e}$	$0{\cdot}85^{a-c}$	$138{\cdot}9^{f-k}$	$58{\cdot}4^{de}$	42^{f-i}
	D	$0{\cdot}36^{a-e}$	$0{\cdot}67^{a-c}$	$155{\cdot}6^{i-k}$	$51{\cdot}1^{c-e}$	31^{e-g}
Laccaria	A	$0{\cdot}33^{a-d}$	$0{\cdot}45^{ab}$	$70{\cdot}1^{a-e}$	$17{\cdot}4^{ab}$	25^{c-f}
	B	$0{\cdot}33^{a-d}$	$0{\cdot}81^{a-c}$	$133{\cdot}4^{f-k}$	$71{\cdot}1^{ef}$	54^{h-k}
	C	$0{\cdot}39^{a-e}$	$0{\cdot}52^{ab}$	$158{\cdot}4^{jk}$	$96{\cdot}4^{f}$	62^{i-k}
	D	$0{\cdot}38^{a-e}$	$0{\cdot}65^{a-c}$	$147{\cdot}7^{h-k}$	$63{\cdot}1^{de}$	43^{f-i}
Hebeloma	A	$0{\cdot}31^{a-c}$	$0{\cdot}60^{a-c}$	$174{\cdot}7^{k}$	$94{\cdot}7^{f}$	53^{h-k}
Descolea	A	$0{\cdot}41^{b-e}$	$0{\cdot}83^{a-c}$	$257{\cdot}8^{l}$	$169{\cdot}5^{g}$	64^{jk}
	B	$0{\cdot}40^{b-e}$	$0{\cdot}74^{a-c}$	$257{\cdot}4^{l}$	$178{\cdot}7^{g}$	70^{k}

Values within columns followed by the same letter are not significantly different ($P < 0{\cdot}05$; Duncan's New Multiple Range Test).

into halves and these were placed onto separate microscope slides and stained with lactic glycerol Trypan blue overnight. Each slide was covered with a cover slip and the length of hyphae on the filter paper determined using a grid intersection method (Newman, 1966) at 250× magnification. Thirty random fields of view were examined on each filter half.

RESULTS

Dry weights

Dry weights of inoculated plants at day 87 ranged from −50% to 350% of the dry weights of uninoculated plants, depending on the fungal isolate (Fig. 1). Plants also responded to inoculation at 63 d, but at this earlier stage of growth responses were much smaller than those observed at the later harvest (data not presented).

Some fungal species (*Descolea maculata*, *Hebeloma westraliense*, *Laccaria laccata* and *Pisolithus tinctorius*) appeared to be more effective in increasing plant growth than others, although there was also marked variation between isolates of the same species. Isolates of ectomycorrhizal fungi which were collected from Tasmania were no more effective in increasing the growth of *E. globulus* (which is native to Tasmania) than isolates collected from other regions of Australia (Fig. 1).

There was little effect of ectomycorrhizal inoculation on the partitioning of dry matter between roots and shoots at either harvest (Harvest 2 data presented in Table 2). For most treatments, there was also little effect of inoculation on the ratio of fine roots to coarse roots at either harvest (Harvest 2 data presented in Table 2). Inoculated plants which were colonized extensively had a higher fine root/coarse root ratio than uninoculated plants in some instances.

Mycorrhizal colonization

Inoculated plants were generally ectomycorrhizal by 87 d, although in many cases 10% or less of their fine root length was colonized (Table 2). This level of colonization is comparable with that observed for the roots of uninoculated control plants and, in some cases, could therefore have been a contaminant in these treatments.

There was large variation, both between fungal species and between isolates of the same species, in the ability of fungi to colonize plant roots (Harvest 2 data presented in Table 2). The most effective ectomycorrhizal fungi were generally those which colonized roots extensively, so that there were positive relationships ($r^2 = 0{\cdot}79$–$0{\cdot}84$) between mycorrhizal colonization and plant growth (Fig. 2). However, for some fungi plant growth responses to inoculation could not be related to the length of colonized root. For example, some *Cortinarius*, *Hysterangium* and *Amanita* isolates increased plant growth yet did not appear to colonize roots extensively. Conversely, two *Hydnangium* isolates developed extensive mycorrhizas yet failed to increase plant growth.

