MYCORRHIZAL ASSOCIATIONS OF *Eucalyptus camaldulensis* Dehnh.

by

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Declaration

I hereby declare that this thesis has been composed by me and that the work recorded is my own, except where acknowledged to the contrary, and no part of it has been presented for a higher degree.

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DEDICATION

This thesis is dedicated to my mother Manoara Begum
who died during its preparation, but who would have been the happiest person
known to me to see it being completed.

ABSTRACT

The species *Eucalyptus camaldulensis* Dehnh. is of great importance in Mediterranean, sub-tropical and tropical countries for the production of domestic products, such as poles, posts, timber and fuelwood. Some members of the genus *Eucalyptus* are reported to form both arbuscular- (AM) and ecto-mycorrhizas (EM). The main objectives of this study were to look at the host-symbiont interactions between *E. camaldulensis* and AM and EM fungi, and interactions between the two mycorrhizal types. The initial aim of the project was to determine suitable experimental conditions for the formation of both types of mycorrhizas on *E. camaldulensis* seedlings. Two experiments, the first with AM fungi and the second with EM fungi, were set up successively using vermiculite-peat (VP) and sand-perlite (SP) as growth media, and 10 mg l^{-1} and 30 mg l^{-1} phosphorus (P) Ingestad's nutrient solution in each case. *Glomus intraradices* Schenck and Smith isolate UT143-2 and *Pisolithus tinctorius* (Pers.) Coker and Couch isolate PTE were used as the test AM and EM fungus respectively. Results showed that both AM and EM colonisation were very low (1-6%), but even so AM inoculation had a significantly depressive growth effect on seedlings of *E. camaldulensis*. In both experiments VP was found to be the best medium for both the growth of seedlings and the formation of mycorrhizas. A subsequent experiment using one nutrient concentration (5 mg l^{-1} P) and three AM and six EM isolates with VP as the growth medium resulted in colonisation of up to 20% by two AM fungi (*Glomus clarum* Nicolson and Schenck isolate BR148-1 and *Gigaspora rosea* Nicolson and Schenck isolate FL105-5) but none of the EM fungi used in that experiment formed any mycorrhizas. The fourth experiment using three AM inocula (including two from the previous experiment and one from a trap culture of Bangladeshi soil) and four nutrient regimes (Ingestad's 2.5, 5.0, 10 and 20 mg l^{-1} P) resulted in 30-50% colonisation; most colonisation was by *G. clarum* BR148-1 and was greatest at 10 mg l^{-1} P (>50%). AM colonisation again resulted in a negative growth response of *E. camaldulensis* seedlings. In a similar experiment using five isolates of *P. tinctorius*, only isolate K55 resulted in colonisation >15% most of which occurred at 2.5 mg l^{-1} P (>25%) while the other isolates resulted in <1% colonisation. Like AM fungi, colonisation by *P. tinctorius* K55 reduced growth in terms of shoot dry mass in *E. camaldulensis* seedlings. In a further experiment, colonisation by AM and EM fungi were found to be not affected by each other in the AM-EM inoculated seedlings although EM colonisation was much lower (<10%) than AM colonisation (up to 40%). Again, inoculation reduced growth compared with the uninoculated controls, with AM <(AM+EM) <EM in terms of the extent of reduction in dry mass.

Microscopy indicated that the AM structures produced by *E. camaldulensis* had appressoria formation, coiled hyphae and arbuscules which were typical of normal arbuscular mycorrhiza but the EM structures appeared to be incomplete or incompatible having little Hartig net formation, little elongation of epidermal cells and phenolic-filled vacuoles in the epidermal cells.

This study showed that *E. camaldulensis* tended to be colonised more readily by arbuscular- than ecto-mycorrhizal fungi in the initial period of seedling growth, but that such colonisation may have a negative impact on growth. There were indications that the species could become ectomycorrhizal after 16 weeks of growth in glasshouse, but that proper care should be taken to select appropriate and compatible EM inoculants.

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CHAPTER 1

Introduction

1.1 Characteristics of *Eucalyptus* Species

1.1.1 *General Information About the Genus Eucalyptus*

Eucalyptus is native to Australia and parts of South East Asia (Indonesia). More than 600 species of *Eucalyptus* are known (Blake, 1953). The genus *Eucalyptus* was named in 1788 by Charles-Louis L' Heritier de Brutelle, a citizen of France working in London at the time (Boland *et al.*, 1984). The genus grows under a wide range of rainfall and temperature in its native range; in high rainfall areas, some of the giant trees have a top height of 90 m, whereas dwarf forms of *Eucalyptus* are quite common in open scrub and low rainfall areas (FAO, 1979). It is the most important genus of forest trees in Australia because of its wide geographic distribution and economic importance.

Eucalypts are widely planted in tropical, sub-tropical and temperate regions of the world, with the plantation area likely to exceed 10 million hectares by the year 2000 (Florence, 1996). When planted outside their natural habitat many species of *Eucalyptus* have shown promising growth and a high degree of tolerance to extremes of latitude and altitude, having been planted between latitudes of 40°N to 45°S (Eldridge *et al.*, 1993). On moderate planting sites, in temperate and tropical countries, eucalypts have a mean annual increment ranging from 10 to 15 m^3 ha^{-1} y^{-1} , while on the best sites mean annual increments of 70 to 90 m^3 ha^{-1} y^{-1} have been obtained at the age of 6-8 years in Africa, Brazil and Papua New Guinea (Eldridge *et al.*, 1993). Where it is grown as an exotic, its wide range of adaptations to different planting sites, simplicity of management system, ability to grow even on wastelands and high yield compared to other species make *Eucalyptus* one of the most widely propagated genera throughout the world (Eldridge *et al.*, 1993). It is believed that

Eucalyptus was probably first grown as an exotic in Portugal about 400 years ago and thereafter planting activities were expanded to many tropical and Mediterranean countries. Large-scale plantations of *Eucalyptus* have been established in Brazil, India, South Africa, Portugal, Spain, Angola, China, Ethiopia, Argentina, Morocco, Uruguay, Peru, and Chile, totalling 6 million hectares in 1985 (Eldridge *et al.*, 1993). Out of these, the greatest area occurs in Brazil (2,500,000 ha) followed by India (550,000) and South Africa (470,000).

Eucalypts are grown mainly as sources of pulpwood, fuelwood and charcoal, mining timber, fibreboard and essential oils. The eucalypt has become one of the world's major sources of fibre for the production of the bleached kraft pulp used in the manufacture of finer quality printing and writing papers (Florence, 1996). However, eucalypts have not been widely harvested as sawlogs in countries where they are planted as exotics, although this is changing as conventional sources of sawlogs decline, and as specialised sawmilling and other technologies are developed for processing eucalypt logs with small diameters (Florence, 1996).

Though it is still widely planted throughout the world, there has recently been adverse reaction from both the general public and some scientific quarters against the planting of *Eucalyptus* in many countries. The main arguments against *Eucalyptus* are: 1) it removes too much water from stream or underground water supplies (Poore and Fries, 1985; Florence, 1996); 2) it degrades the soil through excessive depletion of nutrient reserves, and generates soil erosion (Florence, 1996); 3) it directly inhibits understorey species and adjacent crops (Florence, 1996); 4) it conflicts with the principle of conserving native species (Poore and Fries, 1985) and 5) it has low recreational and aesthetic values (Poore and Fries, 1985). Most of these criticisms would equally apply to all fast growing exotic species (Eldridge *et al.*, 1993) and the merits and demerits of *Eucalyptus* should be judged rationally from the social, economic and ecological points of view.

The first thorough classification of *Eucalyptus* was by George Bantam (1863-78) in the *Flora Australensis* (Florence, 1996). The classification placed primary emphasis on the morphology of the anther. W. F. Blakely (1934, *A Key to Eucalypts*) extended Bentham's antheral classification, but did not significantly advance the orderly classification of species. However, based on the most recent taxonomic classification of Johnson (1971) and Briggs (1983), the genus *Eucalyptus* contains 8 sub-genera out of which the sub-genera *Monocalyptus* (with one operculum) and *Symphyomyrtus* (with two opercula) represent the largest number of species (see Eldridge *et al.*, 1993).

The sub-genus *Symphyomyrtus* consists of nine sections out of which section *Transversaria* (*E. grandis, E. saligna,* and *E. urophylla*), section *Exsertaria* (*E. camaldulensis, E. exserta,* and *E. tereticornis*) and section *Maidenaria (E. dalrympleana, E. globulus, E. gunnii, E. maidenii, E. nitens*, and *E. viminalis*) are the most commonly planted *Eucalyptus* species throughout the world.

1.1.2 ***General Description of Eucalyptus camaldulensis*** **Dehnh.**

1.1.2.1 ***Taxonomic classification and morphological characteristics***

Eucalyptus camaldulensis Dehnh. is classified in the sub-genus *Symphyomyrtus,* and section *Exsertaria* (*E. camaldulensis, E. exserta* and *E. tereticornis*). It is a medium sized to tall tree. It is commonly up to 20 m tall, but occasionally exceeding 45 m. Its trunk is often short and crooked and reaches a basal diameter of 0.6 to 1.0 m. The crown is large and in open formation the tree usually has a short, thick bole (Boland *et al.*, 1984). Blake (1953) describes *E. camaldulensis* as follows:

"tree of varied habit with smooth, deciduous, white or pale grey bark over the greater part, often with patches of darker grey, and sometimes with a variable amount of grey, flaky, bark persisting on the lower part; branchlets often long and pendulous. Juvenile leaves opposite for a few pairs, petiolate, ovate to broadly lanceolate, glaucous. Intermediate leaves broadly lanceolate to lanceolate, up to about 16 x 7.5 cm, glaucous. Adult leaves dull and often pale-coloured, more or less dropping, alternate, prominently petiolate or narrowly lanceolate, acute or acuminate, 6-30 cm long, 0.8-2 cm wide, about 8-20 times as long as wide, with between 20 and 40 pairs of lateral veins at an angle of 40° to 50° with the midrib, the intramarginal vein about 0.9-1.5 mm from the margin. Inflorescence of axillary, 5-1--flowered umbles; peduncles slender, 6-15 mm long; pedicels slender, 3-8 mm long. Buds broadly or occasionally narrowly ovoid, rostrate, acuminate, or acute, rarely obtuse, 6-10 mm long, 4-5 mm wide with a short and broad calyx-tube and a longer (up to 3 times as long), rostrate-acuminate or apiculate (rarely bluntly rounded) operculum. Anthers versatile, obovoid-oblong with parallel cells opening in longitudinal slits. Ovary prominently domed. Fruits subglobose to ovoid in outline, 5-8 mm long, slightly narrower to slightly wider than long, with a short calyx-tube, a high domed disc, and stout, deltoid valves strongly incurved from a slightly spreading base.''

