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Effects of soil pH on the ectomycorrhizal response of *Eucalyptus urophylla* seedlings

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SUMMARY

To examine the effects of soil pH on ectomycorrhizal formation and function on *Eucalyptus urophylla* S. T. Blake, seedlings inoculated with nine ectomycorrhizal fungi (seven isolates of *Pisolithus* spp., *Scleroderma cepa* and *Laccaria laccata* collected under eucalypt stands in Australia and the Philippines) were transplanted into pots containing a non-sterile acid (pH 4·6) sandy loam amended with four levels of $CaCO_3$ that raised the soil pH from 4·6 to 6·6 (5 mM $CaCl_2$). Pots were placed in temperature-controlled water baths (28 ± 2 °C) inside an evaporatively cooled glasshouse for 9 wk.

Increase in soil pH from 4·6 to 6·6 significantly decreased plant d. wt and shoot nutrient content of uninoculated and inoculated seedlings. Inoculation with four *Pisolithus* spp. (H445, H2144, M56 and H4003) significantly increased the growth of *E. urophylla* seedlings at pH 4·6. At pH 6·6, eight ectomycorrhizal isolates significantly improved total d. wt compared with those of the uninoculated seedlings. *Pisolithus* isolates stimulated seedling growth more than *L. laccata* whereas *S. cepa* was ineffective at all pH levels. Total d. wt of H445 inoculated plants grown in P-deficient (8 mg P kg^{-1} soil) soil was 147 % more than that of uninoculated plants given the same P rate and was 70 % that of plants fertilized with 64 mg P kg^{-1} soil (P64) at pH 4·6. At soil pH 5·8 and 6·6, M56 was the best growth-promoter for *E. urophylla*. These results indicate that soil pH can significantly alter the development and function of ectomycorrhizal fungi. Soil pH did not significantly affect mycorrhizal formation by the different ectomycorrhizal fungi. However, the percentages of mycorrhizal root tips formed by the different ectomycorrhizal fungi differed significantly. *Pisolithus* isolate H445 formed the highest percentage of colonized roots and highest total d. wt at pH 4·6 and 5·2, implying its potential for commercial use in acidic conditions.

Key words: *Eucalyptus urophylla*, *Laccaria laccata*, ectomycorrhiza, *Pisolithus* spp., *Scleroderma cepa*, soil pH.

INTRODUCTION

Some fast-growing species of eucalypts are grown as multipurpose plantation trees and have also been used to rehabilitate degraded lands (Turnbull, 1994). Many of these plantings occur on strongly acidic soils in the tropics (Haridasan, 1985). The major growth-limiting factors associated with acid soil infertility include toxicities of Al and Mn, pH *per se*, and deficiencies or low availability of certain essential elements including Ca, Mg, P and Mo (Foy, 1984). These factors can directly or indirectly restrict plant growth through interference in the development and functioning of symbiotic associations with soil micro-organisms (Edwards & Bell, 1989).

Ectomycorrhizal symbioses can play an important role in increasing tree growth in acid soils where the availability of essential nutrients, particularly P, are low (Mengel & Kirkby, 1987). Eucalypts are naturally associated with ectomycorrhizal fungi (Chilvers & Pryor, 1965) and are strongly dependent on mycorrhizal symbionts for growth in soils of low nutritional status (Malajczuk, McComb & Loneragan, 1975). Ectomycorrhizal fungi from the genus *Pisolithus* can substantially increase the P content and growth of eucalypt seedlings (Malajczuk *et al.*, 1975; Heinrich, Mulligan & Patrick, 1988; Burgess, Dell & Malajczuk, 1994). Under controlled glasshouse conditions, growth improvement of *E. diversicolor* was greatest in acid soil where the P supply was moderate to severely limiting, and there was no growth response to inoculation where the P supply was adequate (Bougher, Grove & Malajczuk, 1990). Ectomycorrhizal fungi access soil P through the network of hyphae extending from the root which enlarges the volume of soil explored and hence they facilitate P uptake by the host (Mengel & Kirkby, 1987). However, soil acidity can affect ecto-

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mycorrhiza formation and hyphal development, to a degree dependent on the tree species and the mycorrhizal fungus involved (Sharpe & Marx, 1986).

Soil pH can adversely affect the ability of the root to grow or the ability of the mycorrhizal fungi to colonize roots and to take up nutrients (Lehto, 1994*a*). Generally, increase in soil pH through liming has been shown to inhibit ectomycorrhizal development. For example, Lehto (1994*a*, *b*) found that raising the pH (with $CaCO_3$) of acid (pH 3·6 and 4·6, 0·01 M $CaCl_2$) soils to pH 7·0 increased the percentage of dead mycorrhizas on *Picea abies*. Furthermore, the ability of *P. tinctorius* inoculum to infect plant roots declined when the pH (in water) of a nursery soil was raised (with $Ca(OH)_2$) from 4·8 to 6·8 (Marx, 1990). At the end of the growing season, the number of ectomycorrhizas on *Pinus sylvestris* seedlings at pH 6·8 was *c.* 25 % of those growing in more acid soil. However, Erland & Söderstrom (1990) found that infection of five mycorrhizal types on *P. sylvestris* rose from 70 % to nearly 100 % with an increase (with CaO) in pH (in water) of pine-forest soil from 4 to 5, with a corresponding increase in plant d. wt. The number of mycorrhizal root tips declined beyond pH 5. They also reported that infection levels of different mycorrhizal fungi varied with pH.

