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The ability of 16 ectomycorrhizal fungi to increase growth and phosphorus uptake of *Eucalyptus globulus* Labill. and *E. diversicolor* F. Muell.

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Abstract

The effectiveness of 16 fungal isolates in forming ectomycorrhizas and increasing the growth and phosphorus uptake of *Eucalyptus globulus* Labill. and *E. diversicolor* F. Muell. seedlings was examined in the glasshouse. Seedlings were grown in yellow sand at 2 phosphorus levels (4 and 12 mg P kg⁻¹ sand). At the time of harvest (100 days), the non-inoculated seedlings and seedlings inoculated with *Paxillus muelleri* (Berk.) Sacc. and *Cortinarius globuliformis* Bougher had a low level of contamination from an unknown mycorrhizal fungi. Seedlings inoculated with *Thaxterogaster* sp. nov. and *Hysterangium inflatum* Rodway had developed mycorrhizas of the superficial type whereas *Hydnangium carneum* Wallr. in Dietr., *Hymenogaster viscidus* Masee & Rodway, *Hymenogaster zeylanicus* Petch, *Setchelliogaster* sp. nov., *Laccaria laccata* (Scop. ex. Fr.) Berk., *Scleroderma verrucosum* (Vaillant) Pers., *Amanita xanthocephala* (Berk.) Reid & Hilton, *Descolea maculata* Bougher and Malajczuk and *Pisolithus tinctorius* (Pers.) Coker & Couch formed typical pyramidal ectomycorrhizas. The dry weight of non-inoculated and inoculated *E. globulus* seedlings at 12 mg P kg⁻¹ sand did not differ, whereas several isolates caused growth depression of *E. diversicolor*. By contrast, at 4 mg P kg⁻¹ sand growth increases ranged from 0-13 times above that of non-inoculated seedlings. *P. tinctorius* produced the largest growth increase on both eucalypt species. In general, isolates which developed more extensive mycorrhizas on roots produced the largest growth responses to inoculation. Isolates which increased plant growth also increased phosphorus uptake by the plant. Seedlings inoculated with *L. laccata* and *S. verrucosum* retained more phosphorus in their roots than plants inoculated with the other fungal isolates.

Introduction

Eucalypts have ectomycorrhizal fungi associated with their roots. These fungi can improve tree growth in nutrient deficient soils primarily by increasing the uptake of phosphorus (Bougher et al., 1990; Heinrich et al., 1988; Malajczuk et al., 1975). In the low P soils characteristic of much of Australia (Wild, 1957), ectomycorrhizal associations may be essential for the survival and growth of trees in their natural environment.

The numerous species of ectomycorrhizal fungi associated with eucalypts in their natural habitats cover a wide range of environments and host species. Large differences in the effectiveness of specific fungal isolates in stimulating seedling growth have been observed (Bougher et al., 1990; Heinrich and Patrick, 1986; Malajczuk et al., 1975).

Fast growing eucalypt species, in particular karri (*Eucalyptus diversicolor* F. Muell.) and the Tasmanian blue-gum (*E. globulus* F. Muell.), are being planted in the south of Western Australia; the

potential to enhance their productivity by incorporation of superior isolates of ectomycorrhizal fungi in a nursery inoculation program is being examined.

Recent collections of ectomycorrhizal fungi from beneath native and planted stands of *E. globulus* in Tasmania and native stands of *E. diversicolor* in Western Australia have provided cultures of a large number of isolates of different species from a range of fungal genera. This experiment aimed to compare effects on seedling growth and phosphorus uptake of sixteen fungal isolates inoculated onto seedlings of *E. globulus* and *E. diversicolor* grown in the glasshouse.

Materials and methods

Experimental design

In a fully randomised factorial design, two tree species (*E. globulus* and *E. diversicolor*) were inoculated with 16 fungal isolates at two levels of applied phosphorus with 4 replicates of each treatment combination. Details of the origin of the fungal isolates and coding are given in Table 1. Isolates will be referred to by their codes in all Tables and Figures.

Soil preparation

A yellow sand (pH 5.5, Bray extractable P less than 2 mg P kg⁻¹) collected from the Spearwood dune system north of Perth, Western Australia was steam sterilised at 70°C for 1 hour and then air dried before sieving through a 2.5 mm mesh. Plastic pots (140 mm diameter) lined with plastic bags were filled with 2.5 kg of the sand and nutrient solutions applied to the surface at rates of 48mg K, 35.5 mg S, 17.6 mg N, 9 mg Ca, 4 mg Mn, 3.2 mg Mg, 2.3 mg Cu, 2 mg Zn, 0.58 mg Fe, 0.25 mg Mo, 0.12 mg B, 0.08mg Co kg⁻¹ sand. After air drying, these nutrients and the phosphorus treatments (4 and 12mg P kg⁻¹ sand applied as aerophos Ca(H₂PO₄)₂H₂O) were mixed throughout the sand. Pots were watered with 250 ml of deionised water, the surface of each covered with aluminium insulation foil (to prevent water loss and algal growth) and then left to equilibrate for 2-3 weeks before planting the seedlings.

