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A STUDY ON THE DEVELOPMENT OF ARBUSCULAR MYCORRHIZAS IN *Eucalyptus camaldulensis* Dehnh. SEEDLINGS AT DIFFERENT AGES AND THEIR EFFECTS ON SEEDLING GROWTH.

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Abstract: Host-symbiont interactions between *Eucalyptus camaldulensis* and arbuscular mycorrhizal fungi in terms of colonisation and growth responses in seedlings at 14 and 24 weeks were investigated. The experiment was set up using vermiculite-peat as a growth medium. The treatment combinations consisted of three AM inocula (*Glomus clarum* Nicolson and Schenck. isolate BR148-1, *Gigaspora rosea* Nicolson and Schenck. isolate FL105-5 and a mixed trap culture from Bangladeshi soil) and two nutrient regimes (Ingestad's 2.5 and 10 mg l^{-1} Phosphorus). This study showed that *E. camaldulensis* had more arbuscular mycorrhizal colonisation in the 14-week harvest (>50%) compared with that in the 24-week harvest (<40%). AM colonisation resulted in a negative growth response of *E. camaldulensis* seedlings at both the harvests.

Keywords: *Eucalyptus camaldulensis* Dehnh., *Glomus clarum* Nicolson and Schenck, *Gigaspora rosea* Nicolson and Schenck, arbuscular mycorrhizal fungi.

Introduction

Mycorrhizas are symbiotic associations between roots of higher plants and some groups of soil-inhabiting fungi (Smith and Read, 1997). In 1887, Frank classified mycorrhizas into two distinct groups: (a) endomycorrhizas or arbuscular mycorrhizas (AM) and b) ectomycorrhizas (EM). The AM grow inter-and intra-cellularly forming specific fungal structures such as arbuscules, hyphal coils and/or vesicles within the cortical cells. In the EM, the fungus forms an external hyphal mantle surrounding the root and an internal structure called the Hartig net (Smith and Read, 1997). Most species of the genus *Eucalyptus* predominantly form EM associations in native forests (Chilvers, 1973; Malajczuk and Hington, 1981) and plantations (Chu-Chou and Grace, 1982; Brundrett *et al*., 1996). However, there are reports of the AM associations of *Eucalyptus* species in their native forests in Australia (Malajczuk *et al*., 1981; Brundrett and Abbott, 1995), and in exotic plantations (Bakshi, 1966; Bala *et al*., 1989; Coelho *et al*., 1997; Gong, *et al*., 1997; Jamaluddin and Chandra, 1997; Oliveira *et al*., 1997). Bellei *et al*. (1992) reported that eucalypts remain colonised by AM fungi in their early stage of establishment, and as the seedlings mature, the AM fungi gradually get replaced by EM fungi. *Eucalyptus camaldulensis* has been reported to form AM both in the field and in the controlled environment (Jamaluddin and Chandra, 1997 and Vishwakarma and Singh, 1996). There is dearth of information to see if in absence of EM inocula the extent of AM colonisation drops down over time in the controlled environment. The overall objective of the present study was to assess the development of AM in the root systems of *E. camaldulensis* seedlings at various nutrient concentrations over different stages of seedling maturity and its effect on growth.

Materials and Methods

For all AM fungi, the source inoculum used in the pot culture was a mixture of colonised roots and soil. Approximately 20 g of inoculum was placed 1-2 cm below the surface of the plastic pots (250 ml) filled with the sterilised mixture of loam, sand and grit in the proportions 2:2:1. The pots were then sown with seeds of cowpea (*Vigna unguiculata* L.) and millet (*Pennisetum typhoides* L.), and then covered with a layer of sterilised grit. Pot cultures were maintained for four months in a growth cabinet at temperature of 15-25°C and under fluorescent light (16 h of irradiance; at 240-260 μmol m^{-2} s^{-1}). Pot cultures were harvested and root samples which were found to be ≥60% were used along with soils from the respective pots as inoculum.

The experiments involved a range of arbuscular mycorrhizal (AM) fungi. The isolates that were cultured for inoculation purposes are listed in Table 1.