Eucalyptus urophylla S. T. Blake is one of the most important timber-producing eucalypts (Eldridge *et al.*, 1993). Its adaptability in acid soils might depend on the presence of symbiotic mycorrhizal associations. The aim of this study was to examine the effects of soil pH (through liming) on the formation of ectomycorrhizas by the different fungal symbionts. *Pisolithus* spp., *Scleroderma cepa* and *Laccaria laccata* were chosen because these fungi are early ectomycorrhizal root-colonizers, and some isolates have been reported to be effective in promoting growth of eucalypts (Bougher & Malajczuk, 1990; Burgess, Malajczuk & Grove, 1993; Burgess *et al.*, 1994).

MATERIALS AND METHODS

Experimental design

A two-factor experiment consisting of 10 inoculation treatments and four soil pH levels (with addition of lime) with three replicates was set up following a randomized complete block design in a glasshouse. The inoculation treatments were: seven isolates of *Pisolithus* spp., a *Scleroderma cepa*, a *Laccaria laccata* (Table 1) and a control, hereafter referred to as *Pisolithus* (followed by the isolate code), *Scleroderma*, *Laccaria* and uninoculated, respectively. The soil pH (1:2 soil–5 mM $CaCl_2$ ratio) at planting were (mg $CaCO_3$ kg^{-1} soil): pH 4·6 (no lime added), pH 5·2 (171·4), pH 5·8 (400) and pH 6·6 (771·4), pH in H_2O equivalent to: 4·8, 5·3, 5·8 and 6·4, respectively. The experiment was conducted from June to September. Pots were maintained in root tanks at 28 ± 2 °C. This temperature was chosen to relate to the temperature commonly observed on surface soils in grasslands in the Philippines, where field trials will be established. The average maximum and minimum glasshouse temperatures were 23 ± 2 and 10 ± 2 °C, respectively.

To investigate the additional aspect of the effect of P-availability on non-mycorrhizal plants in the pH gradient, pots with four P levels were set up concurrently with non-mycorrhizal plants at the four different lime levels. The P levels were: 8, 16, 32 and 64 mg P kg^{-1} soil.

Soil collection and preparation

An acidic (pH 4·6, 1:2 soil–5 mM $CaCl_2$ ratio) sandy loam soil (0–20 cm depth) was collected from a remnant stand of woodland in the wheatbelt near Bodallin, approx. 350 km east of Perth, Western Australia. The site was dominated by mixed species in the following genera: *Eucalyptus*, *Acacia*, *Cassia*, *Allocasuarina* and *Santalum*. Air-dried soil was sieved through a 5 × 5 mm stainless steel screen, thoroughly mixed, and three kg portions were placed into undrained 3 l plastic pots lined with polythene bags.

Lime was applied as powdered $CaCO_3$. The rates were selected from a pH incubation curve obtained from a preliminary experiment in which the soil was incubated for 2 wk at field capacity (12 % (w/w)) following the method of Dewis & Freitas (1970).

All pots received the following basal nutrients (mg kg^{-1} soil): 32·5 $Ca(H_2PO_4)_2.H_2O$ (equivalent to 8 mg P kg^{-1} soil), 202 NH_4NO_3 (applied in mg per pot once a week: 28·8 during the addition of basal nutrients, 14·4 in weeks 3 and 4, 28·8 from week 5 to week 9), 233 K_2SO_4, 71·3 $CaCl_2$, 21·4 $MgSO_4.7H_2O$, 10 $ZnSO_4.7H_2O$, 5 $CuSO_4.5H_2O$, 0·36 $CoSO_4.7H_2O$, 0·7 H_3BO_3 and 1·62 $Na_2MoO_4.H_2O$. Manganese was omitted owing to a high concentration in the soil (Jongruaysup, 1993). The nutrients (except P) were applied in solution to the soil surface of each pot and allowed to dry. Before thorough mixing by shaking in plastic containers, the P fertilizer and the lime were added into the soil. The pots were incubated for 2 wk at field capacity before the seedlings were planted.