Seed germination and fungal inoculation

Seeds of *E. globulus* and *E. diversicolor* were surface sterilised in a solution of H₂O and absolute alcohol (1:1 v/v) for 12 minutes, rinsed in distilled water and transferred aseptically to tap water agar plates containing 0.2% glucose. Seeds were germinated at 25 °C with 16 hrs of light (100 uEm⁻² s⁻¹)

Table 1. Origin of ectomycorrhizal isolates giving the code, the fungal species, the area where the isolate was found (TAS. = Tasmania, W.A. = Western Australia) and the *Eucalyptus* sp. it was associated with

Code	Species	Site	Vegetation
CORT	<i>Cortinarius globuliformis</i> Bougher	TAS.	<i>E. globulus</i>
PAX	<i>Paxillus muelleri</i> (Berk.) Sacc	W.A.	<i>E. diversicolor</i>
HYST1	<i>Hysterangium inflatum</i> Rodway	W.A.	<i>E. globulus</i>
HYST2	<i>Hysterangium inflatum</i> Rodway	TAS.	<i>E. globulus</i>
THAX	<i>Thaxterogaster</i> sp nov.	TAS.	<i>E. globulus</i>
AMAN	<i>Amanita xanthocephala</i> (Berk.) Reid & Hilton	W.A.	<i>E. diversicolor</i>
HYM1	<i>Hymenogaster zeylanicus</i> Petch	TAS.	<i>E. globulus</i>
HYM2	<i>Hymenogaster viscidus</i> Masee & Rodway	TAS.	<i>E. globulus</i>
HYM3	<i>Hymenogaster zeylanicus</i> Petch	W.A.	<i>E. globulus</i>
SETCH	<i>Setchelliogaster</i> sp nov.	TAS.	<i>E. globulus</i>
DESC	<i>Descolea maculata</i> Bougher & Malajczuk	W.A.	<i>E. marginata</i>
HYDN	<i>Hydnangium carneum</i> Wallr. & Dietr.	TAS.	<i>E. globulus</i>
LAC1	<i>Laccaria laccata</i> (Scop. ex Fr.) Berk.	W.A.	<i>E. marginata</i>
LAC2	<i>Laccaria laccata</i> (Scop. ex Fr.) Berk.	TAS.	<i>E. globulus</i>
SCLER	<i>Scleroderma verrucosum</i> (Vail.) Persoon	W.A.	<i>E. diversicolor</i>
PISOL	<i>Pisolithus tinctorius</i> (Pers.) Coker & Couch	W.A.	<i>E. marginata</i>

per day. Following germination, seedlings were transferred to polycarbonate jars (65 mm diameter, 80 mm height) containing single fungal isolates actively growing on a reduced carbohydrate (0.5% glucose, 0.3% malt extract), modified Melin-Norkans medium (Marx, 1969). Non-inoculated seedlings did not receive inoculum but were otherwise treated the same as the inoculated seedlings. After 7-10 days in a growth cabinet (25°C, 16 hours of light), the seedlings were transplanted into pots in the glasshouse.

Planting and maintenance

Four seedlings were planted into each pot through holes in the aluminium foil. A small inoculum plug of approximately 0.2 g (a plug of agar only for non-inoculated seedlings) was also placed into the hole. Pots were covered with transparent plastic to maintain humidity until the seedlings were established. After 1 week the plastic was removed and the seedlings thinned to 3 per pot. Pots were randomised on benches and the benches rotated every 2-3 days. The pots were maintained with water to 10% w/w when required (every 3 days initially and daily after 8 weeks). Nitrogen, as NH_4NO_3 , was added to the surface of pots fortnightly (17.8 mg N kg^{-1} sand) to give a final level of applied N of 89 mg N kg^{-1} sand. Height measurements were recorded fortnightly beginning 3 weeks after planting.

Harvesting

Seedlings were harvested 100 days after planting. Shoots were cut at soil level, dried in an oven at 70°C and weighed. Roots were washed free from the soil and divided into coarse and fine root fractions (fine roots were less than 0.5 mm diameter). A sub-sample of fine roots was used for the determination of mycorrhizal root length by the method of Newman (1966). Remaining root fractions were dried at 70°C and dry weights recorded. Dried plant fractions of *E. globulus* and *E. diversicolor* from 8 fungal treatments (CONT, PAX, THAX, AMAN, HYM3, SETCH, HYDN, LAC1, SCLER, and PISOL) and the non-inoculated controls (CONT), grown at 4 mg P kg^{-1} sand were ground and the phosphorus concentration determined by automated colorimetric methods (Technicon Industrial Systems, Tarrytown, NY) following Kjeldahl digestion.

Statistical analysis

Treatment effects were assessed by the analysis of variance of raw data and treatment means compared using Duncan's new multiple range test (Duncan, 1955). Statistical analysis of percent mycorrhiza was performed on raw and transformed data. There was no difference in the significance level and therefore all data sets are untransformed. The LSD value is presented in the Figures.

