

A STUDY OF THE EFFECTS OF ECTOMYCORRHIZAL INOCULATION WITH FIVE ISOLATES OF *Pisolithus* ON GROWTH OF *Eucalyptus camaldulensis* DEHNH. SEEDLINGS AT FOUR NUTRIENT REGIMES.

K. Misbahuzzaman^a and J. Wilson^b

^a*Forestry and Wood Technology Discipline, Khulna University, Khulna-9208, Bangladesh*

^b*Centre for Ecology and Hydrology, Bush Estate, Penicuik, Midlothian EH26 0QB, Edinburgh, UK.*

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Abstract: In this study, host-symbiont interactions between *Eucalyptus camaldulensis* seedlings and five isolates of ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch in terms of colonisation and growth responses in vermiculite-peat at the end of 18 weeks were investigated. The treatment combinations consisted of five isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and four nutrient regimes (Ingestad's 2.5, 5.0, 10 and 20 mg l⁻¹ P). Only isolate K55 resulted in considerable colonisation (up to 27% root tips mycorrhizal) that occurred at 2.5 mg l⁻¹ P while the other isolates resulted in <1% colonisation. Colonisation by *P. tinctorius* K55 reduced growth in terms of shoot dry mass in *E. camaldulensis* seedlings. Regression of total dry mass of seedlings on the extent of colonisation was found to be negative.

Keywords: *Eucalyptus camaldulensis* Dehnh.; *Pisolithus tinctorius* (Pers.) Coker and Couch; ectomycorrhiza.

Introduction

Eucalypts predominantly form ectomycorrhizal (EM) associations in native forests (Chilvers, 1973; Malajczuk and Hington, 1981) and plantations (Chu-Chou and Grace, 1982; Brundrett *et al.*, 1996). There are many studies on various aspects of *Eucalyptus* EM, including detailed analysis of colonised root systems (Chilvers and Gust, 1982a, 1982b), phosphate accumulation (Ashford *et al.*, 1975, 1986), mycorrhizal type characterisation (Chilvers, 1968; Seviour *et al.*, 1978 and Rose *et al.*, 1981), synthesis of EM between compatible and incompatible fungi with a number of *Eucalyptus* species (Malajczuk *et al.*, 1982, 1984), and some studies dealing with the details of the development of EM (Chilvers and Gust, 1982a and Massicote *et al.*, 1987). There have been reports of growth stimulation of *Eucalyptus* species inoculated with EM fungi in nurseries, plantations and glasshouse experiments (Garbaye *et al.*, 1988; Grove *et al.*, 1991; Burgess *et al.*, 1993, 1994). Studies on *E. camaldulensis* EM are, however, very few. Malajczuk and Hartney (1986) compared EM (by using *Pisolithus tinctorius*, *Hydnangium carneum*, *Scleroderma verrucosum* and *Laccaria laccata*) formation on micropropagated plantlets and seedlings of *E. camaldulensis* and observed much more uniform mycorrhizal formation on micropropagated plantlets than seedlings. Abouelkhair *et al.* (1986) inoculated *E. camaldulensis* seedlings with *Hymenogaster alba* (Klotzsch.) Bark. et Br. and *P. tinctorius* in a glasshouse using four concentrations of Ingestad's nutrient solution (Ingestad, 1971) and found that seedlings inoculated with *P. tinctorius* had significantly higher dry mass as compared to *H. alba* inoculated or non-mycorrhizal seedlings. They, however, did not report whether mycorrhiza formation and growth responses occurred at all four nutrient concentrations. Dixon and Hiol-hiol (1992) found that, despite similar leaf stomatal conductance and more negative plant water potential at the peak of the drought, *P. tinctorius* inoculated seedlings of *E. camaldulensis* were able to maintain a higher rate of photosynthesis than plants treated with *Thelephora terrestris*. There is therefore a need to identify appropriate nutrient regimes so that interactive effects of nutrient concentration and EM colonisation can be studied on growth of *E. camaldulensis* seedlings. The objective of the present study was to assess the effects of EM colonisation on growth of *E. camaldulensis* seedlings at various nutrient concentrations.

Materials and Methods

Inocula and inoculum preparation: The experiments involved five different isolates of the ectomycorrhizal fungus *Pisolithus tinctorius* (Pers.) Coker and Couch. Isolates PT7 and PT8 were cultured from young sporocarps produced by *E. globulus* inoculated with isolate PT3 in the pot experiments at a glasshouse in the Institute of Terrestrial Ecology (ITE), Edinburgh, UK while Isolates PTE and K55 were supplied by the University of Kent, Kent, UK which were deposited under International Mycological Institute (IMI, Surrey, UK) collection (Table 1).