P uptake

For treatments which were analyzed for P, growth increases in response to ectomycorrhizal inoculation corresponded with a higher P content in the plant at

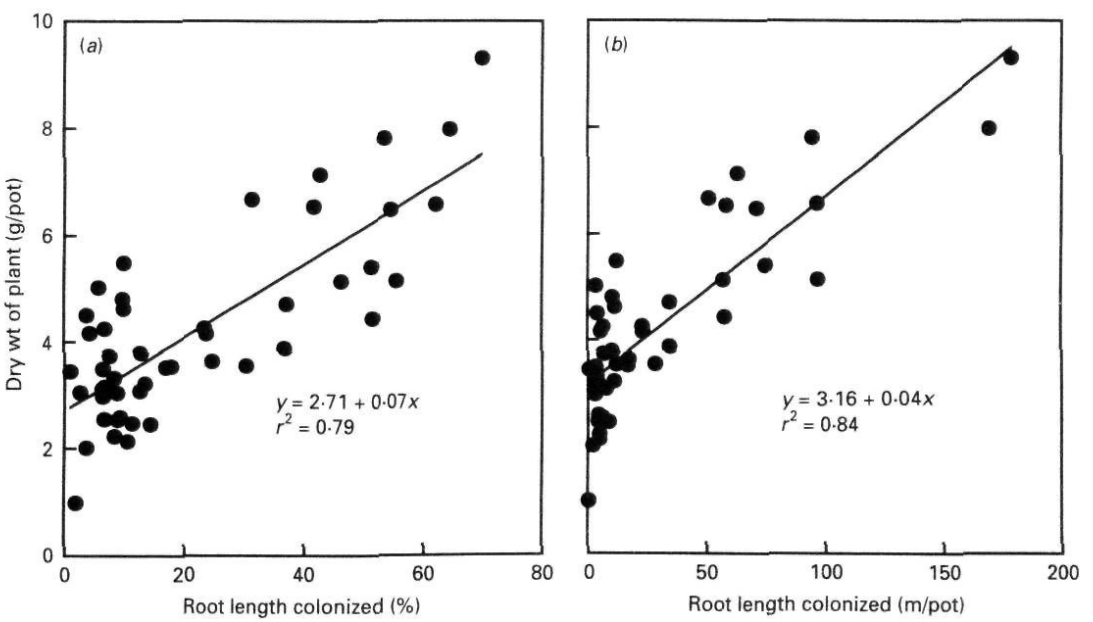

Figure 2. Relationships at 87 d between the length of colonized fine root expressed as a percentage (*a*) and as m/pot (*b*), and the dry weights of inoculated plants.