1.1.2.2 *Natural distribution and habitat*

This species is called river red gum in its country of origin, Australia. It is the most widely distributed of all eucalypts in Australia (Boland *et al.*, 1984; Florence, 1996). With the exception of the southern parts of western Australia, the Nullerbor Plain and the coastal fringe of most of Victoria, New South Wales and eastern Queensland, it is found throughout mainland Australia. It occurs along or near almost all of the seasonal watercourses in arid and semi-arid areas and it is found along many other streams and rivers (Boland *et al.*, 1984).

The range in latitudes is approximately 12.5-38° S and that for altitude is from about 20 to 700 m (Boland *et al.*, 1984). This species grows under a wide range of climatic conditions from warm to hot, sub-humid to semi-arid, with the mean maximum temperature for the hottest month in the range 27-40°C and the mean minimum for the coldest around 3-15°C. The mean annual rainfall range is mostly 250-600 mm.

Soils are typically sandy alluvial (Boland *et al.*, 1984). It can tolerate drought of 4-8 months or more (Eldridge *et al.*, 1993).

1.1.2.3 ***The form of the seedling root***

A relationship between characteristics of the seedling root system and the natural habitat also appears to be a feature of the eucalypt (Florence, 1996). This may be expressed through the root:shoot ratio of the plant, or a tendency to develop a strong tap root. The rapid development of a strong tap root may be an important characteristic of growing in dry environments. *E. camaldulensis* is, perhaps, a specialised example, but it is well known for its capacity to produce a strong tap root enabling it to penetrate quickly the clay layer of the flood plain on which it sometimes occurs (Florence, 1996). By growing seedlings of *E. camaldulensis* and seven other species in 1 m long tubes from a forest on the south coast of New South Wales (Australia), Neave and Florence (1994) found that only *E. camaldulensis* produced a strong tap root and a greater root weight towards the base of the tube in a dry soil as well as in a moist soil. The ability of the seedlings to develop a strong, deeply descending root system in drying soil is undoubtedly an important adaptive feature under drought conditions (Florence, 1996). Moreover, it may be the production of a strong tap root, rather than a large root:shoot ratio, which is the best index of drought adaptation (Florence, 1996).

1.1.2.4 ***Tolerance to waterlogging and salinity***

While waterlogging and poorly drained soils are generally unsuitable for eucalypts, there are some species such as *E. camaldulensis* and some other *Symphyomyrtus* species which can tolerate periodic flooding, and hence are more competitive on poorly aerated or waterlogged soils (Florence, 1996). *E. camaldulensis* is reported to be able to tolerate soil salinity but there is considerable variation in salt tolerance between its provenances. Sands (1981) has shown a gradient in salt tolerance within southern Australian provenances of the species. Seedlings grown from the Lake

Albacutya were the most tolerant, those from Shepparton the least tolerant, and those from Port Lincoln, intermediate. A wider evaluation of provenances of this species (see Midgely *et al.*, 1989) suggests that salt tolerance does not conform to any geographic pattern.

1.1.2.5 *Growth and yield of E. camaldulensis*

E. camaldulensis is one of the most extensively planted species of *Eucalyptus* in the world (FAO, 1979). In countries where the correct provenance is planted, it is an efficient source of timber, yielding maximum biomass production under short rotations. In drier countries, typical plantation yields are 5-10 m^3 ha^{-1} y^{-1} on a 10-20 year rotation, whereas in an area of sufficient moisture for its growth, 30 m^3 ha^{-1} y^{-1} has been recorded (Champion and Brasnett, 1958; Evans, 1992).

1.1.2.6 *Wood properties and utilisation*

The wood of *E. camaldulensis* is strong, hard and heavy, durable and resistant to termites. As noted in the review of Midgley *et al.* (1989), the following basic densities were recorded: (i) 444 to 593 kg m^{-3} at age 10 in Italy; (ii) 487 to 576 kg m^{-3} in Zimbabwe; and (iii) 610 to 640 kg m^{-3} in Sri Lanka for trees grown in plantation. The sap wood is thick and pale red and the heart wood is from reddish to dark red in colour. It is not difficult to saw, but it tends to warp in drying and care has to taken during seasoning. The wood has an average energy content of 19.8 MJ kg^{-1}; it burns quickly and makes good charcoal (Champion and Brasnett, 1958). The only limitation on its use in open fire places is that the wood produces dense smoke.

E. camaldulensis is valuable for many purposes including shelter and honey production. The wood is used for construction materials, railway sleepers and charcoal in many parts of the world. In Australia it is extensively used for railway sleepers, heavy construction timbers, fences, flooring and the foundation of wooden houses (Champion and Brasnett, 1958).

1.1.3 *Ecology of Eucalyptus camaldulensis Planted as an Exotic*

Success or failure for the establishment of *E. camaldulensis* (as well as other eucalypts) plantations in the tropics has always depended on the variability of its provenances. The best *E. camaldulensis* (and of *Eucalyptus tereticornis* Smith) provenances in terms of survival and growth were those of more of tropical origin, that is, from the far north of the Australian continent (Florence, 1996). Provenance variation in northern Australia may reflect the adaptation of *E. camaldulensis* to sites varying in rainfall and soil water status. The water use efficiency of seedlings from the outstanding Petford provenance (720-mm rainfall predominantly in summer) is greater than that of the Tennant Creek (dry region) provenance. The latter provenance appears to have an opportunistic strategy of rapid growth during the limited period in which water is readily available- at the expense of water use efficiency (Hubick and Gibson, 1993). Seedlings of the species from semi-arid environments may adjust morphologically to the onset of drought by developing leaves in which water is limited to the small patches of lamina (Gibson *et al.*, 1991). These patches maintain low levels of photosynthesis, with the greater part of the lamina having no gaseous exchange with the atmosphere at all. In contrast, seedlings from monsoonal environments with more humid, winter mornings, do not restrict gaseous exchange in this way, but close their stomata rapidly and completely after gaseous exchange during the favourable morning hours.

Eucalyptus camaldulensis (as well as *E. tereticornis*) is capable of rapid water use. It has been used successfully in a number of countries (for example, Italy and Turkey) to help control swamps and wetlands (for example, Ghosh *et al.,* 1978). It is circumstances such as these which may have helped to generate the belief that the eucalypts in general and *E. camaldulensis* in particular will rapidly exhaust soil water under most conditions. However, this is not necessarily the case. Where soil water is becoming limiting for rapid growth, or the temperature rises and atmospheric humidity declines, *E. camaldulensis* has the capacity to regulate water use sensitively in order to maintain an essential access to a water supply. It is, perhaps, one of the more remarkable attributes of this widely planted species that it can behave as a highly responsive mesophyte where there is an ample supply of water, yet regulate water use, grow moderately and tolerate relatively long dry periods where there is not (Florence, 1996). Hookey *et al.* (1987) found in their experiment in Western Australia that where growing conditions were favourable in spring, *E. camaldulensis* and another fast growing forest species (*Eucalyptus saligna* Sm.) had a much higher rate of stomatal conductance than typical woodland species. However, stomatal conductance in this species responded very sensitively to a drying atmosphere, even in spring when moisture in the soil was in ample supply. When soil and atmosphere were drier in summer, the stomatal conductance of the species was consistently below that of the woodland species. The similar capacity of *E. camaldulensis* and *E. tereticornis* to regulate water use through stomatal control is illustrated by Roberts *et al.* (1992) in a field study in Southern India. Towards the end of the lengthy dry season, stomata were closed for most of the day after having opened (presumably) for a brief time early in the morning. Under non-stressed conditions in the immediate pre- and post-monsoonal periods, stomatal conductance could be high early in the day, then decline throughout the day. During the monsoon itself, stomatal conductance remained at a moderate and more-or-less constant rate throughout the day.