Results

Mycorrhizal development

At time of harvest, non-inoculated seedlings had a low level (less than 2%) of mycorrhiza, indicating contamination by an unknown mycorrhizal fungus. Inoculation with *Paxillus muelleri* (Berk.) Sacc. and *Cortinarius globuliformis* Bougher was unsuccessful and the contaminant mycorrhiza was also present. Seedlings inoculated with *Thaxterogaster* sp nov. and *Hysterangium inflatum* Rodway had developed mycorrhizas of the superficial type (mantle formation, but no Hartig net) whereas *Hydnangium carneum* Wallr. in Dietr., *Hymenogaster viscidus* Masee & Rodway, *Hymenogaster zeylanicus* Petch, *Setchelliogaster* sp nov., *Laccaria laccata* (Scop. ex Fr.) Berk, *Scleroderma verrucosum* (Vaillant) Pers. *Amanita xanthocephala* (Berk.) Reid & Hilton, *Descolea rnaulata*

Bougher and *Pisolithus tinctorius* (Pers.) Coker & Couch formed typical pyramidal ectomycorrhizas on both *E. globulus* and *E. diversicolor* at both P levels.

Percentage of fine root length colonised and the length of fine root colonised by the different fungal isolates varied substantially (Fig. 1A). At 4 mg P kg⁻¹ sand, 0 to 38% of the total fine root length of both *E. globulus* and *E. diversicolor* was mycorrhizal. *H. inflatum* (HYST1), *Setchelliogaster sp nov.* and *H. carneum* developed more extensive colonisation on *E. globulus* than on *E. diversicolor* whereas the reverse was true for *A. xanthocephala* inoculated seedlings.

The increase in the external supply of P from 4 to 12 mg P kg⁻¹ sand generally decreased the proportion of fine roots that were mycorrhizal. The only exception to this was *P. tinctorius*, which developed more extensive colonisation on *E. diversicolor* at 12 mg P kg⁻¹ sand. *H. zeylanicus* (HYM3) and *L. laccata* (LAC2) produced a greater percentage of ectomycorrhizal roots on *E. globulus* than on *E. diversicolor*, while colonisation by *H. zeylanicus* (HYM1) and *P. tinctorius* was greater on *E.*