All isolates of *Pisolithus tinctorius* were cultured initially on agar plates of modified Melin-Norkran's (MMN) solution at room temperature. These cultures were then used to inoculate 500 ml flasks containing vermiculite-peat soaked in 180 ml MMN solution using the method of Mason (1980). Flasks were maintained at room temperature for four months prior to use as inocula.

Growth conditions: The experiment was carried out between June- October of 1997 in a glasshouse at the Institute of Terrestrial Ecology, Edinburgh, UK. Seeds of *E. camaldulensis* Dehnh. SILVERTON (UMBER.CK) provenance were pre-soaked in 0.1% Thiram (a dithiocarbamate fungicide) for 24 h, dried and sown into sterile trays containing sterilised vermiculite-peat (VP). Seeds were germinated in a growth cabinet at a temperature of 15-25°C and under fluorescent light (16 h irradiance, at 240-260 μmol m⁻² s⁻¹).

*Corresponding author:

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Seedlings were grown in sterilised VP with day/night thermal regime of $20/15\pm 2^\circ\text{C}$ and a light regime ranging between $400\text{--}800\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$. Vermiculite, peat and tap water were mixed in the proportions 45:10:175 by mass and sterilised. The mixture was autoclaved at a temperature of 121°C and a pressure of $1.06\ \text{kg cm}^{-2}$ for one hour. Two litre plastic pots were used for raising the seedlings. One seedling was transplanted from the tray into each pot. Inoculation was carried out by adding 4 g of mycelial VP to the planting hole. Control seedlings received autoclaved portions of the same inoculum. An Ingestad's solution for birch (Ingestad, 1971) was modified according to Mason *et al.* (1999a,b,c) and supplied to the plants. The proportions of N, P and K were 100:16:55. Four nutrient concentrations (Ingestad's 2.5, 5.0, 10 and 20 mg l^{-1} P) were used where all nutrient elements were proportionally adjusted. Plants were supplied twice a week with the solution with a gradually increased dosage every three weeks so that a total of 2, 4, 8, or 16 mg were added to various nutrient treatment pots respectively at the end of 18 weeks.

Table 1. Origin of fungal inocula

Fungus (Isolate code)	Country (place) of origin	Date of collection	Plant of origin
<i>Pisolithus tinctorius</i> (PTE)	Philippines (not known)	1990	<i>Eucalyptus camaldulensis</i>
<i>Pisolithus tinctorius</i> (K55)	Portugal (Obidos)	1993	<i>E. globulus</i>
<i>Pisolithus tinctorius</i> (PT3)	Tasmania (Murdunna)	1990	<i>E. globulus</i>
<i>Pisolithus tinctorius</i> (PT7)	Scotland (Glasshouse)	1993	<i>E. globulus</i>
<i>Pisolithus tinctorius</i> (PT8)	Scotland (Glasshouse)	1993	<i>E. globulus</i>

Mycorrhizal assessment: Pots were soaked overnight in water and roots from each pot were washed off soil by applying a gentle flow of water so that no fine roots were lost. The complete root-system was laid out on top of a graduated glass plate with the root collar set at 0 cm. Three sub-samples, each one cm long, were taken which corresponded with the top, middle and bottom parts respectively of each root system. After washing and sub-sampling, samples were placed in Petri dishes in water for examination of the tips of all short roots under a dissecting microscope. Root tips that had a short root with a mycelial mantle were defined as ectomycorrhizal. When the sub-samples assessed had no colonisation on them, the remaining roots of the root system under investigation were checked for mycorrhizal root tips.

Statistical analysis: Data were analysed by Analysis of Variance (ANOVA). When data were found not to have a normal distribution, necessary transformations (for example, arcsine transformation for mycorrhizal percentages and log transformation for all other variables) were carried out to normalise distributions and enable statistical comparisons of means. Means were compared by Fisher's least significant difference test when the results of Fisher's *F*-test from ANOVA were significant at $P \leq 0.05$. GENSTAT version 5.3 (Lawes Agricultural Trust, Rothamsted, Harpenden, Hertfordshire, UK) was used for statistical analysis and Microsoft Excel 97 for graphics.

Results

Influence of inoculation and nutrient regime on mycorrhizal colonisation: Although five fungal inoculants were used in the experiment, only *P. tinctorius* isolate K55 resulted in significant colonisation (up to 27% of the root tips mycorrhizal) at the end of 18 weeks. The other inoculants resulted in colonisation of only <1% of the root tips. Most colonisation was found to have occurred at 2.5 mg l^{-1} P that was significantly ($P = 0.007$) higher than that at either 10 mg l^{-1} P or 20 mg l^{-1} P (Fig. 1). There was no significant difference among colonisation at 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mg l^{-1} P (Fig. 1).