Ectomycorrhizal synthesis and transplanting

The fungal isolates, which were collected under eucalypt stands in Australia and in the Philippines, were provided by the CSIRO Forestry & Forest Products, Wembley, Western Australia and Murdoch University (Table 1). For mycorrhizal synthesis, plugs of 3 mm diameter were cut at the edge of a 3–4-wk-old fungal colony and grown on tubes (8 cm height and 6·8 cm diameter) containing slanting Modified Melin Norkrans (MMN) (Marx, 196[illegible])

Table 1. *Isolate code, species, host association and origin of ectomycorrhizal fungi*

Isolate code	Species	Associated host	Origin
H445	*Pisolithus* sp.	*Eucalyptus*	Jarrahdale, Western Australia
M56	*Pisolithus* sp.	*Eucalyptus*	Kalbarri, Western Australia
H2144	*Pisolithus* sp.	*Eucalyptus*	Southwest, Western Australia
H4320	*Pisolithus* sp.	*Eucalyptus*	Southwest, Western Australia
H4003	*Pisolithus* sp.	*Eucalyptus*	Cairns, Queensland, Australia
H495	*Pisolithus* sp.	*Eucalyptus*	Bega, New South Wales, Australia
H615	*Pisolithus* sp.	*Eucalyptus*	Nueva Ecija, Luzon, Philippines
H603	*Scleroderma cepa*	*Eucalyptus*	Manjimup, Western Australia
E766	*Laccaria laccata*	*Eucalyptus*	Manjimup, Western Australia

solid medium with reduced glucose concentration (1·75 g l^{-1}).

Seeds of *E. urophylla* (seedlot no. 18094 from Mt Egon, Indonesia) were surface-sterilized with 10% sodium hypochlorite (v/v) for 5 min, rinsed with four changes of sterile water and were plated onto MMN agar. After 10 d, aseptically germinated seedlings were laid onto the edge of 14-d-old fungal mats left for 2 wk in the light (80 μmol m^{-2} s^{-1} irradiance) at 25 °C. Uninoculated seedlings and seedlings with developing ectomycorrhizas were transplanted into pots, and the soil was covered with aluminium foil to minimize contamination from airborne micro-organisms and water loss. Seedlings were thinned to two seedlings per pot 4 wk after planting.

Harvest and assessment of mycorrhizal infection

The seedlings were harvested when the largest plants were 60 cm tall (10 wk) to avoid overcrowding of roots in the pots. Shoots were cut 1 cm above the soil surface and the root systems were gently washed. The fine roots (diameter less than 0·5 mm) were separated from the coarse roots. A subsample of fine roots (0·2 g f. wt) was cut into 2–3 mm lengths and fixed in 70% ethanol for estimation of root infection. A preliminary examination of unstained roots indicated the presence of distinctive yellow *Pisolithus* mycorrhizas. *Scleroderma* formed white clustered ectomycorrhizas; those of *Laccaria* were silvery white and solitary. Fine roots were cleared and stained as described by Phillips & Hayman (1970). Stained roots were spread evenly over a Petri dish and mycorrhizal and non-mycorrhizal infected roots were examined under a stereomicroscope. Fully colonized root tips were scored as mycorrhizal. Dry weights of shoots, coarse and fine roots were measured after 48 h at 70 °C.

Nutrient analyses

Oven-dried shoots were ground in a stainless steel hammer mill. For nitrogen, samples of 100 mg were digested with concentrated H_2SO_4 and H_2O_2 at 400 °C (Dalal, Sahrawat & Myers, 1984). Samples were pre-digested with salicylic acid to reduce nitrate to ammonium. Nitrogen was determined spectrophotometrically using the Berthelot reaction of Issac & Johnson (1976) replacing the phenol with sodium salicylate (Searle, 1984).

For other nutrients (P, K, Ca, Mg, S, B, Al, Mn, Zn, Fe and Cu), samples were analysed in an Inductive Coupled-Plasma (ICP) spectrometer after digestion with HNO_3 (Zarcinas, Cartwright & Spouncer, 1987). Standard reference materials (eucalypt and citrus leaves) obtained from the State Chemistry Laboratory, Department of Agriculture, East Melbourne, Victoria, Australia, and two blanks were included in each digest batch, and concentrations were within the standard deviation of the published means.

Statistical analysis

All data collected were subjected to either one-way or two-way analysis of variance. Treatment means were compared using Duncan's new multiple-range test and least significant difference at $P < 0{\cdot}05$ (Duncan, 1955).

RESULTS

Mycorrhizal infection

Percentages of root tips colonized in the inoculated treatments were not affected by the increase in soil pH from 4·6 to 6·6 through the addition of lime. There were, however, significant ($P < 0{\cdot}001$) differences in the percentages of root tips colonized by the different ectomycorrhizal fungi. All *Pisolithus*- and *Scleroderma*-inoculated plants had significantly greater (21–32%) ectomycorrhizal development than the uninoculated plants (12%) and those inoculated with *Laccaria* (17% of infected root tips) (Fig. 1). Plants inoculated with *Pisolithus* H445 and H4003 had the highest percentages (32% and 31%, respectively) of roots colonized. Infection (12%) observed in the uninoculated seedlings might have come from ectomycorrhizal propagules present in the unsterile soil used in the experiment. Ecto-