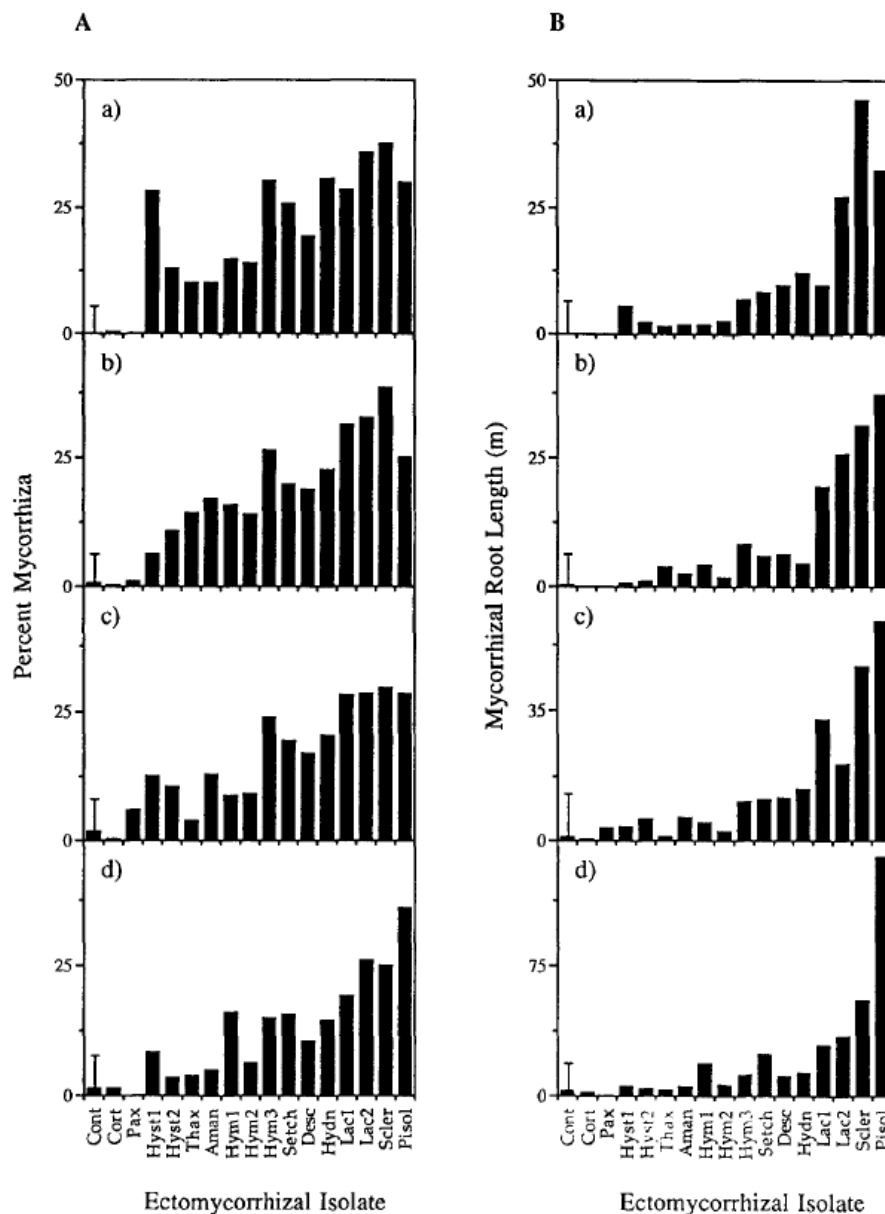


Fig. 1. A) Percent mycorrhiza and B) mycorrhizal fine root length (m) for a) *E. globulus* – 4 mg P kg⁻¹ sand, b) *E. diversicolor* – 4 mg P kg⁻¹ sand, c) *E. globulus* – 12 mg P kg⁻¹ sand, d) *E. diversicolor* – 12 mg P kg⁻¹ sand. Bar indicates LSD value ($p < 0.05$).

E. diversicolor.

The mycorrhizal root length of both eucalypts at 4 mg P kg⁻¹ sand inoculated with by *L. laccata* (LAC2), *S. verrucosum* and *P. tinctorius* (and LAC1 on *E. diversicolor*), was more than twice that of other isolates (Fig. 1B). The root length colonised by all mycorrhizal isolates increased between 4 and 12mg P kg⁻¹ sand, which implies that at least part of the decrease in the proportion of root length colonised by isolates with an increase in the supply of P was due to increased root growth.

Seedling growth

At 4 mg P kg⁻¹ sand, seedlings colonised only by the contaminant mycorrhizal fungus displayed typical P deficiency symptoms (small purplish leaves) for both eucalypt species. From earlier data on plant growth in relation to P supply (Bougher et al., 1990), 4mg P kg⁻¹ would be expected to produce 10% maximum growth for non-inoculated plants while 12 mg P kg⁻¹ would be nearly adequate for maximum growth.

At 4 mg P kg⁻¹ sand, seedlings of *E. globulus* inoculated with 8 fungal isolates (*H. carneum*, *H. zeylanicus* (HYM3), *Setchelliogaster* sp nov., *L. laccata* (LAC1 and LAU2), *S. verrucosum*, *D. maculata* and *P. tinctorius* significantly increased plant dry weight from 2.5 to 9 times that of non-inoculated seedlings (Fig. 2Aa). The same isolates produced increases in the biomass of *E. diversicolor* seedlings ranging from 4 to 13 times that of non-inoculated seedlings (Fig. 2Ab). Although increases in biomass were generally less for *E. globulus* than *E. diversicolor*, absolute increases were generally greater for *E. globulus* as non-inoculated seedlings tended to be larger than non-inoculated *E. diversicolor* seedlings. *L. laccata* (LAC1 and LAC2) and *P. tinctorius* relatively greater growth increase on *E. diversicolor* than on *E. globulus*, while for *H. carneum* this was reversed.

At 12 mg P kg⁻¹ sand, dry weight on non- inoculated and inoculated *E. globulus* seedlings did not differ (Fig. 2Ac). By contrast *P. muelleri*, *C. globuliformis*, *Thaxterogaster* sp nov., *H. inflatum* (HYST1), *H. zeylanicus* (HYM1), *L. laccata* (LAC1) inoculated into *E. diversicolor* significantly decreased seedling dry weight at this higher level of applied P (Fig. 2Ad).

Inoculation with ectomycorrhizal fungi did not significantly alter the partitioning of dry weight between roots and shoots at either P level for either eucalypt species. Root/shoot ratios were larger at 4 mg P kg⁻¹ (*E. globulus*, 0.412 and *E. diversicolor*, 0.519) than 12 mg P kg⁻¹ (*E. globulus*, 0.203 and *E. diversicolor*, 0.473) and *E. diversicolor* had a larger root/shoot ratio than *E. globulus*. In contrast, partitioning between fine roots and coarse roots varied markedly (Fig. 2B). At 4 mg P kg⁻¹ sand, *E. globulus* seedlings inoculated with *L. laccata* (LAC2), *S. verrucosum*, *D. maculata* and *P. tinctorius*, and *E. diversicolor* seedlings inoculated with *H. zeylanicus* (HYM3), *L. laccata* (LAC1 and LAU2) and *D. maculata*, increased the proportion of fine roots above that of non-inoculated seedlings. At 12 mg P kg⁻¹ sand, the ratio of fine/coarse roots increased for the same isolates on *E. globulus*, but only for *P. tinctorius* on *E. diversicolor*.

The relationship between the percent of root length colonised and total dry weight for all fungal isolates at 12 mg P kg⁻¹ sand was poor, but at 4 mg P kg⁻¹ sand (where P was limiting) the relationship was significant for both eucalypts (Fig. 3) being marginally better for *E. diversicolor* (Fig. 3B) than for *E. globulus* (Fig. 3A). The relationships indicate that *P. tinctorius* required a shorter root length to produce an equivalent dry weight than either *S. verrucosum* or *L. laccata* (LAC2).