1.2 Mycorrhizas

The term mycorrhiza was first used in 1885 by Frank (cited in Nicolson, 1967) to describe non-pathogenic associations between fungi and the roots of higher plants. Both partners usually derive benefit from the association (Wheeler *et al.,* 1991). By enhancing access to the growth-limiting nutrients, mycorrhizas can significantly increase carbon fixation (Smith and Read, 1997). This gain occurs primarily via increased photosynthetic rates (Allen *et al.*, 1981; Reid *et al.*, 1983). This symbiotic association improves the uptake of a range of macronutrients, especially immobile phosphorus (P). The mycorrhizal association also improves uptake of micronutrients such as zinc and copper (Gilmore, 1971). This increased uptake has been attributed to factors such as reduced spatial diffusion of the nutrient to the plant, increased rate of absorption and concentration at the absorbing surface, and chemical alteration of the nutrient making it more available for uptake (Smith and Read, 1997). Mycorrhizas can also transfer nutrients between plants of the same and different species (Francis and Read, 1984). However, the mycorrhizal symbiosis can play an important role in generally improving plant viability (Perrin, 1990; Schönbeck and Dehne, 1981). In 1887, Frank classified mycorrhizas into two distinct groups: (a) endomycorrhizas which grow inter- and intra-cellularly forming specific fungal structures (such as arbuscules, hyphal coils and/or vesicles which are defined later- see section 1.3.1) within the cortical cells, and (b) ectomycorrhizas where the fungus forms an external hyphal mantle surrounding the root and an internal structure (called Hartig net which is defined later- see section 1.4). Endomycorrhizas are formed by the fungal group Zygomycetes and ectomycorrhizas are formed by the fungal group Basidiomycetes (Smith and Read, 1997). In spite of their profound morphological and taxonomic differences, some of the effects of AM and EM fungi on host physiology are similar (Tinker *et al.*, 1992; Jones *et al.*, 1998). Endomycorrhizas will here be referred to as Arbuscular Mycorrhizas or AM and the term Ectomycorrhizas will be abbreviated to EM throughout the thesis.

1.2.1 *Mycorrhizal Associations of Plants*

Mycorrhizal associations are widespread among plant families and appear to have evolved and spread with the earliest land plants (Allen, 1991). Evidence from the fossil record demonstrates an early appearance of mycorrhizas in roots (Kidstone and Lang, 1921; Wagner and Taylor, 1981; Stubblefield *et al.*, 1987). These observations led to the proposal that invasion of the land by plants depended in part on the evolution of mycorrhizas (Pirozynski and Mulloch, 1975; Pirozynski, 1981). Baylis (1975) has pointed out that plant species with a poor development of root hairs tend to be more mycotrophic; that is, dependent upon mycorrhizal fungi for nutrient uptake, than those with good development of root hairs.

The concept that plants have varying degrees of dependence on mycorrhizal associations has gained acceptance (see Janos, 1980a; Brundrett, 1991). Detailed examinations of plants in natural ecosystems often show consistent differences between host plants occurring in a particular habitat in both the intensity and consistency of mycorrhiza formation (Brundrett *et al.*, 1996c). These observations have shown that species generally either have consistently high mycorrhizal colonisation, intermediate or variable degrees of mycorrhizal colonisation, or are not mycorrhizal at all (Janos, 1980a; Brundrett and Kendrick, 1988). In the field, plant species can be obligately mycotrophic or facultatively mycotrophic (Janos, 1980b). Obligately mycotrophic species replace facultatively mycotrophic species as fertility declines, provided that suitable inoculum is available.

Amaranthus and Perry (1989) and Borchers and Perry (1990) provide evidence that conifer and hardwood shrub communities interact below-ground through a shared rhizosphere microflora. The tropics, where EM-dominated forests are less common than AM-dominated forests provide striking examples of interactions among mycorrhizal types (Janos, 1980a). Tropical forests dominated by EM hosts are less rich in tree species, compared with AM-dominated tropical forests. Janos (1980b) hypothesised that this was due to competitive exclusion of AM hosts by EM hosts combined with greater host specificity of EM fungi. EM species become more competitive at very low fertility (Janos, 1980b). Alexander (1987), Newbery *et al.* (1988), Newbery and Gartlan (1996) and Newbery *et al.* (1997) observed that three EM caesalpinoid leguminous tree species (*Microberlinia bisculata* A. Chev., *Tetraberlinia bifoliata* (Harms) Hauman and *T. moreliana* Aubrév) form distinct stands amongst a diverse matrix of AM species in the Korup National Park in Cameroon, and these stands tend to be associated with low phosphate supply. This pattern of local dominance of EM trees may reflect the ability of EM fungi to use sources of nitrogen and phosphorus (Abuzinadah and Read, 1986), enabling them to outcompete AM species which may not have this capability (Janos, 1983).

Plant communities may also be affected by mycorrhizal interactions between and among host plants that are connected by shared mycorrhiza mycelia. Transport of assimilates through an intact hyphal network has been demonstrated in both AM and EM systems (Francis and Read, 1984; Read *et al.*, 1985; Whittingham and Read, 1982; Fitter *et al.*, 1998) which in AM systems tend to promote species richness (Grime *et al.*, 1987) and in EM systems leads to dominance of a few species which are susceptible to EM colonisation (Newbery *et al.*, 1988).

1.2.2 *Dual Mycorrhizal Associations*

Surveys of mycorrhizal literature have established that plants within a genus usually have the same type of mycorrhizas, that is, EM, AM, etc., or else they remain non-mycorrhizal (Brundrett *et al.*, 1996c) and these relationships are generally also consistent within a family (Harley and Harley, 1987; Newman and Reddell, 1987; Brundrett and Abbott, 1991). This high correlation between plant phylogeny and mycorrhizal relationships has been observed for families with EM, as well as those containing species that are usually non-mycorrhizal, but there are also many exceptions (Smith and Read, 1997; Testier *et al.*, 1987; Brundrett, 1991). Molina *et al.* (1992) listed 42 plant genera known for their ability to form both EM (ericoid) and AM. In the northern hemisphere, *Populus* spp. often form both EM and AM (Brundrett, 1991). Many Australian trees and shrubs such as *Eucalyptus* and *Acacia* species have been reported to have both EM and AM associations (Warcup, 1980; Malajczuk *et al.*, 1981; McGee, 1986; Chilvers *et al.*, 1987; Reddell and Warren, 1987; Brundrett and Abbott, 1991). Besides *Populus* and *Acacias*, *Alnus* and *Salix* and some woody legumes can also form both AM and EM (Harley and Harley, 1987). However there is some controversy about the relative importance of EM and AM associations in plants which have both (Brundrett *et al.*, 1996c). For example, in northern and eastern Australia, *Acacia* species were reported to have dual AM and EM associations or AM only (Warcup, 1980; Reddell and Warren, 1987; Bellgard, 1991), while acacias from south-western Australia had AM, but not EM (Jasper *et al.*, 1989; Brundrett and Abbott, 1991). In the family *Casuarinaceae*, the genus

Casuarina apparently has AM while *Allocasuarina* has EM and AM (Brundrett *et al.*, 1996c).

There have also been reports that eucalypt seedlings may initially have AM associations, which are replaced by EM associations as they mature (Lapeyrie and Chilvers, 1985; Chilvers *et al.*, 1987; Gardner and Malajczuk, 1988). Similar succession from AM to EM in the same root system has been described for *Helianthemum* (Read *et al.*, 1977), and *Alnus* (Molina *et al.*, 1994; Arveby and Granhall, 1998). Lapeyrie and Chilvers (1985) suspected that predominantly EM tree species may be capable of brief AM episodes in the seedling stage and the AM may be important to the early establishment of plants in low nutrient or calcareous soils. Chilvers *et al.* (1987) considered AM fungi to be well-adapted to rapid primary colonisation and perpetuation within individual roots but inferior to EM fungi for secondary colonisation because of slow hyphal spread via root branches. Cázares and Smith (1996) hypothesised that AM fungi readily colonise typically EM hosts that establish early in plant community succession, in areas where EM propagules are sparse or absent. However, hosts that establish later in plant community succession are less readily colonised by AM fungi. Although these different hypotheses exist, the understanding of this AM-EM succession is still not very clear.

Similar fungal succession but within EM was reported by several authors (Mason *et al.*, 1982; Fleming *et al.*, 1984; Last *et al.*, 1984) in *Betula* spp. EM fungal species *Laccaria* and *Hebeloma* colonised Scottish birch early in the succession, while *Lactarius pubescens* (Fr. ex Krombh.) Fr. and *Leccinum* spp. colonised it later (Deacon *et al.*, 1983). Fleming *et al.* (1986) suspected that these late-stage fungi may have required a food base provided by a mature tree in order to have sufficient inoculum potential to colonise birch seedling roots. Although these late-stage fungi formed few mycorrhizas on glasshouse-grown seedlings compared to early-stage fungi (Fleming *et al.*, 1986), Mason *et al.* (1983) observed in an earlier study that both early- and late-stage fungi formed mycorrhizas on seedlings growing in axenic (aseptic) conditions. Fleming *et al.*, (1986), therefore, suggested that soil or other site factors can modify the behaviour of a mycorrhizal fungus and that the concept of early- versus late-stage must be viewed in relation to soil and other biotic and abiotic variables. A similar trend is likely to prevail in the AM-EM succession of the dual mycorrhizal plant species. Soil rich in organic matter may favour EM fungi to establish and form mycorrhiza (Smith and Read, 1997) which under mature trees are obviously abundant because of accumulation and decomposition of litter. However, recent studies suggest that inoculum availability (Newton, 1992; Oliveira *et al.*, 1997; Taylor, 1998; Moyersoen and Fitter, 1999) and identity of partners with respect to habitat (Newton, 1992; Moyersoen and Fitter, 1999) are the main factors that control the occurrence of mycorrhizal type(s).

Eucalyptus arbuscular mycorrhizas were first described by Asai (1934) and then by Maeda (1954), but the first synthesis in controlled conditions (Malajczuk *et al.*, 1981) as well as the first ultrastructural studies (Boudarga and Dexheimer, 1989) are fairly recent (Lapeyrie *et al.*, 1992). In pot experiments, with natural (calcareous) soil, the AM were more prevalent on young seedlings of *E. dumosa* A. Cunn. ex Schau. whereas EM took over as the plant aged (Lapeyrie and Chilvers, 1985). However, both types of symbionts can be present simultaneously in the same root apex: the AM fungus colonises the inner part of the cortex while the EM is restricted to the outer cell layer. In a southern Brazilian *Eucalyptus* (*E. viminalis*) plantation, the AM-EM succession occurred during the first year (Bellei *et al.*, 1992). In another study of the same area, however, Oliveira *et al.* (1997) found that *E. viminalis* investigated in the study of Bellei *et al.* (1992) was planted on AM-dominated sites where EM became prevalent later. They found a stronger EM colonisation of *E. dunnii* in a different site of the same area that had previously carried EM-dominated *E. viminalis*. Therefore, they argued that occurrence or succession of AM and EM in eucalypts is mainly controlled by the availability of inoculum although they did not disregard the possibility that also host preference to some fungal strains could play a role in the AM/EM succession. Some evidence suggests that the succession between AM and EM during host plant ageing could be related to competition for colonisation sites (Chilvers *et al.*, 1987). However, none of these studies could reach any conclusion regarding the interaction of AM and EM fungi in the same root system.