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Table 1. Origin of fungal inocula

Inoculum	Isolate code	Country of origin	Place of origin	Date of collection	Plant of origin
Glomus clarum Nicolson and Schenck.	BR148-1*	Brazil	Rain forests	Not known	Not known
Gigaspora rosea Nicolson and Schenck.	FL105.5*	USA	Gainesville	Not known	*Glycine max*
A mixed culture	---------	Bangladesh	Cox's Bazar	March 1996	*Eucalyptus camaldulensis*

* The single species cultures were all provided by INVAM (International Culture Collection of Arbuscular and VA Mycorrhizal Fungi, College of Agriculture and Forestry, West Virginia University, USA).

***Growth Condition*:** The experiment was carried out between May 1998 and October 1998 in a glasshouse at the Institute of Terrestrial Ecology, Edinburgh, UK. In general, seedlings were grown with day/night thermal regime of 20/15±2°C and a light regime ranging between 400-800 µmol photons m^{-2} s^{-1}. Seeds of *E. camaldulensis* Dehnh. of 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}). Seedlings were grown in sterilised vermiculite-peat (VP). Vermiculite, peat and tap water were mixed in the proportions 45:10:175 by mass and sterilised. The mixture was autoclaved at 121°C and 1.06 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 inserting 5 g portions of soil-root mixture into the planting hole. Control seedlings received autoclaved portions of the same inoculum. A nutrient solution modified according to Mason *et al.* (1999a,b,c) from Ingestad's solution for birch (Ingestad, 1971) was supplied to the plants. The proportions of N, P and K were 100:16:55. Two nutrient concentrations (Ingestad's 2.5 and 10 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 mg and 8 mg phosphorus had been added to various nutrient treatment pots respectively by the end of 14 weeks and a total of 3.75 mg and 15 mg of phosphorus at the end of 24 weeks.

***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. Sampled roots were stained using the method of Phillips and Hayman (1970) with modifications given by Koske and Gemma (1989). The percentage of root length colonised was determined under a low power microscope using the grid-line intersect method (Tennant, 1975) modified by Giovanetti and Mosse (1980). Mycorrhizal colonisation was scored by the presence of vesicles, arbuscules or hyphae at the point of intersection between root and grid.

***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: At the end of 14 weeks, extent of colonisation by the three fungi varied between >35- <60% (of the total root length). There was a significant interactive effect of inoculation and nutrient treatments on colonisation of *E. camaldulensis* seedlings by different inoculants (P= 0.028). Colonisation by *Glomus clarum* isolate BR148-1 or the Bangladesh culture varied significantly between 2.5 mg l^{-1} P and 10 mg l^{-1} P. For both the inoculation treatments, the extent of colonisation was significantly higher at 10 mg l^{-1} P compared to that at 2.5 mg l^{-1} P. Colonisation by *Gigaspora rosea* isolate FL105-5 was not significantly different among the two nutrient treatments (Fig.-1).

At 24 weeks, there was no significant (P= 0.913) interactive effect of nutrient regime and inoculation on AM colonisation (Fig.-1). Also there was no significant difference in colonisation between the three AM

inoculation treatments (mean <40% of total root length) (Fig.-1). All three AM fungi had >35% colonisation at 2.5 mg l^{-1} P and <33% at 10 mg l^{-1} P.

Influence of Inoculation and Nutrient Regime on Growth: At the end of 14 weeks, there were significant interactive effects of inoculation and nutrient treatments on total dry mass (P= 0.003). The three fungi did not vary significantly in their effects on total dry mass of the seedlings grown at 2.5 mg l^{-1} P but each of them was significantly different from the uninoculated control at this nutrient regime (Fig.-2). At 10 mg l^{-1} P, *G. clarum* BR148-1 was associated with significantly lower dry mass of seedlings than the other two fungi of which none was significantly different from the uninoculated control.

At the end of 14 weeks, mycorrhizal colonisation resulted in negative growth responses in terms of shoot dry mass (P=<0.001), root dry mass (P=0.002) and total dry mass (P=<0.001) (Table 2). No difference between the three inoculant fungi was recorded in terms of their effect on root dry mass (Table 2). There was no significant difference between the effects of *G. rosea* FL105-5 and the Bangladesh culture on shoot dry mass and total dry mass, but all three fungal inoculants had a significant negative effect on growth (Table 2).