Phosphorus uptake

At 4 mg P kg⁻¹ sand, *L. laccata* (LAC2), *S. verrucosum* and *P. tinctorius* increased the concentration of P in whole plants of *E. globulus*, whereas for *E. diversicolor*, inoculation with *Thaxterogaster* sp. nov., *H. zeylanicus* (HYM3), *Setchelliogaster* sp. nov., *L. laccata* (LAC2), *S. verrucosum* and *P. tinctorius* increased the concentration of P over that of non-inoculated plants (Table 2). For both eucalypts, the total uptake of P at 4 mg P kg⁻¹ sand was increased by inoculation with all fungi examined except *A. xanthocephala* and *Thaxterogaster* sp. nov. on *E. globulus*. P uptake (expressed as total plant P per metre fine roots) was increased by inoculation of both eucalypt species except for *S. verrucosum* on *E. globulus*.

Inoculation did not affect the partitioning of P between roots and shoots, except for *L. laccata* (LAC2) and *S. verrucosum*, which increased the proportion of P in the roots of *E. globulus* seedlings. By contrast, inoculation with ectomycorrhizal isolates altered the partitioning of phosphorus between fine roots and coarse roots. The proportion of total root phosphorus in the fine root component was increased by inoculation of *E. globulus* with *L. laccata* (LAC2) and *S.*

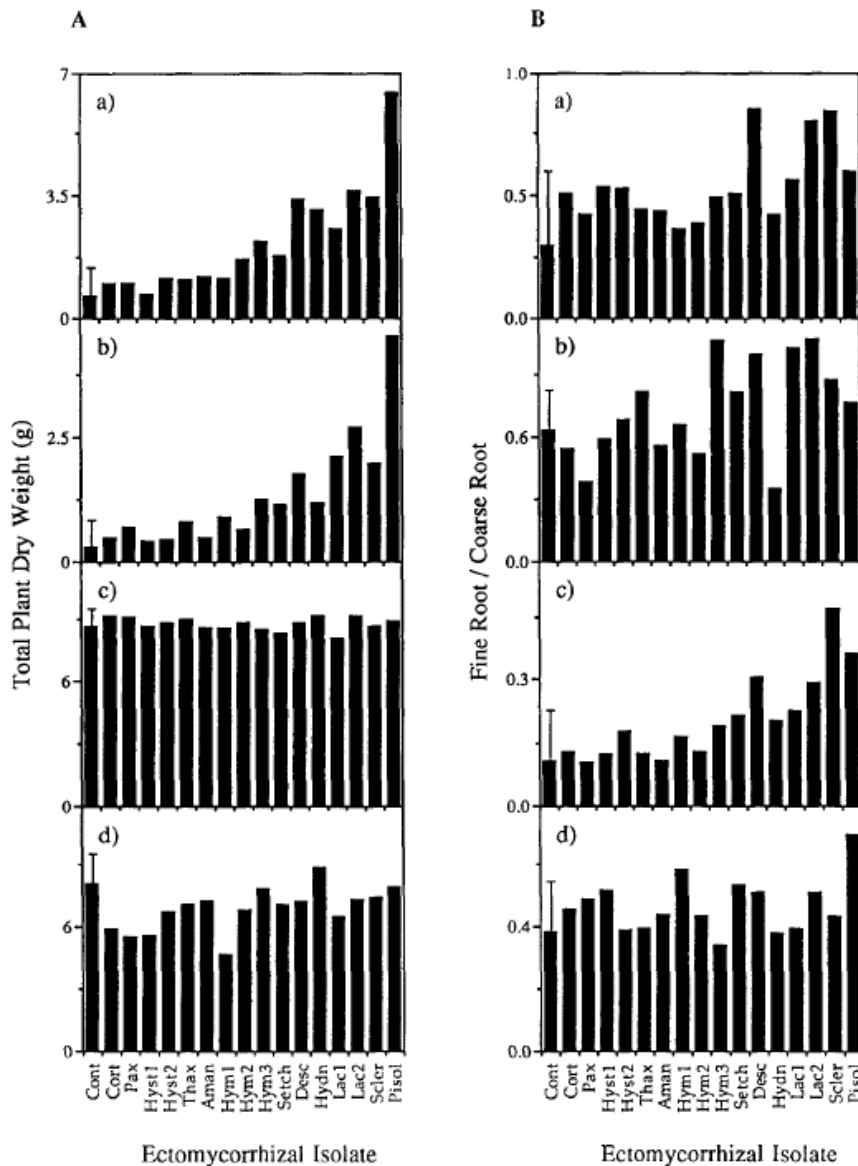


Fig. 2. A) Total plant dry weight (g) and B) Fine root/coarse root dry weight ratio for a) *E. globulus* - 4 mg P kg⁻¹ sand, b) *E. diversicolor* - 4 mg P kg⁻¹ sand, c) *E. globulus* - 12 mg P kg⁻¹ sand, d) *E. diversicolor* - 12 mg P kg⁻¹ sand. Bar indicates LSD value ($p < 0.05$)

Verrucosum and by inoculation of *E. diversicolor* with *A. xanthocephala*, *Thaxterogaster* sp nov., *H. zeylanicus* (HYM3), *Setchelliogaster* sp. nov., *L. laccata* (LAC2), *S. verrucosum* and *P. tinctorius*.