A technique has been recently described for the *in vitro* synthesis of AM and EM on the same root apex (Boudarga *et al.*, 1990). In this technique, an attempt was made to assess the structure and function of both the symbionts. A preliminary ultrastructural study showed that both types of mycorrhiza could be found in an active state, even when they are involved in a dual association. In such dual mycorrhiza the ultrastructure of each individual symbiotic association was indistinguishable from that found in single AM or EM. The AM endophyte was functional within the EM as indicated by the occurrence of a continuous host cell plasma membrane around the arbuscule. However, the EM may not become totally operational until the AM arbuscules are degenerating (Boudarga, 1989; as cited in Lapeyrie *et al.* 1992).

Lopez-Aguillon and Garbaye (1989) found that hybrid poplars inoculated with both AM and EM fungi resulted in increased biomass compared to AM or EM alone. Muchovej and Amorim (1990) (as cited in Lapeyrie *et al.*, 1992) conducted experiments where seedlings of *E. grandis* were inoculated with a mixed AM-EM inoculum. After two months, the extent of colonisation by either symbiont was not dependent on the presence or absence of the co-symbiont. EM inoculation stimulated plant growth compared with the uninoculated control and stimulated growth of seedlings co-inoculated with AM fungal isolates compared with the AM treatment alone. In contrast, AM inoculation had no effect on plant growth compared with uninoculated plants and more surprisingly it had a depressive effect on plant growth when added to EM plants (Muchovej and Amorim, 1990; as cited in Lapeyrie *et al.*, 1992). This unexpected result is difficult to explain and indicates that the interactions between symbionts and the host plant are still poorly understood. However, Vishwakarma and Singh (1996b) recently found that a dual inoculation of *E. tereticornis* and *E. camaldulensis* with both AM and EM fungi resulted in a significantly increased biomass as compared to AM, EM or the uninoculated control. Complementary studies on different soils and in different nurseries with different isolates and *Eucalyptus* species are required before rejecting the potential use of AM for nursery inoculation with or without EM fungi.

1.3 Arbuscular Mycorrhizas

Arbuscular mycorhizal symbioses are the most widespread in the plant kingdom, commonly occurring in the bryophytes (mosses), the pteridophytes (ferns) and the gymnosperms and angiosperms from the tropical to the arctic regions of the world (Jackson and Mason, 1984; Mason and Wilson, 1992; Torrey, 1992). It has been calculated that about 80% of plant families from all phyla of land plants are hosts of AM fungi (Giovannetti and Sbrana, 1998). Published records indicate that 71% of tropical plant species are AM (Sieverding, 1991), and about 95% of the tree species in natural tropical forest ecosystems are mycorrhizal exclusively with AM fungi (Le Tacon, Garbaye and Carr, 1987). AM fungal associations have been recognised on the roots or rhizomes of fossils plants up to 370 million years old but in spite of this long period of co-evolution, modern species of AM fungi are not very specific in their host ranges (Smith and Read, 1997). Most species or isolates of AM fungi will colonise any AM host, but the degree of colonisation and the physiological effect can vary in different host-endophyte combinations. Furthermore, various fungi (Hayman, 1983) and different isolates within the same fungus (Sieverding, 1991) can be affected differently by soil conditions such as pH or fertiliser addition. Since it has been hypothesised that P is the limiting element in most tropical rain forests (Vitousek, 1984, Vitousek and Sanford, 1986), AM might be expected to play an important role in P nutrition. However, recent findings show that P does not always limit the growth of tropical rain forest seedlings (Burslem *et al.*, 1995, Raaimackers and Lambers, 1996). The occurrence of EM in the tropics on extremely poor soil (Högberg and Pearce, 1986; Moyersoen *et al.*, 1998; Newbery *et al.*, 1988 and 1997; Singer and Araujo, 1979 and 1986) suggest that EM are particularly beneficial in these habitats.

A revised classification scheme places all soil-borne fungi that form arbuscules in association with terrestrial plants in the order *Glomales* (Zygomycetes) (Morton and

Benny, 1990). Those taxa that form intraradical vesicles (for example, *Acaulospora, Entrophospora, Glomus* and *Sclerocystis*) are placed in the suborder *Glomineae* and those that form extraradical auxiliary cells and no intraradical vesicles (for example, *Gigaspora* and *Scutellospora*) are placed in the *Gigasporineae.* Taxonomy of AM fungi is based on morphology of the chlamydospores and azygospores. The comprehensive work of Schenck and Perez (1990) provides information for generic and specific identification of these fungi.

1.3.1 *Structure and Development of AM*

In the precolonisation stage, resting spores of the fungus, fungal hyphae in the soil or root fragments with fungal structures are sources (infective propagules) where the fungal development can start (Bowen, 1987). Hyphae of AM fungi play key roles in the formation, functioning and perpetuation of mycorrhizas in natural and disturbed ecosystems (Abbott *et al.*, 1992). Hyphae in soil, originating from either an established hyphal network or from other propagules (spores, vesicles and root pieces) lead to the colonisation and subsequent colonisation of roots (Bowen, 1987). The process is initiated by the germination and development of propagules of the fungi living in the proximity to the feeder roots of the host (Bowen, 1987). The host releases certain substances that produce a remote and selective stimulation of the prospective microsymbionts (Barea and Azcon-Aguilar, 1983). Spore germination and primary growth of the germ tube in the soil are mainly determined by the physical and chemical factors of the soil (Sieverding, 1991). In general, soil conditions conducive for rapid seed germination are often also conducive to the rapid germination of spores of AM fungi (Tommerup, 1983). Spores may require a quiescent period before they germinate (Gemma and Koske, 1988). Temperature or water stress may reduce hyphal growth and increase the time required for colonisation (Daniels and Trappe, 1980; Siqueira *et al.*, 1985). Growth of the germ tube is unaffected by soil solution P availability; growth of germination hyphae close to the host and root penetration appear to be affected by the host tissue P concentration, root exudates, and CO_2 (Becard and Piche, 1989a,b).

In the primary colonisation stage, hyphae may enter roots via root hairs or more commonly between epidermal cells (Barea and Azcon-Aguilar, 1983; Smith and Read, 1997) and a swollen structure known as appressorium is formed in the first cell layers. After this the autotrophic growth of the fungus terminates (Sieverding, 1991).

In the third stage, hyphae spread intercelluraly along the cortical cells and becoming intracellular in the second layer of the cortical cells without passing beyond the endodermis into the root meristems (Bowen, 1987; Gianinazzi-Pearson *et al.*, 1996). Within the inner cortex, growing hyphae develop into arbuscules. Arbuscules are haustoria-like structures consisting of dense clusters that develop by repeated dichotomous branching of the invading hyphae which may occupy the entire lumen of cells (Bowen, 1987). These finely branched hyphae are surrounded by the plasmalemma which provides an extensive area of contact for nutrient exchange between fungus and the host cell protoplasm (Smith and Read, 1997; Barea and Azcon-Aguilar, 1983; Allen, 1991). Arbuscules are often formed within cells shortly after penetration (2-5 days) (Sieverding, 1991). These are ephemeral structures with a life-span of one to three weeks (Wheeler *et al.*, 1991) and they senesce by leaving tannin bodies and the initial dichotomous branch. Vesicles are terminal sac-like swellings of the hyphae (Wheeler *et al.*, 1991) which develop at the time of arbuscule formation or later, generally in the middle and outer cortex appearing either within cells or in an intracellular position. Vesicles are generally regarded as temporary storage organs containing lipids which supply the fungus with metabolites when host plants are stressed and supply to the fungus is reduced (Smith and Read, 1997; Barea and Azcon-Aguilar, 1983). Fungal species belonging to the genera *Gigaspora* and *Scutellospora* never form vesicles, some other AM do, but only rarely.

In the fourth stage, the fungus extends in the roots and in the rhizosphere. The extension of the colonisation in the root is divided into three phases: 1) the initial phase (lag phase) during which the primary colonisation takes place, 2) the exponential phase during which the fungus spreads rapidly in the root and grows faster than it, and 3) the plateau phase during which root and fungal growth proceed at the same rate (Bowen, 1987) . The plant and the fungal species and especially the physical and chemical conditions (as well as the resulting nutrient uptake rates) influence the duration of the lag phase of colonisation, the colonisation spread in the root as well as the plateau phase of colonisation (Bowen, 1987). The primary colonisation during the lag phase was described above. During the exponential phase, the fungus grows inter- and intra-cellularly especially in fine secondary roots (Bowen, 1987). The colonisation spread in the root system takes place also through 'runner hyphae' (thick-walled structures of diameter 5-20 μm) on the root surface (Bowen, 1987). Runner hyphae follow root growth and penetrate the root again at irregular distances. Factors affecting the relative growth rate (RGR) of the root or of the fungus can change the equilibrium between root and fungus development. Arbuscules (and vesicles) are continually formed and degraded during the exponential and the plateau phases of colonisation. Due to this, the individual phases of fungal development are difficult to distinguish when colonised roots are being observed (Bowen, 1987).