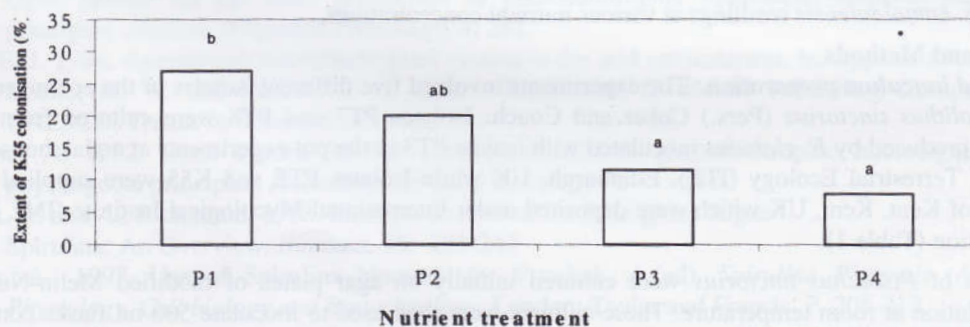


Fig. 1: Mean extent of ectomycorrhizal colonisation (%) by *Pisolithus tinctorius* isolate K55 under four nutrient treatments: P1, 2.5 mg l^{-1} phosphorus (P); P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P; P4, 20 mg l^{-1} P. Bars indicate mean and means with different letters are significantly ($P < 0.05$) different.

Interaction of inoculation and nutrient regime on growth: There was significant interactive effects of nutrient and fungus on shoot dry mass of seedlings ($P = 0.043$). Only *P. tinctorius* isolate K55 resulted in considerable colonisation (up to 27% of the root tips mycorrhizal) in the experiment. Seedlings inoculated

with this isolate showed significantly lower shoot dry mass than those under the uninoculated control as well as from those under the inoculation treatments using *P. tinctorius* isolates PTE, PT8 but not PT3 and PT7 at 2.5 mg l⁻¹ P (Fig. 2). Shoot dry mass of the seedlings inoculated with *P. tinctorius* K55 at the other three nutrient treatments were not significantly different from the uninoculated control.

Mycorrhizal colonisation resulted in negative growth responses in terms of stem diameter only ($P=0.023$). The other variables such as height, leaf area, stem dry mass, leaf dry mass, root dry mass and total dry mass were not affected by mycorrhizal colonisation. Stem diameter in the *P. tinctorius* isolate K55 treatment (which resulted in most of the colonization) was significantly lower as compared to those by isolate PTE or isolate PT7 or the uninoculated control. Isolates K55, PT3 and PT8 did not differ significantly in their effects on stem diameter. Regression between total dry mass and the extent of colonisation (Fig. 3) by the isolate K55 was significant ($P < 0.05$); total dry mass decreased with an increasing extent of colonisation by the isolate K55.

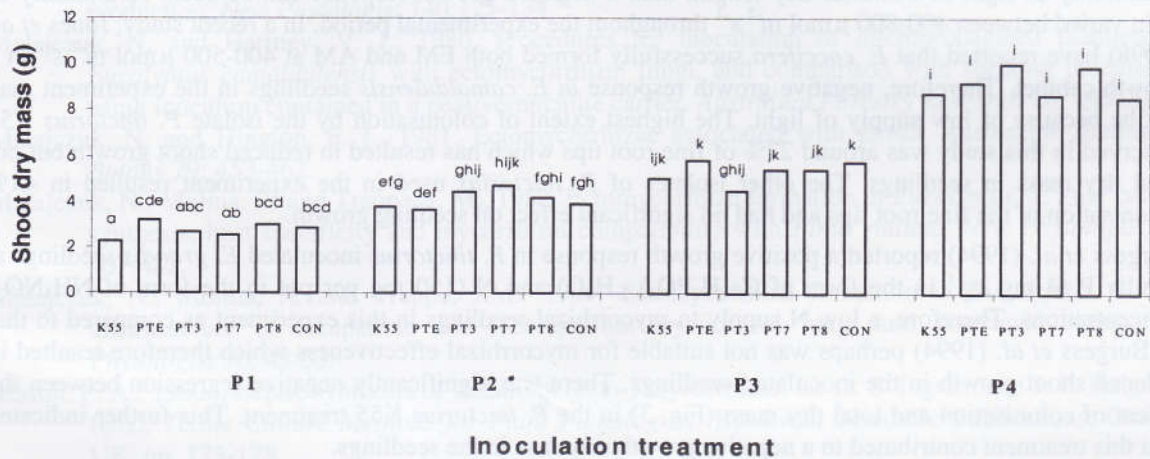


Fig. 2: Mean shoot dry mass of *Eucalyptus camaldulensis* seedlings inoculated with five different isolates of *Pisolithus tinctorius* (K55, PTE, PT3, PT7 and PT8) including control (CON) under four nutrient treatments: P1, 2.5 mg l⁻¹ phosphorus (P); P2, 5.0 mg l⁻¹ P; P3, 10 mg l⁻¹ P; P4, 20 mg l⁻¹ P. Bars indicate mean and means with different letters are significantly ($P < 0.05$) different.