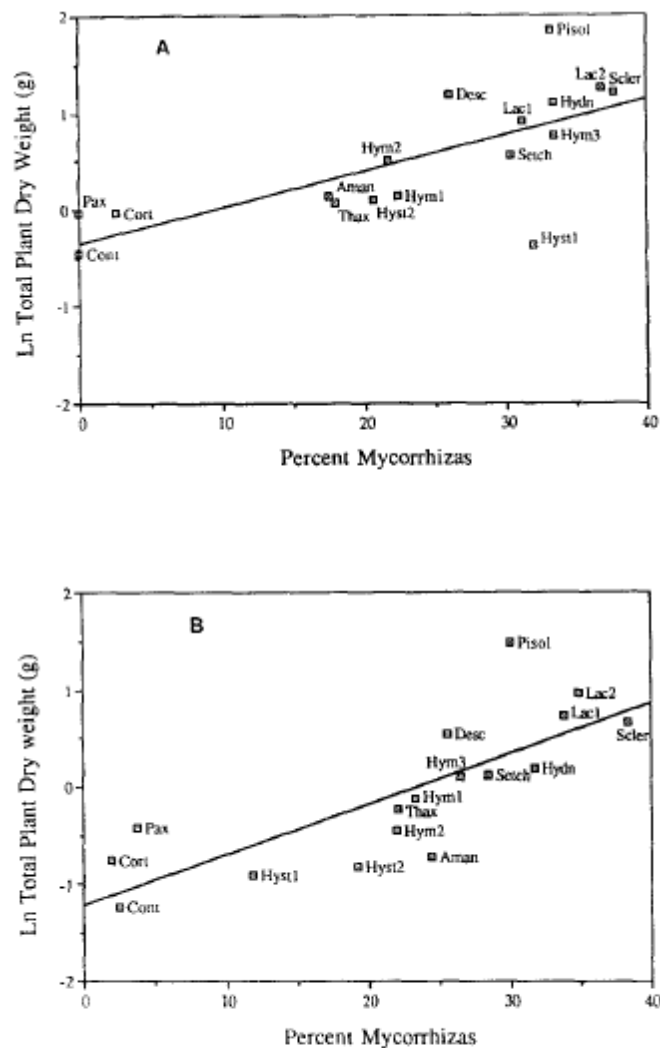


Fig. 3. The relationship between mycorrhizal fine root length (m) and Ln total plant dry weight (g) for A) *E. globulus* - 4 mg P kg⁻¹ sand, B) *E. diversicolor* - 4 mg P kg⁻¹ sand.

Discussion

Inoculating seedlings with ectomycorrhizal fungi generally increases the growth of plants under conditions of limiting soil phosphorus (Bougher et al, 1990; Daughteridge et al 1986; Ford et al., 1985 Harley and Smith, 1983; Heinrich and Patrick, 1986; Heinrich et al., 1988; Mulligan and Patrick, 1985). Our study has shown a large variation in the responses obtained with a range of ectomycorrhizal fungal species that are common associates of eucalypts.

Fungal isolates which formed the pyramidal type of mycorrhizas increased the growth of *E. globulus* and *E. diversicolor*, when P was deficient for plant growth. Growth responses of seedlings to inoculation with different fungi isolates ranged from 1.5 to 13 times that of inoculated seedlings. The largest increases in growth were obtained with *P. tinctorius*, while moderate responses were obtained with *S. verrucosum*, *D. maculata* and *L. laccata* (LAC2) and smaller responses with *H. carneum*,

Hymenogaster spp., *Setchelliogaster* sp. nov., and *L. laccata* (LAC1). *H. inflatum* and *Thaxterogaster* sp nov. formed superficial mycorrhizas of the type described by Malajczuk et al. (1987) and although they developed extensive extrametrical hyphae, this did not result in increased plant growth under the conditions of the experiment.

The dry weight of plants was positively correlated with mycorrhizal root length when P was deficient. Some of the variability in the relationships may be due to variation in morphology of ectomycorrhiza and functional differences between isolates. For example, isolates forming superficial mycorrhizas did not stimulate growth, whereas *P. tinctorius*, which gave the largest growth response, had a lower proportion of mycorrhizas than *S. verrucosum*, which only produced 75% of the maximum growth response.

Inoculation did not affect partitioning of plant dry weight between roots and shoots, as has been observed in other studies (Abbott and Robson, 1984; Bougher et al., 1900; Harley and Smith, 1983; Rousseau and Reid, 1990). However, the effect of inoculation of eucalypts at 4 mg P kg⁻¹ sand on the fine/coarse root ratio varied across the range of growth responses, with some isolates (e.g. *L. laccata*) with high colonisation and corresponding high growth responses having a high fine/coarse root ratio. In other plant-fungus combinations, giving large responses (e.g. with *P. tinctorius*), the ratios were lower. These differences probably result from contrasts in the morphology of the association. Some fungi form abundant, thick ectomycorrhizas and for these the fungal biomass may contribute significantly to the fine root biomass. Other fungi form smaller ectomycorrhizas and produce more prolific extrametrical mycelium.