In the fifth stage, smaller, thin-walled hyphae (1-5 μm) are produced from the runner hyphae which extend into the soils (Bowen, 1987). These 'External' hyphae may develop after internal colonisation has been consolidated. These hyphae are more ephemeral and perform absorption functions (Barea and Azcon-Aguilar, 1983) and are responsible for the transport of elemental nutrients to the root (Sieverding, 1991). Mosse and Hayman (1980) have reported that these fine, thin-walled absorbing hyphae develop septa as they lose their contents and become functionless.

The final stage is characterised by the production of reproductive structures, that is, the formation of resting spores on the coarse, thick-walled external hyphae (Bowen, 1987). The diameter of the spores is dependent on the fungal species and can range from 15 to 800 μm (Sieverding, 1991). Bowen (1987) has observed that spore formation can start very soon, 3-4 weeks after root colonisation, with some fungal species, while other AM fungal species require up to six months before sporulation begins. The fungal species, the host plant, and the soil and environmental conditions all affect the time and extent of sporulation. Fungal sporulation is a dynamic process; so spores are formed and others may germinate at the same time. The fungal mycelium, inside and outside the root, is another reproductive of AM fungi (Bowen, 1987). It can germinate and colonise new roots. However, where spores can survive for up to several years in the soil, the colonising ability of fungal mycelium (separated from the host plant or after the death of the host plants) lasts only 2-4 weeks (Sieverding, 1991).

1.3.2 *Ecology of AM*

In AM, the effects of mycorrhizal fungi on root hair activity are of major importance. Baylis (1975) hypothesised that AM are of greater importance when root hair activity is reduced. St John (1980) surveyed tropical trees and found, among his samples, that extent of AM colonisation correlated with reduced root hairs, supporting Baylis's hypothesis. Other studies, however, have demonstrated extensive and intensive AM activity in grasses (e.g. Davidson and Christensen, 1977; Caldwell *et al.*, 1985), which have prolific development of root hairs. In one axenic system, *Bouteloua gracilis* (H.B.K.) Lag. ex Steud., a common grass on the North American Great Plains, root branching increased substantially with mycorrhizal formation when the phosphate source was predominantly Ca-phytate (Hetrick *et al.*, 1988). When the phosphate source was NaH_2PO_4, no root responses were noted. These data suggest that the AM effects on root structure are conditioned by the surrounding environment. Thus, overall AM effects on root structure probably result from a combination of the coevolutionary history of a plant species and the particular environment in which any given individual of the species resides (Allen, 1991).

The dispersion in space of the external hyphal network, from the scale of an individual hyphal fragment to the distribution of mycorrhizas across a landscape in the field, is poorly understood (Allen, 1991). Evidence is accumulating which supports the hypothesis first substantiated by Sanders and Tinker (1971) for AM fungi that absorption of P by external hyphae of this type of mycorrhiza is more efficient than by the fine roots of its host. Hyphal branching is an effective long- and short-distance fungal strategy to efficiently explore a volume of medium/soil to ensure contact with a root (Nagahashi *et al.*, 1996). The fungus, because of its large surface to volume ratio, can intensively explore a soil volume. The plant explores a large volume less efficiently. However, a single plant may interact with several mycorrhizal fungi, and the fungus with several plants (Allen, 1991). In undisturbed habitats, the mycorrhiza is patchy because of resource distribution associated with individual plants (for example, Allen and McMahon, 1985) and the distribution patterns of the host plants (Allen, 1991). Animal activity appears to be a major regulator of the horizontal distribution of mycorrhizal activity. Several early reports suggested that mycorrhizal fungi were primarily distributed vertically near the soil surface where labile nutrients were being released (either by the mycorrhizal fungi themselves, for example, Herrera *et al.*, 1978, or from newly decomposing organic matter, for example, Allen, 1991). Allen (1988), however, suggested that AM fungi can extend deep into the soil profile.

Hyphae of one plant may form a partnership with adjacent plants and the direct transfer of nutrients between two plants via mycorrhizal hyphae has been demonstrated (Bethlenfalvay *et al.*, 1991; Fitter *et al.*, 1998). AM can affect plant growth and vigour by mechanisms other than improved host nutrition. AM fungi have been shown to improve soil structure through binding and aggregating of soil particles by the external hyphae (Bethlenfalvay *et al.*, 1988; Miller and Jastrow, 1990; 1992), helped by the cementing action of bacterial polysaccharides (Burns and Davies, 1986). Benefits of AM association may also include increased tolerance of environmental stresses in their hosts. AM may help plants withstand root diseases either by protecting the root system against pathogen attack or compensating for root damage (Garcia Garrido and Ocampo, 1989; Sieverding, 1991). AM associations may also produce beneficial alterations of plant growth regulators (Barea and Azcon-Aguilar, 1982; Allen *et al.*, 1982).

Francis and Read (1995) have hypothesised that AM fungi may play an important role in determining plant community composition. They have found that plant species which respond mutualistically to AM fungi establish themselves in closed communities while those (putative hosts or non-hosts) which are antagonised with AM fungi consign themselves to open habitats. AM fungi have been found in many studies to play an important role for growth of early successional species in the tropics, for example, in a tropical sand-dune system in the Gulf of Mexico (Corkidi and Rincon, 1997) and in a southeastern Brazilian woodland (Sequeira *et al.*, 1998).

1.3.3 *The Role of AM in Plant Carbon and Mineral Nutrition, and Water Relations*

The major role of mycorrhizal symbiosis is to provide access to key growth limiting nutrients at crucial stages in a plant's development (Read, 1991). Increased P uptake is the most obvious benefit of AM colonisation, having been demonstrated in many native and agricultural plants (Abbott and Robson, 1984; O'Keefe and Sylvia, 1990). Phosphate, the major form of P available for uptake by plants, is relatively insoluble in the soil solution and therefore, is not readily transported by mass flow (Nye and Tinker, 1977). The benefits of increased P uptake are most evident in soils low in total P, but are also observed in soils with low soluble P, which are found in many of the highly weathered soils of the tropics and subtropics. Tarafdar and Marschner, (1994) have found that AM hyphae can also efficiently utilise organic sources of P (Na-phytate). It is suggested that P is absorbed in the form of orthophosphate and transported actively in the hyphae as polyphosphate (Sieverding, 1991). Translocation of P in hyphae to roots has been calculated at a rate of $1\text{-}2\times10^{-9}$ mol cm^{-2} root s^{-1} (Sieverding, 1991). The major transfer of P from the fungus to the plant occurs in those root cells which contain arbuscules; however, root-internal hyphae too can release P to the host plant (Sieverding, 1991). Hyphal P transport may be considered as the result of three steps: hyphal uptake, translocation in hyphae, and transfer across the symbiotic interface (Jakobsen, 1992). It is generally thought that the P transfer from the fungus to the host takes place in a process of interchange with carbon compound metabolites from the host to the fungus. The host plant appears to control fungal development and activity via regulation of carbohydrate movement to the fungus (Sieverding, 1991).

The role of the hyphae in phosphate uptake and soil stabilisation is dependent on their distribution within the soil matrix in relation to the root surface (Abbott *et al.*, 1992). The ability of the fungus to absorb nutrients may be closely linked with the development of the external hyphae. The external hyphae of the AM fungi are able to affect phosphate absorption beyond the depleted zone up to approximately 10 cm from the root (Rhodes and Gerdemann, 1975; Cooper, 1984; Li *et al.*, 1991a). Increased nitrogen concentration in AM plants has been reported (Smith, 1980). There is also evidence that nitrogen is taken up in $NH4^+$ form by hyphae from inorganic sources of ammonia and transported to the plant (Ames *et al.*, 1983; Frey and Schüepp, 1993). This may be especially important when the N is distributed in discrete patches (Allen, 1991). Uptake of K by AM has also been reported in different plant species (Davies, 1987; Kucey and Janzen, 1987; Ahiabor and Hirata, 1994). Direct evidence of AM fungi in improving zinc, copper, boron, and molybdenum uptake has also been reported (Kothari *et al.*, 1991; Li *et al.*, 1991b). However, it has been argued that levels of AM colonisation are poor indicators of mycorrhizal functioning in terms of nutrient uptake and growth responses in host seedlings (McGonigle *et al.*, 1990; Thingstrup *et al.*, 1998).

AM fungi need plant photosynthate for their metabolic activity. The additional percentage of carbon fixed that is directed below ground is normally some 6-12% greater when the host is colonised by AM fungi (Whipps, 1990). This cost may be a considerable penalty to a plant (Tinker *et al.*, 1992). Normally it is compensated for by the increased photosynthetic rates or physiological changes (Whipps, 1990), but if there were no compensating advantages of colonisation, it could cause major yield depressions (Tinker *et al.*, 1992).

AM roots also aid in water uptake by plants. George *et al.* (1992) stated that the higher specific water uptake rates of AM roots may be due to higher transpirational demand and/or higher hydraulic conductivity of AM roots. Several hypotheses have been put forward to explain the improved water relations of mycorrhizal plants: increased nutrient uptake, increased water uptake by means of either larger root systems or fungal hyphae, and hyphae within the root providing a high conductance pathway for water movement (Safir and Nelsen, 1985). Read and Boyd (1986) suggest that there are several ways for the mycorrhizal colonisation to affect the physiology of the plant, and these are independent, not mutually exclusive.

Several researchers have suggested that varied responses of plant to AM can characterise the mutualism depending on the environmental conditions. For example, AM may improve water uptake in arid to semi-arid habitats that generally contain high levels of soil P (Levy and Krikun, 1980; Bildusas *et al.*, 1986; Trent *et al.*, 1989), hormone balance in response to stress (Allen *et al.*, 1980, 1982) and direct N transport when soils contain predominantly $NH4^+$-N (Ames *et al.*, 1983).