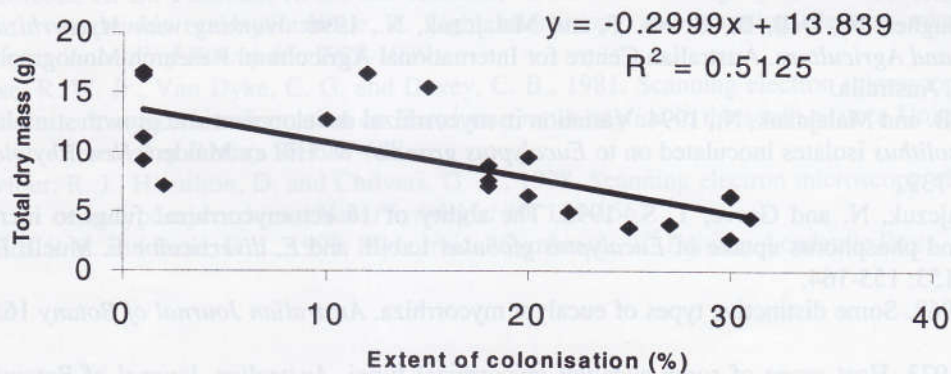


Fig. 3. Regression of the total dry mass of *E. camaldulensis* seedlings on extent of colonisation by *Pisolithus tinctorius* isolate K55.

Discussion

Among the five fungal isolates used in the experiment, only *P. tinctorius* K55 resulted in colonisation (up to 27% of the root tips) while the other isolates resulted in only <1% colonisation of the fine root tips. Colonisation by the isolate *P. tinctorius* K55 led to a negative growth response in *E. camaldulensis* seedlings in terms of shoot dry mass at 2.5 mg l⁻¹ P (Fig. 2). Most of the colonisation was found at 2.5 mg l⁻¹

P. This confirms the other findings where lower P concentrations (2-4 ppm) have been reported to be more favourable for EM formation in *Eucalyptus* by *P. tinctorius* (Burgess *et al.*, 1993; Mason *et al.*, 1999b). EM fungi, in general, require a larger amount of plant photosynthate as compared to arbuscular mycorrhizal fungi for their growth and maintenance (Smith and Read, 1997), and this can be realised from the fact that they need to form extensive mycelial mats or mantle on the root surface before penetrating the root endodermis to establish a functional symbiosis which involves formation of a Hartig net along with internal hyphae. Colpaert *et al.* (1996) stated that reduced seedling growth in response to EM colonisation may be the result of increased below-ground carbon allocation or it may be a consequence of high nutrient retention by the mycobiont.

In many mycorrhizal experiments, an initial growth drain on seedlings by mycorrhizal fungi have often been encountered (Janos, 1996). Often C drain is offset by an increased uptake of nutrient elements from soil/growth-medium by mycorrhizal seedlings. However, when seedlings fail to assimilate sufficient photosynthate to cover for an initial loss due to mycorrhizal association because of, for example, a low availability of light or a shorter day length, then a negative growth response can be seen. Availability of light varied between 400-800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ throughout the experimental period. In a recent study, Jones *et al.* (1998) have reported that *E. coccifera* successfully formed both EM and AM at 400-500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in a growth cabinet. Therefore, negative growth response in *E. camaldulensis* seedlings in the experiment may not be because of low supply of light. The highest extent of colonisation by the isolate *P. tinctorius* K55 observed in this study was around 27% of fine root tips which has resulted in reduced shoot growth but not total dry mass in seedlings. The other isolates of *P. tinctorius* used in the experiment resulted in <1% colonisation of the fine root tips and had no significant effect on seedling growth.

Burgess *et al.* (1994) reported a positive growth response in *P. tinctorius* inoculated *E. grandis* seedlings at similar P (4 mg kg⁻¹ in the form of Ca(H₂PO₄)₂·H₂O) and N (150 mg per pot in the form of NH₄NO₃) concentrations. Therefore, a low N supply to mycorrhizal seedlings in this experiment as compared to that of Burgess *et al.* (1994) perhaps was not suitable for mycorrhizal effectiveness which therefore resulted in reduced shoot growth in the inoculated seedlings. There was significantly negative regression between the extent of colonisation and total dry mass (Fig. 3) in the *P. tinctorius* K55 treatment. This further indicated that this treatment contributed to a negative growth response in the seedlings.

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