Table 2. Means of shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *Eucalyptus camaldulensis* seedlings at the 14-week harvest under four inoculation treatments

Inoculation treatment	SDM (g)	RDM (g)	TDM (g)
GC	2.903*a*	1.376*a*	4.279*a*
GR	3.633*b*	1.521*a*	5.153*b*
MC	3.631*b*	1.590*a*	5.221*b*
CON	4.124*c*	1.900*b*	6.023*c*

GC, *Glomus clarum* isolate BR148-1; GR, *Gigaspora rosea* isolate FL105-5; MC, a mixed culture from Bangladesh; and CON, the uninoculated control. Means with different letters for each variable are significantly different at $P \leq 0.05$ (ANOVA).

However, there was no significant interactive effect of nutrient regime and inoculation on any variable of seedling growth at the 24-week harvest.

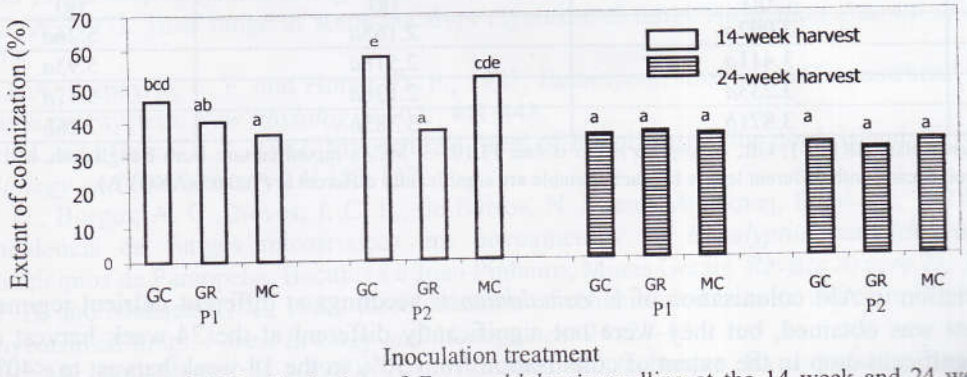

Fig. 1. Mean extent of AM colonization of *E. camaldulensis* seedling at the 14 week and 24 week harvest under three AM inoculation treatments. GC, *Glomus clarum* BR 148-1; GR, *Gigaspora rosea* FL 105-5; MC, a mixed culture from Bangladesh and two nutrient treatments: P1, 2.5 mg/l phosphorus (P); P2, 10 mg/l P. Bars indicate means and means with different letters are significantly ($p<0.05$) different.

The 24 week harvest indicated that mycorrhizal colonisation has resulted in negative growth responses in terms of shoot dry mass (P=0.020), root dry mass (P=<0.001) and total dry mass (P=<0.001) (Table 3).

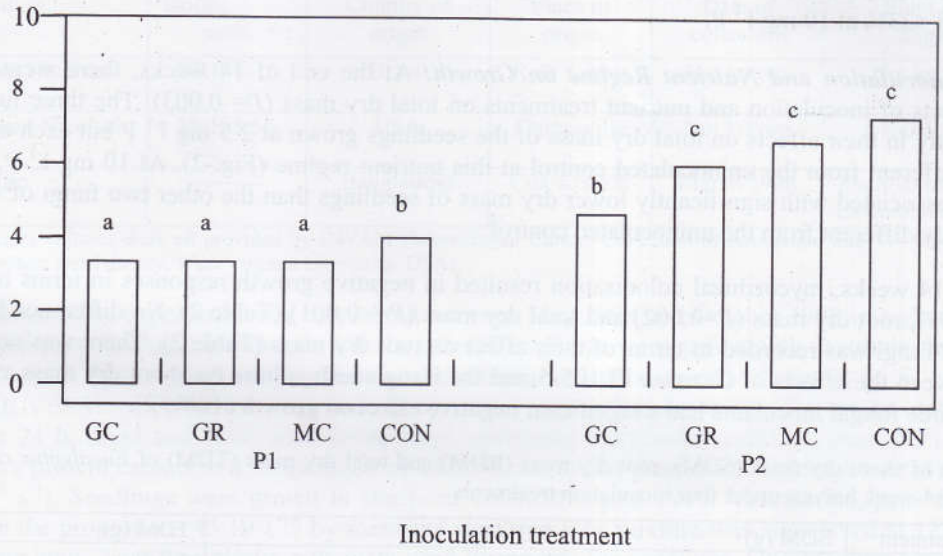

Fig.-2. Mean total dry mass of *E. camaldulensis* seedlings under four inoculation treatments by mycorrhizal fungi at 14 week harvest GC= Glomus clarum BR148-1, GR= Gigaspora rosea FL 105-5 and MC= a mixed AM culture from Bangladesh and two nutrient treatments: 2.5 mg/l phosphorus (P1) and 10 mg/l phosphorus (P2). Bars indicate means and means with different letters are significantly (p<0.05) different.