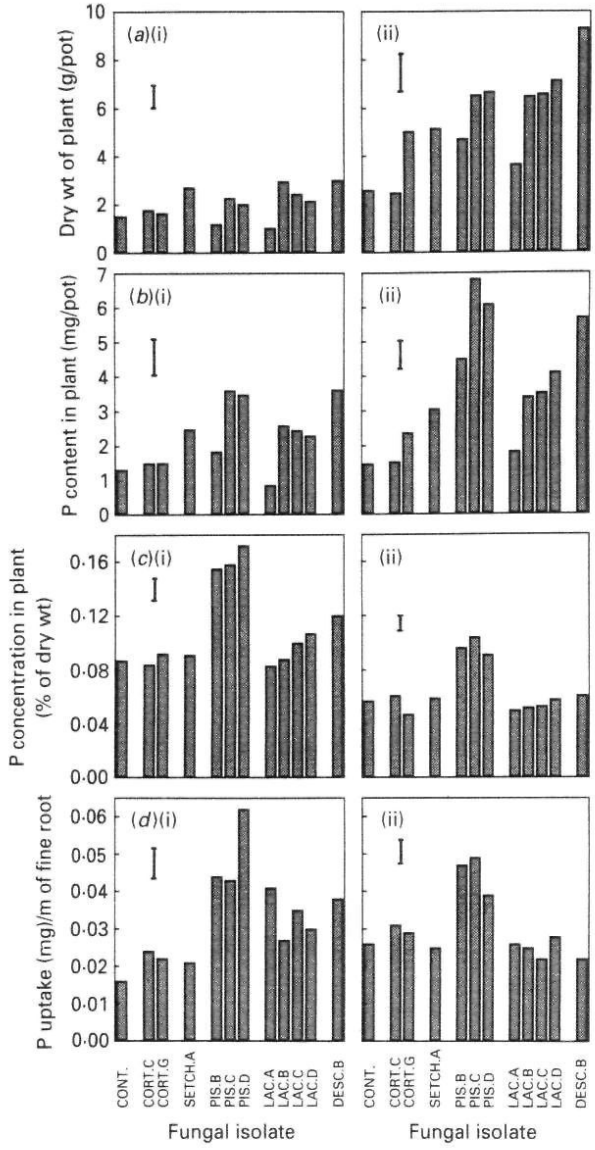

Figure 3. Dry weights (*a*), P contents (*b*), P concentrations (*c*), and P uptake per m of fine root (*d*) of *Eucalyptus globulus* seedlings inoculated with selected ectomycorrhizal fungi (CORT., *Cortinarius*; SETCH., *Setchelliogaster*; PIS., *Pisolithus*; LAC., *Laccaria*; DESC., *Descolea*). Control seedlings (CONT.) were uninoculated. Plants were harvested (i) 63 and (ii) 87 d after planting. Vertical bars represent 2 × SEM.

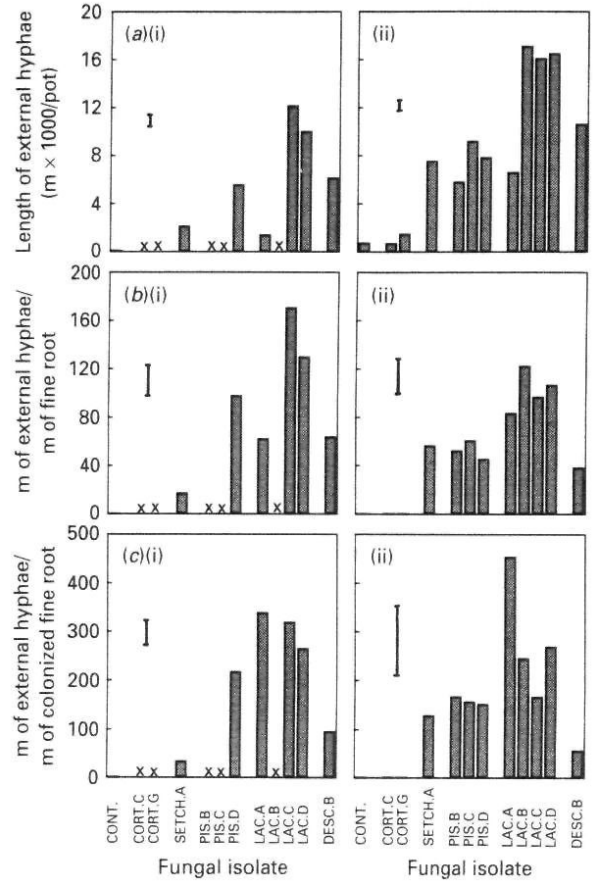

Figure 4. Length of external hyphae per pot (*a*), per m of fine root (*b*) and per m of colonized fine root (*c*) of *Eucalyptus globulus* seedlings inoculated with selected ectomycorrhizal fungi (CORT., *Cortinarius*; SETCH., *Setchelliogaster*; PIS., *Pisolithus*; LAC., *Laccaria*; DESC., *Descolea*). Control seedlings (CONT.) were uninoculated. External hyphae and root colonization in uninoculated pots were subtracted from inoculated pots in the calculation of (*b*) and (*c*). X marks where hyphae in soil were not measured. Plants were harvested (i) 63 and (ii) 87 d after planting. Vertical bars represent 2 × SEM.