1.3.4 *Inoculation with AM Fungi*

Growth response by host may depend on rapid colonisation by the AM fungi (Abbott and Robson, 1985; Graham *et al.*, 1996; Hung *et al.*, 1990), although growth responses do not always correlate with rate of colonisation (Adjoud *et al.*, 1996; Vishwakarma and Singh, 1996a; Jones *et al.*, 1998). Propagule density, which refers to the concentration of infective fungal units in the inoculum or crop growth medium and is best predicted by the Most Probable Number (MPN) assay, may largely contribute to colonisation rate (Pfender *et al.*, 1981; Porter, 1979; Wilson and Trinick, 1983). Propagules that originate in root pieces or active soil-borne hyphae may colonise more quickly and be more numerous than spores (Wilson, 1984). Actively growing mycorrhizas can colonise susceptible roots of transplants in as few as two days (Brundrett *et al.*, 1985), whereas colonisation from spores may take up to 10 days after spore germination (Bevege *et al.*, 1975). Warner and Mosse (1980) and

Hepper (1983) suggested that AM fungi could maintain a limited saprophytic growth phase.

Buwalda and colleagues (1984) found that the rate of colonisation spread in container-grown crops was independent of placement of inoculum in small containers (<100 ml) and in soil-less growth media. However, low levels of colonisation in soil-less growth media may be related to high soluble P concentrations (Biermann and Lindermann, 1983a,b). In nonpasteurised soil-based growth media, AM fungi need to compete with indigenous populations, and inoculum placement may be important (Hepper *et al.*, 1988; Lopez-Aguillon and Mosse, 1987; Wilson, 1984).

1.4 Ectomycorrhizas

Fungi of ectotrophic mycorrhizas enclose the roots in a dense sheath and penetrate the host cells to a very limited extent. EM roots may be either extensively branched or undivided, depending on the host and fungal species (Wheeler *et al.*, 1991). Ectomycorrhizas (EM) are formed by the fungal groups Basidiomycetes, Ascomycetes and Gasteromycetes (Smith and Read, 1997). EM roots are characterised by (1) a fungal sheath or mantle which encloses the root in a fungal tissue, and (2) a Hartig net which is a plexus of fungal hyphae between epidermal and cortical cells (Smith and Read, 1997). Most EM are associated with woody perennials including some temperate broadleaves and conifers, and some tropical trees notably from the families Pinaceae, Fagaceae and Dipterocarpaceae are ectomycorrhizal (Smith and Read, 1997; Malloch *et al.*, 1980 and Meyer, 1973). The EM fungi show various degrees of host specificity. Many can form mycorrhizas with nearly any EM host (Molina and Trappe, 1982a), others are specific to a particular host genus, such as *Pinus*, or even a sub-group within that genus (Molina and Trappe, 1982b).

1.4.1 *Structure And Development of EM*

In nature, it is mainly the shortest roots of trees that become modified into EM (Jackson and Mason, 1984). EM are easily recognised without special staining procedures. They are usually swollen, branched, mantled by fungal tissues, and lack root hairs. Hyphae grow from the root surface between and around cells of the root epidermis and cortex. The hyphal growth between root cells, termed the Hartig net, does not damage root cells and usually indicates a mutualistic symbiosis. When an EM fungus develops a mantle, it stops growing in bundles or in isolated hyphae, and organises a more complicated structure (Bonfante-Fasolo and Scannerini, 1992). Two reasons may account for the dramatically different branching pattern of the fungus during its symbiotic status than in culture. First, the root tissues develop a three-dimensional substrate that differs from the flat medium of a Petri-dish. Second, the root apex, one of the most important sites for hormone production, could influence fungal morphogenesis. Thus the formation of an EM is characterised by the well-known changes in the root morphology (Clowes, 1981) and by less well-known fungal modifications, caused by some potential morphogenetical factor (Nylund, 1988). The strongest impact of such a hypothetical factor can be observed during the Hartig net phase. Here, the longitudinal fungal growth through the intercellular spaces is restricted. In some cases, the fungus sends hyphae into as well as among cortical cells; such colonisations are termed ectendomycorrhizal and seem to function much the same as do EM. Hyphae of both the inner mantle and Hartig net grow into a labyrinthine pattern (Smith and Read, 1997).

The early events in EM colonisation have been studied extensively by light and electron microscopy (Massicotte *et al.*, 1986, Massicotte *et al.*, 1989; Nylund and Unestam, 1982). First, hyphae contact a rootlet and form a loose external weft. Then the hyphae penetrate between outer root cells, soon forming a mantle tightly appressed to the root surface together with the Hartig net, which surrounds outer cortical cells, but leaves intact the plasmodesmata connecting one root cell with another. Ultimately, the labyrinthiform tissues accumulate mitochondria and Golgi bodies, probably indicating high rates of metabolic activity. Often the root cells react, and the walls become invaginated from interaction with the Hartig net (Duddridge and Read, 1984; Massicotte *et al.*, 1986). These alterations in root cells are thought to maximise the area for exchange of materials between symbionts.

Finlay and Read (1986) described the development of hyphal fans growing away from EM root tips in the soil. The fans are an exploratory phase of growth. Upon encountering an uncolonised rootlet or area with a high nutrient concentration, they proliferate and colonise the root tip or nutrient pocket. Mycelial strands or rhizomorphs are composed of parallel hyphae growing in bundles. Often the hyphae in the centre of a strand expand to form conducting tissue. Radiotracer studies show that strands can move nutrients from soil to host or from one host to another (Finlay and Read, 1986).

Because EM fungi vary tremendously in their morphology and physiology, even among genotypes within species, isolates for use in forestry and horticulture should be carefully selected (Trappe, 1977). Thickness and colour of the EM mantle, branching pattern, presence of intracellular hyphae, and the form of external mycelium, all vary from one association to another. Some fungi grow well only in certain substrates, such as mineral soil or decaying wood. The fungi also differ in their physiology [for example, in optimal pH for growth (Hung and Trappe, 1983; Laiho, 1970), in the ability to utilise various C and N sources (Hutchison, 1990) and in spore dormancy (Fries, 1987)]. Some evidence indicates that rhizomorph-forming fungi improve host drought tolerance more than fungi lacking rhizomorphs (Dosskey *et al.*, 1990).

1.4.2 *Ecology of EM*

EM host plants must form mycorrhizas to survive more than a few years in the field (Christy *et al.*, 1982; Mikola, 1970). Two primary sources are spores and the mycelia of the established mycorrhizas. Spores are produced by sexual fruiting bodies, such as those of mushrooms or truffles. Mushroom or puffball spores are discharged to the air- and wind-dispersed; truffle spores depend on being eaten and then excreted by animals. The fruiting bodies are seasonal, but the spores of EM fungi can remain dormant for undetermined lengths of time. Consequently, viable spores may always be available in the soil. Exudates from roots and some soil organisms can increase germination of spores of some EM fungi (Fries, 1982). Because of their dormancy and ability to travel, spores are probably important in primary colonisation of newly exposed substrates (Trappe, 1988), after severe disturbance (Janos, 1980b), and where EM have long been absent (Perry *et al.*, 1987). Mycorrhizas also form from hyphae of established EM. The hyphae grow faster in the presence of plant roots and root exudates (Read *et al.*, 1985) and can grow from an EM root tip to colonise new rootlets. Roots can grow many metres from the stem of the tree, and hyphae may grow beyond that. In a forest where most of the soil mass is occupied by roots and hyphae, understorey seedlings are probably colonised more often by established mycorrhizal hyphae than by germinated spores (Mikola, 1970). Anthropogenic disturbance and events such as landslides, volcanic eruptions, fire and even logging activities (such as clear-felling) affect availability of mycorrhizal propagules. The longer EM hosts are absent from sites, the more recolonisation depends on spores, rather than on pre-existing mycelium as inoculum. Even with the adequate availability of EM propagules, mycorrhizal formation may depend on bacterial activity in ectomycorrhizospheres (Dunstan *et al.*, 1998; Perrotto and Bonfante, 1997). Neal *et al.* (1964) and Rambelli (1973) reported that total number of bacteria could be 10-100 times higher in ectomycorrhizospheres than in soil away from roots. McAfee and Fortin (1988) argued that these bacteria may increase or decrease mycorrhizal formation, depending upon the taxonomic mix.

EM fungal succession has been reported with developing stands of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Frankland, 1998), *Populus balsamifera* ssp. *trichocarpa* (Torr. &Gray) (Helm *et al.*, 1996), *Alnus incana* (L.) Moench. (Arveby and Granhall, 1998) and birch (*Betula* spp.) (Mason *et al.*, 1982; Fleming *et al.*, 1984; Last *et al.*, 1984). For example, as noted earlier (section 1.2.2), EM fungal species *Laccaria* and *Hebeloma* colonised Scottish birch early in the succession, while *L. pubescens* and *Leccinum* spp. colonised it later (Deacon *et al.*, 1983). Fleming (1984) observed that the late-stage fungi colonised young birch seedlings when they were planted in undisturbed positions around mature trees. However, Fleming *et al.*, (1986) found that the late-stage fungi as naturally occurring inocula in soil formed few mycorrhizas on glasshouse grown seedlings whereas early-stage fungi formed many mycorrhizas in equivalent conditions. Fleming *et al.* (1986), therefore, suggested that soil or other (biotic and abiotic) site factors can modify the behaviour of a mycorrhizal fungus.