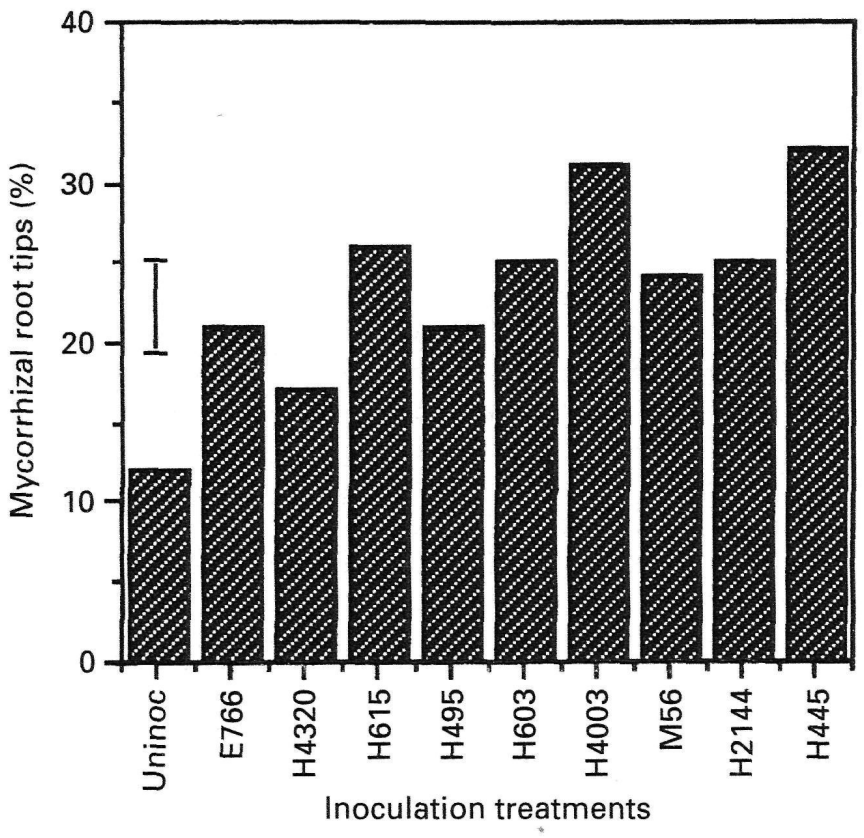

Figure 1. Percentage of mycorrhizal root tips formed by the different ectomycorrhizal fungi on the roots of *E. urophylla* seedlings. Bar represents LSD value for the main inoculation effects of two-way factor analysis at $P < 0{\cdot}05$. There was no significant interaction between inoculation and soil pH on mycorrhizal infection. Ectomycorrhizal isolates are *Pisolithus* spp. except E766 = *Laccaria laccata*, H603 = *Scleroderma cepa* (refer to Table 1).

mycorrhizas on the fine roots of uninoculated seedlings were white and clustered, similar to those colonized by *Scleroderma* H603. Thus, it was not possible to differentiate between ectomycorrhizas formed by any native *Scleroderma* in the Bodallin soil and the inoculant *Scleroderma*. However, no white ectomycorrhizas were observed on seedlings inoculated with *Pisolithus*, which had golden yellow infected roots, or in seedlings inoculated with *Laccaria*, with silvery white ectomycorrhizas.

Growth response to inoculation

The uninoculated seedlings fertilized with 8 mg P kg^{-1} soil grown at soil pH 4·6 (no lime added) exhibited P deficiency symptoms: purplish leaves and stem and stunted growth. Increase in soil pH reduced ($P < 0{\cdot}001$) height and stem diameter of both inoculated and uninoculated plants (data not presented). Inoculation corrected the P deficiency symptoms of plants at pH 4·6 and inoculated plants grew vigorously.

There was a significant ($P < 0{\cdot}001$) interaction between soil pH and inoculation treatments on total dry weight of *E. urophylla* seedlings. At soil pH 4·6, only four *Pisolithus* isolates (H445, H2144, M56 and H4003) significantly increased total d. wt. of inoculated seedlings (Fig. 2). *Pisolithus* H445 and H2144 inoculated seedlings had significantly greater total d. wt than those inoculated with M56 and H4003. At soil pH 6·6, all the ectomycorrhizal fungi, except *Scleroderma* H603, significantly promoted seedling total d. wt. Inoculation increased total dry weight of plants from 14 to 147 % at pH 4·6 and from 109 to 376 % at pH 6·6, relative to their uninoculated counterparts.