both harvests, with an increased P uptake per m of fine root at 63 d, and to a lesser extent with a higher P concentration in the plant at 63 d (Fig. 3). Concentrations of P in plants inoculated with isolates of *Pisolithus tinctorius* were almost double those of other plants at 63 and 87 d. Phosphorus uptake per m of fine root by plants inoculated with the *P. tinctorius* isolates was also almost double that of other plants at day 87.

Plants which responded positively to ectomycorrhizal inoculation retained a greater proportion of absorbed P in their roots than uninoculated plants at both harvests, despite having similar root to shoot dry weight ratios (Table 3). Furthermore, the majority of this root P was associated with the fine root fraction (Table 3). This effect was particularly evident for plants inoculated with the *P. tinctorius* isolates.

External hyphae

There was large inter- and intraspecific variation in the development of external hyphae by the ectomycorrhizal fungi (Fig. 4). The *L. laccata* isolates generally formed a greater length of hyphae in soil than the other fungal isolates at 63 and 87 d, which corresponds with a greater length of external hyphae per m of fine root and per m of colonized fine root for these fungi (Fig. 4). *D. maculata* formed the least amount of external hyphae per m of colonized fine root at 87 d.

For treatments which were assessed for external

Table 3. *Partitioning of P between roots and shoots, and between fine roots and coarse roots, of* Eucalyptus globulus *seedlings inoculated with selected ectomycorrhizal fungi. Control seedlings were uninoculated. Plants were harvested 63 (D63) and 87 (D87) days after planting*

		P in roots/ P in shoots		P in fine roots/ P in coarse roots	
Genus	Isolate	D63	D87	D63	D87
	Control	$0{\cdot}33^{a}$	$0{\cdot}37^{ab}$	$0{\cdot}89^{a}$	$0{\cdot}66^{a}$
Cortinarius	C	$0{\cdot}33^{a}$	$0{\cdot}29^{a}$	$0{\cdot}93^{a}$	$1{\cdot}09^{a-c}$
	G	$0{\cdot}32^{a}$	$0{\cdot}30^{a}$	$1{\cdot}20^{a}$	$0{\cdot}71^{a}$
Setchelliogaster	A	$0{\cdot}57^{a-c}$	$0{\cdot}49^{b-d}$	$2{\cdot}95^{b}$	$1{\cdot}65^{c-e}$
Pisolithus	B	$0{\cdot}68^{bc}$	$0{\cdot}73^{f}$	$4{\cdot}31^{c}$	$2{\cdot}82^{f}$
	C	$0{\cdot}73^{c}$	$0{\cdot}98^{g}$	$4{\cdot}84^{c}$	$3{\cdot}73^{g}$
	D	$0{\cdot}70^{bc}$	$0{\cdot}63^{d-f}$	$4{\cdot}46^{c}$	$2{\cdot}16^{e}$
Laccaria	A	$0{\cdot}42^{ab}$	$0{\cdot}42^{a-c}$	$0{\cdot}80^{a}$	$0{\cdot}88^{ab}$
	B	$0{\cdot}32^{a}$	$0{\cdot}56^{c-e}$	$0{\cdot}64^{a}$	$1{\cdot}41^{b-d}$
	C	$0{\cdot}76^{c}$	$0{\cdot}63^{d-f}$	$2{\cdot}48^{b}$	$1{\cdot}11^{a-c}$
	D	$0{\cdot}55^{a-c}$	$0{\cdot}57^{c-e}$	$2{\cdot}30^{b}$	$1{\cdot}27^{a-d}$
Descolea	B	$0{\cdot}60^{bc}$	$0{\cdot}69^{ef}$	$4{\cdot}17^{c}$	$1{\cdot}85^{de}$