In the tropics, occurrence of EM fungal associations have been observed to be associated with plant succession (Janos, 1980b). Tropical forests dominated by EM hosts are less rich in tree species, compared with AM-dominated, tropical forests. Janos (1980b) hypothesised that this was due to competitive exclusion of AM hosts by EM hosts combined with greater host specificity of EM fungi. AM fungal species probably dominate certain soils because of their ability to outgrow other species in that environment rather than because of selection by a host. Furthermore, AM fungi depend on mycotrophic plants for carbon, and because they produce few spores which may survive only a short time in the lowland humid tropics, plant composition in turn affects the mycorrhizal fungus content of the soil. EM individuals are likely to be locally abundant where they do occur, and if together they occupy much root space, and are therefore likely to influence succession by causing AM fungal populations to decline (Janos, 1980b). Further studies in tropical forests (for example, studies of Alexander, 1987; Newbery *et al.*, 1988;, Newbery and Gartlan, 1996 and Newbery *et al.*, 1997) were supportive of Janos's hypothesis which indicated that EM-dominated forests form distinct matrix.

Plant communities may also be affected by mycorrhizal interactions between and among host plants that are connected by shared mycorrhiza mycelia. Thus achlorophyllous plants such as *Monotropa* spp. obtain carbohydrates from overstorey EM trees by a common mycorrhizal network (Björkman, 1960). As noted in section 1.2.1, Read and co-workers (1982, 1984 and 1985) showed that mycorrhizal fungi can grow from mycorrhizas of one host plant to colonise roots of other species, and that C can be transferred by hyphal links. In the East African Miombo woodland, seedlings of large EM trees have been reported to remain attached to the parent hyphal systems so that they can receive water and nutrients to survive the dry periods (Högburg, 1986).

EM species of *Gautieria* and *Hysterangium* form distinctive hyphal or rhizomorph mats which have been observed in forests ranging from the subtropical (*Eucalyptus* in Australia) to boreal forests in Alaska (Griffiths *et al.*, 1991). These mats are reported to be responsible for accelerated mineral weathering such as advanced clay weathering in presence of elevated oxalate concentrations by *H. setchellii* (Fischer) mats in the Pacific nothwest (Cromack *et al.*, 1979), for processing of detrital nutrient resources such as carbon, nitrogen and phosphorus (Griffiths and Caldwell, 1992), and for establishment of a distinct soil microhabitat by being able to transport organic nitrogen and phosphorus (released during the degradative process of organic polymers by mats) to the host tree (Griffiths and Caldwell, 1992).

1.4.3 *The Role of EM in Plant Carbon and Mineral Nutrition, and Water Relations*

The first direct evidence of nutrient translocation through ectomycorrhizal mycelia was provided by the pioneering studies of Melin and Nilsson (1950) using stable and radioactive isotopes. In these experiments mycelial uptake and translocation of ^{32}P, ^{15}N, ^{45}Ca and ^{22}Na to pine seedlings of different species were demonstrated under sterile conditions using a range of mycorrhizal associations (see Melin *et al.*, 1958 and references therein). Since these early studies a substantial amount of evidence has accumulated to show that not only phosphorus, nitrogen, calcium and sodium, but also the uptake of other mineral elements is enhanced by mycorrhizal structures as opposed to non-mycorrhizal roots (Bowen, 1973; Smith and Read, 1997). In spite of this, there may not always be much difference in the nutrient absorbing power of mycorrhizal and non-mycorrhizal roots. Reid and Bowen (1979) reported very similar phosphate absorbing rates for young non-mycorrhizal and mycorrhizal roots of coniferous seedlings per unit surface area, even though the phosphate absorption was larger in mycorrhizal roots per unit fresh weight. Also, Ingestad *et al.* (1986) were unable to show an inherently greater nutrient uptake capacity of ectomycorrhizal root systems in liquid culture as opposed to non-mycorrhizal, and they concluded that the mycorrhizal enhancement of nutrient uptake is caused by indirect factors. However, Bowen (1973) and Duddridge *et al.* (1980) observed that the high absorbing power of mycorrhizas is more sustained, whereas that of nonmycorrhizal roots in any one place in soil decreases rapidly with time. Mycorrhizas are also able to break down and take up organic compounds; ectomycorrhizal fungi produce phytases (Gianinazzi-Pearson and Gianinazzi, 1986); they can enhance the production of acid phosphatases (Williamson and Alexander, 1975); they are able to produce proteolytic enzymes (Read *et al.*, 1989) and take up amino acids which are inaccessible to host plant roots (Alexander, 1973, 1983). There is also a possibility that mycorrhizal fungi can dissolve inorganic phosphates in some soils by acidifying their environment (Gianinazzi-Pearson and Gianinazzi, 1986). The growth and metabolism of the mycorrhizal fungus induces a sink of carbon to the host plant; the fungus uses current photosynthate from the host (Smith and Read, 1997; Söderström and Read, 1987), and between 10-20% times more photosynthate may be translocated to ectomycorrhizal than nonmycorrhizal roots of the same plants (Smith and Read, 1997). Therefore the photosynthetic rate of the host plant may be expected to increase as a result from colonisation, according to the concept that the sink size is a regulator of photosynthetic rates. This would be an effect parallel to the mycorrhizal effects mediated by mineral nutrition, and hence rather difficult to study experimentally. Much of the experimental work on the carbon balance of EM and nonmycorrhizal plants has been confounded by differences in the mineral nutrition of the plants.

The photosynthetic rates of mycorrhizal (*Suillus granulatus* (L.:Fr.) Kuntze and *Pisolithus tinctorius* (Pers.) Coker and Couch) *Pinus contorta* Dougl, ex Loud. var. latifolia Engelm and *P. taeda* L. seedlings were considerably higher than those of nonmycorrhizal seedlings (Ekwebelam and Reid, 1983, Reid *et al.*, 1983), and allocation of photoassimilated ^{14}C in the root systems as well as root respiration were higher, when the mycorrhizal seedlings had higher P concentrations (Reid *et al.*, 1983). In contrast, Ahrens and Reid (1973) did not find differences in quantities of soluble carbohydrates or their distribution between roots and shoots of mycorrhizal (*Cenococcum geophilum* Fr., *Thelephora terrestris* (Ehrh.) Fr. and *Rhizopogon vinicolor* A.H.Smith) and nonmycorhizal *P. contorta* seedlings after exposure to $^{14}CO_2$, when the shoot and root dry weights were similar in the treatments. Moreover, when similar conditions of mineral nutrition were achieved, there was no difference in the assimilation rates of mycorrhizal (*Paxillus involutus* (Batsch) Fr. and nonmycorrhizal Sitka spruce seedlings (Lehto, 1989). Nylund and Unestam (1987) and Nylund and Wallander (1989) did find an increased photosynthetic rate as a result of colonisation of 'perfectly nourished' Scots pine with *Laccaria* and *Hebeloma* sp., but this was concomitant with a comparative decrease in their relative growth rates (RGRs). Similarly, Ingestad *et al.*, (1986) found lower RGRs in *Suillus bovinus* (L. ex Fr.) O.Kuntze mycorrhizal Scots pine than nonmycorrhizal seedlings of the same nitrogen status. Hence the photosynthetic rates were not increased enough to cover the carbon sink to the fungus, when plants were supplied with soluble nutrients. Recent work indicates that EM plants may photosynthesise at lower water potentials than nonmycorrhizal ones (Dosskey *et al.*, 1990; Nylund and Wallander, 1989). One explanation of this phenomenon is that increased C flow to mycorrhizal roots delays CO_2 saturation in leaf mesophyll because of delayed accumulation of starch, which in turn, delays stomatal closure. Alternatively or additionally, hyphae of mycorrhizal fungi may have more access to soil water than roots themselves by penetrating smaller pores or by retrieving water beyond the root zone (Nylund and Wallander, 1989).

The conclusion that mycorrhizal colonisation may not increase growth unless mycorrhizas can substantially increase nutrient uptake, has also been reached in experiments with containerised coniferous seedlings (Molina, 1982; Molina and Chamard, 1983). However, in natural environments the carbon sink in the fungus may be compensated for by the lower maintenance of respiration of mycorrhizal root systems (Marshall and Perry, 1987), the relative longevity of functional mycorrhizas (Bowen, 1973), and increased carbon assimilation due to improved nutrition.

EM fungi may improve the water relations of trees (Smith and Read, 1997). Dixon *et al.* (1983), Walker *et al.* (1982) and Walker *et al.* (1989) studied the water balance of bare-root and container-grown seedlings of *Quercus velutina*, *Pinus virginiana* and *P. taeda* respectively which had been inoculated with *P. tinctorius* or left uninoculated. They found that container-grown plants with extensive mycorrhiza development had a significantly improved water balance following transplantation, pre-dawn shoot water potential values being significantly higher in the mycorrhizal plants during mild drought. However, these results are at variance with those of some other studies (Lehto, 1989; Coleman *et al.*, 1990) which report either no impact of EM colonisation upon pre-dawn water potential or a negative effect. However, the potential of mycelial system to provide conduits for the transport of water was indicated by Boyd *et al.* (1986). Also the importance of the extraradical phase for water absorption was emphasised by Lamhamedi *et al.* (1992).

1.4.4 ***Inoculation with EM Fungi***

Growth response by host may depend on rapid colonisation by the EM fungi (Smith and Read, 1997). Various inoculation techniques have been developed for use in seedling production (Smith and Read, 1997). The most successful have involved the growth of vegetative mycelium in vermiculite-peat mixtures moistened with liquid nutrient medium (Marx and Kenny, 1982; Mason *et al.*, 1999a,b,c). Vermiculite provides a well-aerated laminated substrate, within which the mycelium is protected, and addition of peat in different ratios enables adjustment of pH to the required range, usually 4.8-5.5. The recommended nutrient solution has a C:N ratio of between 50 and 60 and is added in volumes sufficient to ensure that all free C is utilised by the fungus in the course of its development in the medium. The presence of available C at the time of inoculation leads to competitive exclusion of the mycorrhizal fungus by saprophytes (Smith and Read, 1997). Mycelia can also be applied in liquid media or as encapsulated in alginate beads (Brundrett *et al.*, 1996c). Advantages of mycelial forms of inoculum are that they can be more effective than spores in colonising roots and they allow large scale production of single isolates of fungi (Cordell *et al.*, 1988). Their increased effectiveness is due to their presence in an active physiological state when applied as inoculum (Brundrett *et al.*, 1996c; Smith and Read, 1997).