Table 3. Means of shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *Eucalyptus camaldulensis* seedlings at the 24-week harvest under four inoculation treatments

Inoculation treatment	SDM (g)	RDM (g)	TDM (g)
GC	3.092*a*	2.168*a*	5.26*a*
GR	3.411*a*	2.518*a*	5.93*a*
MC	3.233*a*	2.136*a*	5.37*a*
CON	3.871*b*	3.385*b*	7.26*b*

GC, *Glomus clarum* isolate BR148-1; GR, *Gigaspora rosea* isolate FL105-5; MC, a mixed culture from Bangladesh; and CON, the uninoculated control. Means with different letters for each variable are significantly different at $P \leq 0.05$ (ANOVA).

Discussion

Significant variation in AM colonisation of *E. camaldulensis* seedlings at different nutrient regimes at the 14-week harvest was obtained, but they were not significantly different at the 24-week harvest (Fig.-1). There was a significant drop in the extent of colonisation from 50% in the 14-week harvest to <40% in the 24-week harvest. There have been reports that eucalypt seedlings may initially have AM associations because of the rapid primary colonisation potential of AM fungi, which are replaced by EM associations as they mature (Lapeyrie and Chilvers, 1985; Chilvers *et al.*, 1987; Gardner and Malajczuk, 1988; Cázares and Smith, 1996). Therefore, the drop in the extent of colonisation from the 14-week harvest to the 24-week harvest may be attributable to the inability of the AM fungi for secondary colonisation. Although AM colonisation by *G. clarum* and the mixed culture varied significantly between the two nutrient regimes in the 14-week harvest they leveled off at the end of 24 weeks of growth (Fig.-1). There are reports that AM colonisation best occurs in moderate availability of nutrients (Smith and Read, 1997). In this experiment, however, accumulation of nutrients over time in the high phosphorus (10 mg l^{-1}) treated seedlings perhaps tended to inhibit the extent of AM colonisation in them. Extent of colonisation by any mycorrhizal fungus decreases with an increasing availability of soluble P in the growth medium, a trend that has been observed in a number of mycorrhizal experiments (Smith and Read, 1997; Misbahuzzaman, 1999).

In this study, AM colonisation led to a negative growth response mainly in the low P nutrient regime, that is, at 2.5 mg l^{-1} P (Fig.-2) where each of the three inoculant fungi had significantly lower total dry mass than the uninoculated control at the end of 14 weeks. At 10 mg l^{-1} P nutrient treatment of the 14-week harvest, only *G. clarum* BR148-1 was responsible for a significant growth depression in *E. camaldulensis* seedlings, and the other two fungi were not significantly different from the uninoculated control. Unlike the other fungi

used in the experiment, *G. clarum* BR148-1 produced structures such as large internal and external spores that could have caused a higher carbon drain on the seedlings (Misbahuzzaman and Ingleby, 1999). Carbon drain was considered to occur as a result of colonisation by *Glomus* species on a variety of hosts (Varma and Schüepp, 1994) in controlled conditions.

At both harvests, AM colonisation resulted in significantly lower shoot dry mass, root dry mass and total dry mass of the seedlings compared to the controls (Table 2 and Table 3). The growth depression may occur because of C drain from seedlings (Smith and Read, 1997). The large spores of AM fungi, in general, act as energy reserves (Janos, 1996) thus storing C in them. Moreover, growth depression may, sometimes, occur in pot-grown seedlings because of their increased root density in restricted volume of growth media (Khaliq and Sanders, 1998). However, positive growth responses in *E. camaldulensis* seedlings from AM inoculation have been reported in a study done by Vishwakarma and Singh (1996) but no account on the nutrient regimes used in that experiment was reported in their work. However, one of the other differences between their study and this study is that they used different AM fungal inoculants. Therefore, a variety of AM fungal species could be tried using a broad range of nutrient regimes and different volumes of growth media to assess the growth performance of mycorrhizal *E. camaldulensis* seedlings.

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