Values within columns followed by the same letter are not significantly different ($P < 0{\cdot}05$; Duncan's New Multiple Range Test).

hyphae, plant growth responses to ectomycorrhizal inoculation at 87 days did not relate more closely with the length of hyphae in soil ($r^2 = 0{\cdot}50$) than with the length of colonized fine root expressed as either m/pot ($r^2 = 0{\cdot}77$) or as a percentage ($r^2 = 0{\cdot}69$). Furthermore, differences in the P content and the P uptake per m of fine root of plants at 87 d (which are perhaps more direct measures of the effectiveness of ectomycorrhizal fungi in increasing P uptake) correlated poorly with the length of hyphae in soil ($r^2 = 0{\cdot}18$ and $0{\cdot}05$ respectively), the length of external hyphae per m of fine root ($r^2 = 0{\cdot}30$ and $0{\cdot}17$ respectively), and the length of external hyphae per m of colonized fine root ($r^2 = 0{\cdot}44$ and $0{\cdot}03$ respectively) for these fungi. The ability of a *Cortinarius* sp. isolate (CORT.G) to increase plant growth without colonizing roots extensively could not be related to the development of hyphae in soil by this fungus.

DISCUSSION

We tested a large number of ectomycorrhizal fungi for their ability to increase the growth of *E. globulus* at a deficient supply of P. Growth responses to inoculation ranged from $-50\,\%$ to $350\,\%$ of uninoculated controls, which is comparable with responses observed for eucalypts in other pot studies (Heinrich & Patrick, 1986; Bougher *et al.*, 1990). The increased growth of inoculated plants could be attributed to increased P uptake by the plant, and this was reflected in a higher P content, an increased P uptake per m of fine root and in some instances a higher P concentration in mycorrhizal plants (cf. Heinrich & Patrick, 1986; Bougher *et al.*, 1990; Jones *et al.*, 1990). Growth depressions following mycorrhizal inoculation are generally attributed to the carbohydrate drain of the mycorrhizal fungus (Abbott & Robson, 1984).

The ectomycorrhizal fungi which were most effective in increasing the uptake of P and growth of *E. globulus* were those which colonized roots extensively (isolates of *D. maculata*, *H. westraliense*, *L. laccata* and *P. tinctorius*). These fungal species have been identified as early colonizers of eucalypts in the field (Gardner & Malajczuk, 1988) and may be the most appropriate species for increasing the growth of plantation eucalypts after outplanting. We are currently testing this theory in the field. The large variation in effectiveness which occurred between isolates of the same fungal species indicates the gains that can also be made from screening and selecting inoculant ectomycorrhizal fungi within a species. We compared the effectiveness of mycorrhizal isolates collected from Tasmania (*E. globulus* is native to Tasmania) with isolates collected from other regions of Australia, but there appeared to be no additional benefit to the plant in inoculating with these Tasmanian isolates.

Growth responses of *E. globulus* to inoculation with ectomycorrhizal fungi were positively correlated with colonized root length. A similar relationship between plant growth and ectomycorrhizal colonization has been observed by others (Cline & Reid, 1982; Heinrich & Patrick, 1986), and implies that ectomycorrhizal fungi can be screened for effectiveness on the basis of their ability to colonize plant roots. Not all studies, however, have been able to relate the effectiveness of mycorrhizal