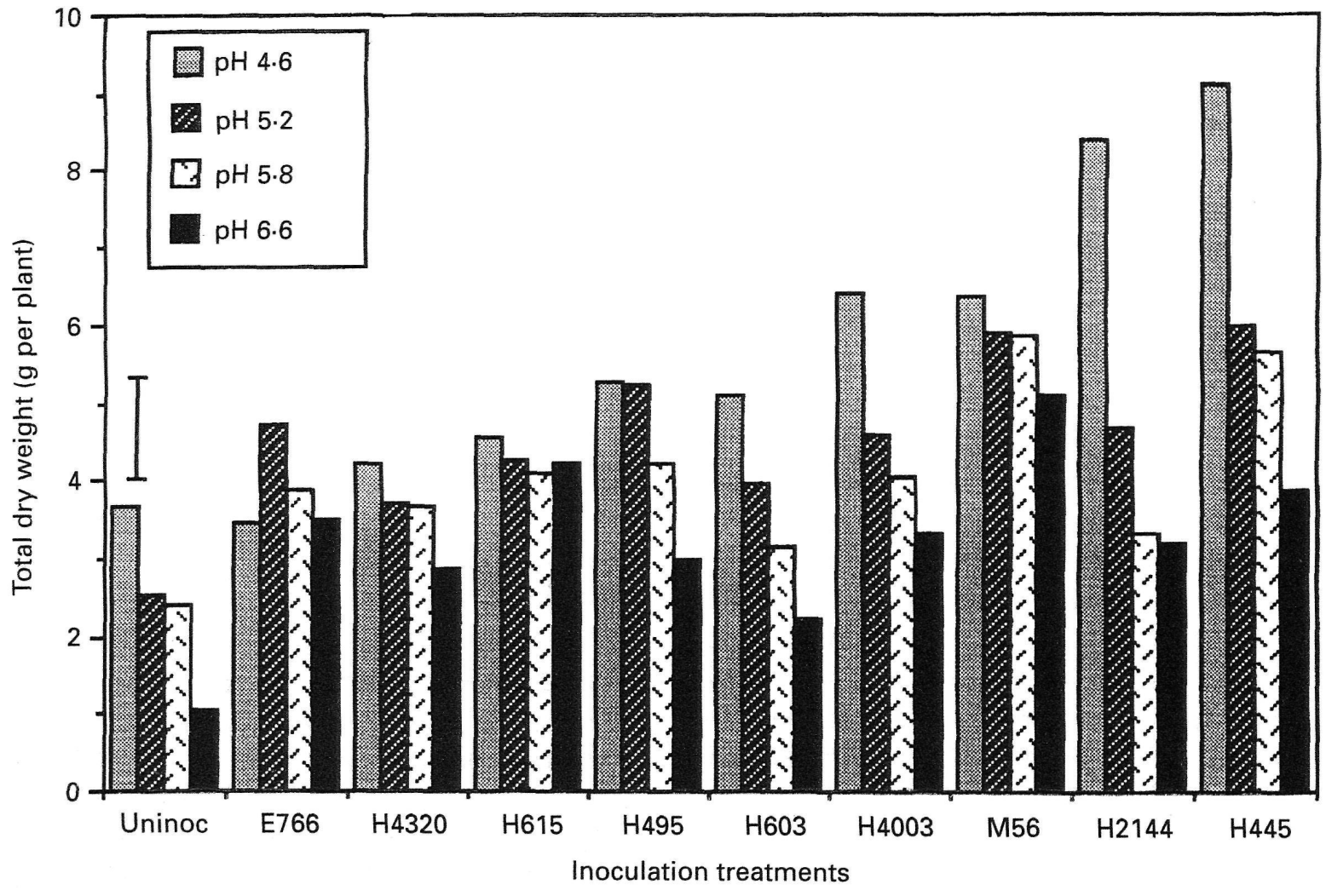

Figure 2. Total dry weight of *E. urophylla* seedlings inoculated with different ectomycorrhizal fungi and grown in soil with increasing pH due to lime application. All treatments received 8 mg kg^{-1} soil as basal P. Bar represents LSD value for the interaction between soil pH and inoculation treatments of two-way factor analysis at $P < 0{\cdot}05$. Ectomycorrhizal isolates are *Pisolithus* spp. except E766 = *Laccaria laccata*, H603 = *Scleroderma cepa* (refer to Table 1).

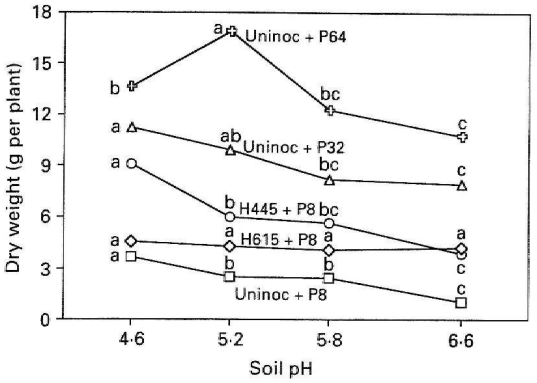

Figure 3. Total dry weight of *E. urophylla* seedlings inoculated with two *Pisolithus* isolates (at 8 mg P kg^{-1} soil) and grown at four soil pH (1 : 2 soil–5 mM $CaCl_2$) compared with uninoculated seedlings fertilized with 8, 32 and 64 mg P kg^{-1} soil. Symbols with the same letter(s) in each line are not significantly different from each other using Duncan's new Multiple Range Test at $P < 0{\cdot}05$. *Pisolithus* H445 = Western Australia and H615 = Philippines.