At a lower level of applied P, inoculated seedlings of both eucalypts generally had a greater amount and a higher concentration of P and greater P uptake per unit length of fine root than the non-inoculated plants. Some fungi increased the relative amount of phosphorus in the fine roots compared with the coarse roots. This is generally attributed to storage of P in mycorrhizas as polyphosphate

Table 2. Concentration, uptake and partitioning of phosphorus in whole plants of (a) *E. globulus* and (b) *E. diversicolor* at the lower rate of applied P (4 mg P kg⁻¹ sand). Means within columns with same superscripts are not significantly different ($p < 0.05$) by Duncan's new multiple range test. Isolate codes refer to the fungal species shown in Table 1 and CONT refers to non-inoculated control seedlings

	P concentration (%)	Total plant P (mg)	Fine root/ Coarse root	Root/Shoot	P uptake Total plant P/m fine roots)
a) <i>E. globulus</i>					
CONT	0.0403 ^a	0.261 ^a	0.521 ^a	0.345 ^a	23.3 ^a
THAX	0.0463 ^{abc}	0.491 ^a	0.762 ^a	0.377 ^a	44.8 ^c
AMAN	0.0455 ^{abc}	0.522 ^a	1.091 ^a	0.371 ^a	34.7 ^{bc}
HYM3	0.0493 ^{abc}	1.081 ^{bc}	0.884 ^a	0.482 ^a	66.5 ^d
SETCH	0.0480 ^{abc}	0.851 ^b	1.173 ^a	0.469 ^a	41.9 ^c
HYDN	0.0448 ^{abc}	1.346 ^c	0.894 ^a	0.488 ^a	56.2 ^d
LAC2	0.0545 ^{cd}	1.942 ^d	1.923 ^{bc}	0.865 ^b	41.6 ^c
SCLER	0.0610 ^d	2.109 ^d	2.330 ^c	0.940 ^b	28.6 ^{ab}
PISOL	0.0500 ^{bc}	3.218 ^e	1.317 ^{ab}	0.579 ^a	44.9 ^c
b) <i>E. diversicolor</i>					
CONT	0.0350 ^a	0.122 ^a	0.734 ^a	0.774 ^{ab}	10.0 ^a
THAX	0.0550 ^{de}	0.472 ^{bc}	1.754 ^b	0.513 ^a	21.7 ^b
AMAN	0.0445 ^{bc}	0.223 ^{ab}	1.400 ^b	0.884 ^b	20.7 ^b
HYM3	0.0482 ^{cd}	0.613 ^c	1.742 ^b	0.681 ^{ab}	29.7 ^{bc}
SETCH	0.0483 ^{cd}	0.625 ^c	1.814 ^b	0.681 ^{ab}	26.4 ^{bc}
HYDN	0.0373 ^{ab}	0.400 ^{bc}	0.614 ^a	0.941 ^b	29.1 ^{bc}
LAC2	0.0590 ^e	1.586 ^d	3.023 ^c	0.935 ^b	31.5 ^{bc}
SETCH	0.0803 ^f	1.509 ^d	2.558 ^c	0.834 ^b	33.0 ^c
PISOL	0.0553 ^{de}	2.480 ^e	1.512 ^b	0.513 ^a	22.6 ^{bc}

(Lapeyrie et al., 1984; Mulligan and Patrick, 1985; martin et al., 1985). However, only *L. laccata* (LAC2) and *S. verrucosum* on *E. globulus* increased the relative amount of phosphorus in roots compared with shoots.

For most ectomycorrhizal isolates, increased growth of eucalypt seedlings was associated with improved P uptake. However, storage of P in line roots and translocation to the rest of the plant differed between isolates. *P. tinctorius* produced the largest increase in seedling growth but contained relatively less P in fine roots than *L. laccata* (LAC2) and *S. verrucosum*. *Setchelliogaster* sp. nov., *H. zeylanicus* (HYM3) and *H. carneum* gave moderate growth responses without an apparent retention of P in the roots.

When P was applied at a rate which was nearly adequate for maximum growth (i.e. 12 mg P kg⁻¹ sand), a number of fungal isolates decreased the growth of *E. diversicolor* seedlings. This effect has been observed previously where the external phosphorus supply has been adequate for maximum plant growth but not high enough to prevent colonisation (Bethlenfalvay et al., 1983; Bougher et al., 1990; Thomson et al., 1986). The effect can usually be attributed to the fungus acting as a net carbohydrate drain (Bougher et al., 1990). In our study, the growth depression did not significantly alter the root/shoot ratio, but rather was reflected over the whole plant. Growth depression was not observed for *E. globulus* seedlings grown at 12 mg P kg⁻¹ sand suggesting physiological differences between the two eucalypts in nutrient uptake.