fungi to their colonization capacity (Graham *et al.*, 1982; Jones *et al.*, 1990). In our study also, differences in effectiveness between fungi could not always be related to colonized root length. Jones *et al.* (1990) attributed differences in the effectiveness of two ectomycorrhizal fungi which colonized the same length of root, to differences in the development of hyphae in soil by these fungi. We related the growth of plants to hyphal development in soil, but found that the most effective fungus in increasing plant growth (*D. maculata*) formed the least amount of external hyphae per m of colonized root. Furthermore, isolates of *L. laccata* developed more external hyphae per m of root than other fungal isolates without any apparent additional benefit to the plant. Hyphal development in soil therefore appeared to be a poor indicator of mycorrhizal effectiveness. The spread of hyphae in soil (Jakobsen, Abbott & Robson, 1992) and the proportion of metabolically active hyphae in soil (Hamel, Fyles & Smith, 1990) may be better indicators of mycorrhizal effectiveness than total hyphal length (measured in our study), and these possibilities need to be tested. Other functional differences between ectomycorrhizal fungi may also affect their ability to increase plant growth. For example, plants inoculated with isolates of *P. tinctorius* retained a greater proportion of P in their roots than plants inoculated with other isolates. This storage of P may be an important attribute in environments where the growth period is seasonal or intermittent. Further work is required to identify characteristics of ectomycorrhizal fungi which can be used in the screening and selection of inoculant fungi.

Our observation that *L. laccata* isolates formed more external hyphae per unit of colonized root than other fungal species is consistent with the results of Jones *et al.* (1990) and Wallander & Nylund (1992) who also demonstrated that *Laccaria* spp. tend to form more hyphae in soil than other ectomycorrhizal fungi.

ACKNOWLEDGEMENTS

We would like to thank Janine Catchpole for excellent technical assistance. We are also grateful to Bunnings Treefarms Pty Ltd for providing financial support for this research.

REFERENCES

Abbott LK, Robson AD. 1984. The effect of VA mycorrhizae on plant growth. In: Powell CL, Bagyaraj DJ, eds. *VA mycorrhiza*. Boca Raton, Florida: CRC Press, 113–130.

Abbott LK, Robson AD, De Boer G. 1984. The effect of phosphorus on the formation of hyphae in soil by the vesicular–arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *New Phytologist* **97**: 437–446.

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 Phytologist* **114**: 77–85.

Cline ML, Reid CPP. 1982. Seed source and mycorrhizal fungal effects on growth of containerized *Pinus contorta* and *Pinus ponderosa* seedlings. *Forest Science* **28**: 237–250.

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

Gardner JH, Malajczuk N. 1988. Recolonisation of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. *Forest Ecology & Management* **24**: 27–42.

Graham JH, Linderman RG, Menge JA. 1982. Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to root colonization and growth of troyer citrange. *New Phytologist* **91**: 183–189.

Hamel C, Fyles H, Smith DL. 1990. Measurement of development of endomycorrhizal mycelium using three different vital stains. *New Phytologist* **115**: 297–302.

Harley JL, Smith SE. 1983. *Mycorrhizal symbiosis*. London: Academic Press.

Heinrich PA, Patrick JW. 1986. Phosphorus acquisition in the soil-root system of *Eucalyptus pilularis* Smith seedlings. II. The effect of ectomycorrhizas on seedling phosphorus and dry weight acquisition. *Australian Journal of Botany* **34**: 445–454.

Jakobsen I, Abbott LK, Robson AD. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* **120**: 371–380.

Jones MD, Durall DM, Tinker PB. 1990. Phosphorus relationships and production of extramatrical hyphae by two types of willow ectomycorrhizas at different soil phosphorus levels. *New Phytologist* **115**, 259–267.

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.

Newman EI. 1966. A method for estimating the total length of a root in a sample. *Journal of Applied Ecology* **3**: 139–145.

Nye PH, Tinker PB. 1977. *Solute movement in the soil-root system*. Oxford: Blackwell Scientific Publications.

Salt PD. 1968. The automatic determination of phosphorus in extracts of soils made with 0·5 M sodium hydrogen carbonate and 0·01 M calcium chloride. *Chemistry and Industry* **4**: 584–586.

Tinker PB. 1975. The soil chemistry of phosphorus and mycorrhizal effects on plant growth. In: Sanders FE, Mosse B, Tinker PB, eds. *Endomycorrhizas*. London: Academic Press, 353–372.

Wallander H, Nylund JE. 1992. Effects of excess nitrogen and phosphorus starvation on the extramatrical mycelium of ectomycorrhizas of *Pinus sylvestris* L. *New Phytologist* **120**: 495–503.