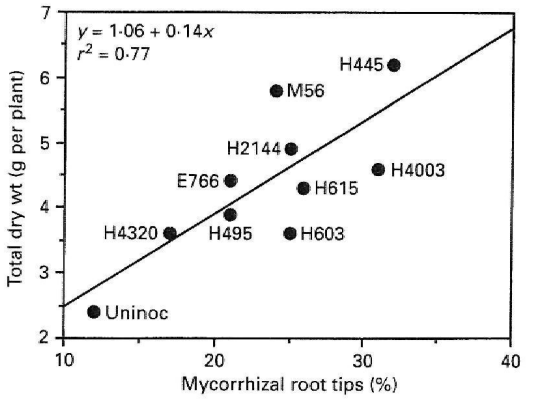

Figure 4. Relationship between mycorrhizal infection and total dry weight of *E. urophylla* seedlings inoculated with different ectomycorrhizal fungi. Ectomycorrhizal isolates are *Pisolithus* spp. except E766 = *Laccaria laccata*, H603 = *Scleroderma cepa* (refer to Table 1).

Increasing soil pH from 4·6 to 6·6 likewise reduced the total d. wt of uninoculated plants fertilized with P (32 and 64 mg P kg^{-1} soil) (Fig. 3). Ectomycorrhizal responses lay in between those of the uninoculated plants and those fertilized with 16 mg P kg^{-1} soil. Where P was limiting (8 mg P kg^{-1} soil), the total d. wt of plants inoculated with H615 was not significantly affected by the increase in soil pH from 4·6 to 6·6. At pH 4·6, total d. wt of plants inoculated with *Pisolithus* H445 and H615 were 80% and 50%, respectively, those of seedlings fertilized with 32 mg P kg^{-1} soil (Fig. 3). Irrespective of pH levels, there was a strong positive relationship between the percentage of mycorrhizal root tips and total dry weight ($r^2 = 0{\cdot}77$) (Fig. 4).

Nutrient concentration in shoot

Except for P, Al and Mn, increase in soil pH did not affect the nutrient concentration in the shoot of uninoculated seedlings fertilized with 8 mg P kg^{-1} soil (data not shown). Increase in soil pH from 4·6 to 5·2 increased ($P < 0{\cdot}001$) P concentration and reduced ($P < 0{\cdot}001$) Al and Mn concentrations. Further increase in soil pH from 5·2 to 6·6, reduced shoot P, Al and Mn concentrations, but differences were not statistically significant. There was a significant interaction between soil pH and inoculation on P, Mn, Zn and Cu concentration in the shoots of *E. urophylla* seedlings (Table 2). At pH 4·6, P, Mn, and Cu concentrations of *Pisolithus* H615 inoculated plants were significantly higher (P (28%), Mn (36%), and Cu (34%)) than those inoculated with *Pisolithus* H445. However, at pH 6·6, the P concentration of H445 inoculated plants were significantly higher than those inoculated with H615.

Nutrient content in shoot

Generally, all nutrient contents were lower in shoots of plants grown at pH 6·6 than in those grown at pH 4·6 (Table 2). There was a significant interaction between effects of soil pH and inoculation on the content of all nutrients except Al, Zn and Cu (Table 2). Inoculation with *Pisolithus* H445 significantly improved shoot content of all nutrients at pH 4·6. By contrast, at pH 4·6, H615 significantly increased shoot P content and significantly decreased Mn content compared with those of the uninoculated plants. At pH 6·6, inoculation with *Pisolithus* H445 or H615 significantly increased the contents of all nutrients. Nutrient contents of plants inoculated with the two fungi were similar except that H615-inoculated plants had significantly higher Mg content than the H445-inoculated plants.

DISCUSSION

Effect of soil pH on growth of uninoculated plants

Most eucalypts grow naturally on acid soils (Turnbull & Pryor, 1984) and do not thrive on soils that are alkaline with free calcium carbonates or sulphates in the profile. *E. urophylla* fits this pattern since growth of both the P-deficient and P-adequate seedlings declined in parallel with the increase in soil pH (Fig. 3). Reduced growth of eucalypts in a pallid zone clay due to liming (with $CaCO_3$) the soil from pH 4 to 7·2 was reported by Dell, Loneragan & Plaskett (1983). By contrast, Jongruaysup (1993) using the same acidic Bodallin soil as reported here, found that liming improved the growth of crop legumes. This improvement was reported to have been due to the improved Mo status of the plants as well as to reduced Mn toxicity. Crops grown on Australian soils, following the application of lime, can show