The geographic disjunction between Tasmania and Western Australia, and consequently of the natural range of *E. globulus*, *E. diversicolor* and the fungi associated with them, provided a unique opportunity to study host-fungus specificity at the functional level. It may be expected that isolates from Tasmania would have been more effective with *E. globulus* and those from Western Australia more effective on *E. diversicolor*. However, isolates from both regions produced a similar range of responses. Comparison of responses of the two eucalypt species showed that the Western Australia isolate of *L. laccata* was more effective in increasing the growth of *E. diversicolor* while the isolate of *H. carneum* from Tasmania was more effective on *E. globulus*. However, this trend was not evident for other isolates, suggesting that the effectiveness of an isolates in stimulating growth was generally not related to the source of the isolate.

Some of the Western Australian isolates had been collected beneath *E. marginata* and perhaps were more suited to this eucalypt. However there appeared to be no host specificity especially since the isolate, which gave the highest growth stimulation, was collected beneath *E. marginata* in a native stand in Western Australia. These results are therefore consistent with observations of Chilvers (1973) indicating little evidence of host specificity at the eucalypt sub-genus level.

This experiment clearly demonstrates the range of differences that can be obtained in the ability of ectomycorrhizal fungi to colonise roots and increase the growth and P uptake of eucalypt seedlings in a simple sand system under controlled growing conditions. The poor performance of some of the isolates may be due to inability to grow in different soil types. In future experiments the effectiveness of these isolates in increasing the growth of eucalypt seedlings will be examined in natural soils and in the field.

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References

- Abbott L K and Robson A D 1984 Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 99, 245-255.
- Bethlenfalvay G J, Bayne H G and Pacovsky R S 1963 Parasitic and mutualistic associations between mycorrhizal fungus and soybean: The effect of phosphorus on host plant-endophyte interactions. *Physiol. Plant.* 57, 543-546.
- Bougher N L, Grove T S and Malajczuk N 1990 Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor*) seedlings inoculated with ectomycorrhizal fungi in relation to soil phosphorus supply. *New Phytol.* 114, 77-85.
- Chilvers G A 1973 Host range of some eucalypt mycorrhizal fungi. *Aust. J. Bot.* 21, 103-111
- Daughteridge A T, Pallardy S G, Garrett H G and Sanders I L 1986 Growth analysis of mycorrhizal and non-mycorrhizal black oak (*Quercus velutina* Lam) seedlings. *New Phytol.* 103, 473-480.
- Duncan D B 1955 Multiple range and multiple F-tests. *Biometrics* 11, 1-24
- Ford V L, Torbert J L, Burger J A and Miller O K 1985 Comparative effects of four mycorrhizal fungi on Loblolly pine seedlings growing in a greenhouse in Piedmont soil. *Plant and Soil* 83, 215-221.
- Harley J E and Smith S E 1983 *Mycorrhizal Symbiosis*. Academic Press. London. 483 p.
- Heinrich P A and Patrick J W 1986 Phosphorus accumulation in the soil-root system of *Eucalyptus pilularis* Sm. seedlings. II. The effect of ectomycorrhizas on the pattern of phosphorus acquisition. *Aust. J. Bot.* 34, 445-454.
- Heinrich P A, Mulligan D R and Patrick J W 1988 The effect of ectomycorrhizas on the phosphorus and dry weight acquisition of *Eucalyptus* seedlings. *Plant and Soil* 109, 147-149
- Lapeyrie F F, Chilvers G A and Douglas P A 1984 Formation of metachromatic granules following phosphorus uptake by mycelial hyphae of an ectomycorrhizal fungus. *New Phytol.* 98, 345-360
- Malajczuk N, McComb A J and Loneragan J F 1975 Phosphorus uptake and growth of mycorrhizal and non-mycorrhizal seedlings of *Eucalyptus calophyllai* R. Br. *Aust. J. Bot.* 23, 231-238.
- Malajczuk N, Dell B and Bougher N L 1987 Ectomycorrhizal formation in *Eucalyptus* III. Superficial ectomycorrhizas initiated by *Hysterangium* and *Cortinari* species. *New Phytol.* 105, 421-428.
- Martin F, Marchal J P, Timiniska A and Carnet D 1985 The metabolism and physical state of polyphosphates in ectomycorrhizal fungi. A ³¹P nuclear magnetic resonance study. *New Phytol.* 101, 275-290
- Marx D H 1969 The influence of ectotrophic mycorrhizal fungi on the resistance of pine root pathogenic infection. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* 59, 153-163.
- Mulligan D K and Patrick J W 1985 Growth and phosphorus partitioning in *Eucalyptus pilulari* Smith seedlings raised in phosphorus deficient soil. *Aust. J. Bot.* 33, 245-259.
- Newman E I 1966 A method for estimating the total root length in a sample. *J. Appl. Ecol.* 3, 139-145.
- Rousseau J V D and Reid C P P 1990 Effects of phosphorus and ectomycorrhizas on the carbon balance of Loblolly pine seedling. *For. Sci.* 36, 101-112.
- Thomson B D, Robson A D and Abbott L K 1986 Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytol.* 103, 751-765.
- Wild A. (1957) The phosphate content of Australian soils. *Aust. J. Agric. Res.* 9, 194-204.