Table 2. *Nutrient concentrations and content in the shoot of* E. urophylla *seedlings as affected by soil pH and inoculation with two* Pisolithus (*H445, Western Australia and H615, Philippines*) *isolates*

	pH 4·6			pH 6·6			
Nutrients	Uninoc	H445	H615	Uninoc	H445	H615	*F* test
	Nutrient concentration (mg g^{-1})						
N	32·1	32·4	38·0	33·5	35·6	34·7	n.s.
P	1·10 c	1·16 c	1·48 a	1·35 b	1·52 a	1·32 b	*
K	17·8	28·6	22·2	26·3	26·6	24·7	n.s.
Ca	5·92	5·14	5·65	7·62	7·49	9·00	n.s.
Mg	2·05	2·12	2·39	2·08	2·01	2·04	n.s.
S	2·2	2·30	2·73	2·80	2·88	2·82	n.s.
	Nutrient concentration (μg g^{-1})						
B	22·4	25·4	26·6	26·6	27·3	28·9	n.s.
Al	27·6	22·8	22·2	4·8	18·6	12·8	n.s.
Mn	514 b	519 b	701 a	290 c	292 c	299 c	*
Fe	60·2	54·6	73·0	71·0	69·6	63·4	n.s.
Zn	41·3 ab	43·6 ab	51·3 a	35·2 bc	37·5 abc	24·4 c	*
Cu	11·4 b	11·3 b	15·2 a	15·6 a	15·8 a	12·1 b	**
	Nutrient content (mg per shoot)						
N	97 b	233 a	133 b	23 c	116 b	124 b	**
P	3·4 c	8·5 a	5·3 b	1·0 d	5·0 bc	4·9 bc	*
K	54 b	211 a	77 b	17 c	85 b	90 b	*
Mg	23 b	44 a	24 b	6 c	30 b	40 a	***
Ca	7·6 b	18·0 a	10 b	1·6 c	7·5 b	8·6 b	***
S	8·1 c	19·2 a	11·2 bc	2·3 d	11·9 b	13·1 b	**
	Nutrient content (μg per shoot)						
B	83 b	217 a	113 b	19 c	95 b	115 b	**
Al	88	167	78	3	85	48	n.s.
Mn	1573 c	3732 a	2448 b	182 d	948 c	1081 c	**
Fe	187 b	394 a	256 b	48 c	222 b	228 b	*
Zn	160	380	221	28	153	112	n.s.
Cu	34	83	53	10	51	44	n.s.

Means in each row with the same letter(s) are not significantly different from each other using Duncan's new Multiple Range Test at $P < 0{\cdot}05$.

*, **, ***, significant at 5 %, 1 % and 0·1 %, respectively.

n.s., not significant.

growth promotion or suppression (Cregan, Hirth & Conyers, 1989). These authors pointed out that most of the yield depressions observed in pot trials occurred at pH levels lower than neutrality, illustrating the highly leached and weakly buffered nature of Australian soils and the likelihood that liming might induce nutrient deficiencies.

Effect of soil pH on the growth of inoculated plants

Eucalypts growing in acid soils are commonly characterized by the presence of ectomycorrhizas within the root systems (Brundrett & Abbott, 1991). Although inoculation with some ectomycorrhizal isolates (e.g. H445, H2144 and M56) improved plant growth at all pH levels, growth of all inoculated seedlings declined with the increase in soil pH (Fig. 2). A similar decrease in the growth of *P. tinctorius* inoculated pecan (*Carya illinoensis*) seedlings due to increasing soil pH brought about by liming was observed by Sharpe & Marx (1986).

The nine ectomycorrhizal fungi exhibited differential effectiveness in stimulating growth of *E. urophylla* seedlings. At pH 4·6, four *Pisolithus* isolates (H445, H2144, M56 and H4003) were effective in increasing plant growth while at pH 6·6, all seven *Pisolithus* isolates (H445, H2144, M56, H4003, H495, H615 and H4320) stimulated plant growth in relation to their uninoculated counterparts (Fig. 2). *Laccaria laccata* was less effective than *Pisolithus* spp. in stimulating growth at all pH levels, whereas *Scleroderma cepa* was ineffective at all pH levels, relative to the uninoculated control treatments. These results indicate that *Pisolithus* spp. can be more effective growth-promoters for *E. urophylla* than *Laccaria laccata* and *Scleroderma cepa* on non-sterile acid soils. However, not all strains/isolates of *Pisolithus* are growth-promoters. For example, Malajczuk, Lapeyrie & Garbaye (1990) found that isolates of *P. tinctorius* from under pine are ineffective for eucalypts, and not all isolates of *P. tinctorius* collected from under eucalypts are equally effective in promoting growth of eucalypts (Burgess *et al.*, 1994; Tonkin, Malajczuk & McComb, 1989). Burgess *et al.* (1994) rated 16 isolates of *Pisolithus* spp. from under eucalypts according to their effect on *E*

grandis in yellow sand as poor (2–10 times the growth of uninoculated seedlings), moderate (15–20 times), good (25–35 times) and superior (45 times) growth-promoters. By contrast, in the present study, growth of *E. urophylla* seedlings due to inoculation with *Pisolithus* (H445, H2144, M56 and H4003) at pH 4·6 was 2–3 times, whereas at pH 6·6 it was 3–5 times greater than the uninoculated treatments. However, comparison of the two experiments is complicated because Burgess *et al.* (1994) used pasteurized sand and the plants were more severely P-deficient (4 mg P kg^{-1} sand) than those in the Bodallin loam (8 mg P kg^{-1} soil). A more meaningful comparison is to compare the growth of inoculated plants relative to the maximum yield of non-mycorrhizal plants fertilized with 64 mg P kg^{-1} soil (Fig. 3). On this criterion, total dry weights of the best inoculation treatments were comparable in the two experiments.

Effect of soil pH on mycorrhizal infection

Although the percentage of *E. urophylla* roots colonized by the nine fungal isolates decreased with the increase in soil pH from 4·6 to 6·6, there was no significant interaction between effects of inoculation with ectomycorrhizal fungi and of soil pH. This observation agrees well with those of Sharpe & Marx (1986) who reported that there was no significant interaction between inoculation with ectomycorrhizal fungi and soil pH in spite of the 50 % reduction in the amount of roots colonized by *P. tinctorius* on *C. illinoensis* seedlings resulting from a rise in soil pH from 5·5 to 6·5. They suggested that the higher ectomycorrhizal development at low soil pH was associated with the natural ecological adaptation of *P. tinctorius* to acid soils. *P. tinctorius* has been found in great abundance associated with tree species growing in strip-mined coal spoils with soil pH (in water) of 2·8–3·8 (Berry, 1982) and on other adverse sites exposed to excessive drought and low soil fertility (Marx *et al.*, 1984).

The lower (15–33 %) percentage of roots colonized by *Pisolithus* spp. in this experiment, compared to the 40–65 % reported by Burgess *et al.* (1994) might be related to the use of non-sterile soil in the former and pasteurized sand in the latter. The presence of soil micro-organisms in the non-sterile Bodallin loam might have positively or negatively affected ectomycorrhizal formation (Garbaye, 1983; Fitter & Garbaye, 1994). In a fumigated nursery soil, Sharpe & Marx (1986) observed an average *P. tinctorius* infection of 33 % on *C. illinoensis* seedlings, the top range in non-sterile soil. The fumigated soil became contaminated with naturally occurring fungi (Sharpe & Marx, 1986) which might have had an effect on the level of colonization. Under field conditions, Thomson *et al.* (1996) reported < 20 % root colonization by the inoculant ectomycorrhizal fungi on the fine roots of *E. globulus* planted on a gravelly yellow duplex soil, and 30–50 % in a yellow sandy earth, 6 months after outplanting (Thomson *et al.*, 1996). However, at 1 yr after outplanting, root colonization by the inoculant fungi in the yellow sandy earth was reduced to 10 % by colonization by the resident ectomycorrhizal fungi (Thomson *et al.*, 1996).

Effect of soil pH and inoculation on nutrients in the shoot

Decreased growth of seedlings fertilized with high rates of P (32 and 64 mg P kg^{-1} soil) in limed soil is probably not caused by an induced nutrient deficiency even though liming significantly reduced the concentrations of Mn and Zn in the shoot. Except for P, nutrient concentrations in the shoots were probably not limiting plant growth (Dell, Malajczuk & Grove, 1995). Although nutrient concentrations vary with plant age and plant organ (Mengel & Kirkby, 1987), the nutrients in young shoots of seedlings (this experiment) are not greatly different from those in the youngest fully expanded leaves used by Dell *et al.* (1995). The uninoculated plants grown at pH 4·6 (unlimed) showed P deficiency symptoms but did not show other nutrient deficiency or toxicity symptoms.

Generally, inoculation with H445 increased the shoot content of all macro- and some micronutrients (B, Mn and Fe) of plants grown at pH 4·6. By contrast, inoculation with H615 increased P and Mn contents in the shoot only in some lime treatments. A similar increase in macro- and some micronutrient (Cu and Mn) contents due to inoculation with *Pisolithus* was reported by Sharpe & Marx (1986).

CONCLUSION

Mycorrhizal formation was not affected by the change in soil pH but the effectiveness of the different ectomycorrhizal fungi in promoting plant growth at increasing pH levels was reduced. These observations suggest that soil pH might have affected the development of the nutrient-absorbing external hyphae. The different ectomycorrhizal fungi showed differential preference to soil pH. *Pisolithus* isolate H445 formed the highest number of ectomycorrhizas and promoted the best growth at the lowest pH (4·6) in a non-sterile soil in the present experiment. The superiority of *Pisolithus* H445 to other ectomycorrhizal fungi used in this study was related to its ability to form ectomycorrhizas and, as a consequence, to stimulate plant growth in non-sterile acidic soil. This is very important because this implies the persistence and survival of the fungus in the presence of indigenous soil micro-organisms. Most of the reforestation areas in Asia are characterized by acidic pH, thus, screening for acid-tolerant ectomycorrhizal symbionts is a necessity before embarking on inoculation programmes on such soils.

However, it must be considered that soil pH interacts with the many biotic and abiotic soil factors which might affect the effectiveness of an ectomycorrhizal isolate to improve plant growth. Thus, future work should be directed at matching isolates with particular acid soil conditions that might be site-specific (e.g. acid sulphate soils, soils high in Al and other metals).

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