Basidiospore inoculum of *P. tinctorius* has been used on an experimental basis in the USA and elsewhere. This can yield growth responses, but rarely produces as many mycorrhizas per plant as does the 'super-strain' vegetative inoculum and so is less effective (Smith and Read, 1997). Major drawbacks to the use of spore inoculum are the restrictions to the fungal species that produce abundant sporocarps (for example, *Pisolithus* and *Scleroderma*), the poor germination or low viability of spores of some species and that large spore numbers are often required for colonisation of roots (Fries, 1983; Cordell *et al.*, 1988).

A recent molecular study established that there are considerable geographical and host-linked variation in *Pisolithus* populations, for example, isolates of *Pisolithus* from the Philippines were genetically very homogeneous but distinct from less related isolates from Europe, Scandinavia and North America (Sims *et al.*, 1999). It may therefore be important to consider geographic origin in selecting fungal isolates before inoculation.

1.5 Compatibility and Incompatibility in Mycorrhizal Associations

The term 'compatibility' in the sense of Gianinazzi-Pearson and Gianinazzi (1983) is used to emphasise the merging of symbionts to form a structurally defined mycorrhiza in which physiological activity and exchange of metabolites between the partners improve the nutrition of host and fungus. Ecological benefits toward one or both partners indicate functional compatibility, particularly with reference to mycorrhizal linkages in ecosystems. Incompatibility produces deleterious effects on fungus or host or both. For example, the production of phenolics in root tissue or tissue disorganisation in response to fungal penetration may be considered signs of incompatibility (Molina *et al.*, 1992).

A compatible AM association is established when colonisation by AM fungus results in the formation on the root surface of an appressorium, from which the hypha penetrates the root tissue, and culminates in the intense proliferation of mycelium (inter- and intra-radical hyphae, coiled hyphae) in the cortical parenchyma and the differentiation of specialised, highly branched arbuscules (Gianinazzi-Pearson *et al.*, 1996). In contrast, typical defence responses such as abnormally thick wall appositions in epidermal and hypodermal cell walls adjacent to appressoria (Gianinazzi-Pearson *et al.*, 1991) are associated with incompatibility in plant genotypes resistant to AM fungi and non-mycorrhizal plants, in which root interactions and fungal development are arrested at the stage of appressorium formation on the root surface (Gianinazzi-Pearson *et al.*, 1996). The wall thickenings often indicate phenolic accumulation (Gollotte *et al.*, 1993).

Microscopic study of the mycorrhizal root samples from aseptically (Molina and Palmer, 1982) or semi-aseptically (Fortin *et al.*, 1980) grown seedlings which are inoculated with single isolates of known fungi can be useful in documenting compatible and incompatible responses (Molina *et al.*, 1992). A typical angiosperm EM forms layers of fungal hyphae covering the host root surface; the epidermal cells show a rapid response to the presence of EM fungus in the form of a considerable radial elongation, and a Hartig net is formed which is restricted to root epidermis (Brundrett *et al.*, 1996c). In contrast, a typical gymnosperm EM forms a very thin layer of mantle with no elongation of epidermal cells but Hartig net hyphae penetrate hypodermal and cortical cells (Brundrett *et al.*, 1996c). An incompatible angiosperm EM, for example, the associations between *Paxillus involutus* (Batsch.) Fr. and *Alnus* spp., is characterised by a limited Hartig net formation and poor elongation of epidermal cells (Molina, 1981; Massicotte *et al.*, 1999). Similar structural features have been reported for incompatible *Eucalyptus* EM (Burgess *et al.*, 1994). In addition, deposition of phenolic compounds in plant cell walls and vacuoles frequently indicates an incompatible interaction between EM fungi and host roots (Ling-Lee *et al.*, 1975; Nylund and Unestam, 1982; Malajczuk *et al.*, 1984; Duddridge, 1986; Horan *et al.*, 1988; Massicotte *et al.*, 1999).

1.6 Mycorrhizal Formation and Functioning in Pot Experiments and Its Field Implications

Dependence on mycorrhizas for nutrient uptake is probably greater in seedlings than mature perennials (Baylis, 1962; Harley, 1978). In pot experiments, type of soil as well as volume of soil available to the plant (de Vries, 1980; McGreevy, 1996) and soil moisture regime (de Vries, 1980; Bougher and Malajczuk, 1990; Mason *et al.*, 1999a), and the amount of readily available nutrients (Ross, 1971; Hall, 1976; Tawaraya *et al.*, 1994) can greatly affect the outcome such as mycorrhizal formation and its effect on growth responses in seedlings. The method of inoculation can also influence the outcome of mycorrhizal experiments (Johnson, 1977). However, pot experiments may be necessary when the experimental objectives include any one or a combination of the following (Brundrett *et al.*, 1996c):

1) confirmation of the mycorrhizal status of host plants and mycorrhizal fungi and their compatibility (mycorrhizal synthesis);
2) comparison of mycorrhizal and non-mycorrhizal plant growth responses to mycorrhizal inoculation at different soil fertility levels (mycorrhizal dependency);

3) estimation of the inoculum potential of compatible mycorrhizal fungi in a particular soil (bioassays);
4) selection of superior mycorrhizal fungal isolates by comparing growth responses of host in particular soils (isolate screening);
5) determininination of critical concentrations of trace elements for mycorrhizal and non-mycorrhizal plants (micronutrient research);
6) provision of mycorrhizal root material of known fungi for microscopy (histology); and
7) testing of plant/fungal response to various soil factors (physiology).

The capability of mycorrhizal fungi to compete with indigenous soil microbes in a particular soil can be established by comparing their colonisation ability and capacity to promote plant growth in pasteurised and unsterile soil (Aggangan *et al.*, 1995). However, field trials provide the ultimate opportunity to evaluate the persistence and performance of fungi in the field (Brundrett *et al.*, 1996c). The demonstration of a plant growth response in controlled conditions is a valuable step, but only a first step, in evaluating the importance of the symbiosis in nature (St. John and Coleman, 1983). Results from pot experiments can therefore be important in designing large-scale field experiments keeping in mind that longer time-scale, spatial variability and climatic factors could affect the experimental results (Brundrett *et al.*, 1996c). The ultimate success of both pot and field experiments will depend on the survival and persistence of mycorrhizal seedlings in the field where, many factors (for example, competition with other plants) other than mycorrhizas influence plant ecology.

1.7 Effects of Different Types of Nutrient and Nutrient Application on Mycorrhiza Formation in Pot Expeirments.

In general, growth and phosphorus uptake from insoluble sources are enhanced by mycorrhizal colonisation, but only at higher rates of application (Alexander, 1989). There are several disadvantages of using solid fertilisers in the growing medium (Brundrett *et al.*, 1996c). Firstly, it is difficult to evenly distribute the fertiliser through the media while mixing because fertiliser particles will adhere to moist constituents of the media. Secondly, if all the fertiliser for growth of the plant were to be added before planting, the availabilities of N and P in the soil solution would most likely be unfavourable for mycorrhizal development. The best way to supply a carefully regulated amount of nutrients is as a liquid (Brundrett *et al.*, 1996c). Liquid fertilisers can be applied continuously each time the plants are watered or periodically according to a fixed schedule. However, readily available nutrient elements in excessive amounts can have deleterious effect on mycorrhizal formation and resulting growth response in the host seedlings (Ross, 1971; Hall, 1976). In a programmed nutrition addition, however, the amount of fertiliser applied is kept low when the seedlings are small and is increased when the seedlings are larger (Brundrett *et al.*, 1996c). This is defined as 'exponential fertiliser delivery' which involves progressively increasing nutrient applications that correspond closer to the relative growth rate of seedlings during their exponential phase of growth (Ingestad and Lund, 1979; Ingestad, 1982; Ågren, 1985). Exponentially increasing fertiliser additions are reported to have stimulated mycorrhizal development compared with conventional constant-rate fertiliser addition (Ingestad *et al.*, 1986; Quoreshi and Timmer, 1998).

1.8 Effect of Environmental Conditions on Mycorrhiza Formation

Björkman (1942) published a theory on the decisive role of low N (and P) levels in plants leading to ‘surplus’ carbohydrates in roots which make it possible for mycorrhizal fungi to colonise them. Since then there has been a lot of controversy about the subject (Marx *et al.*, 1977, Nylund, 1988). A second major theory has been promoted by Slankis (1973, 1974), suggesting that fungal auxins increase transport of carbohydrates to roots. Hence the high levels of soluble carbohydrates found in mycorrhizal roots would be the consequence rather than the cause of colonisation, and the inhibition of mycorrhizal formation by high nutrient levels would be an interaction between N and hormones: high N concentration suppress formation of fungal auxins (Slankis, 1973). This theory has been increasingly supported by experimental data (Mudge, 1987; Nylund, 1988; Nylund and Wallander, 1989). However, in a later study of various Scots pine mycorrhizas, Wallander and Nylund (1992) found that although high N application had deleterious effect on the production of external mycelium, it did not affect mantle and Hartig net formation.

The effect of a particular environmental factor on mycorrhizal formation may be mediated in different ways depending on the host and fungal species involved, their developmental stage and environmental conditions. As soil temperature influences the whole pattern of root development, hence the number of short roots available for colonisation is affected as well as the growth of the fungus in the rhizosphere (Wilcox and Ganmore-Neumann, 1975). The physiological status of the host plant is dependent on the environment, and so are rates of processes such as carbohydrate translocation and root exudation, which affect mycorrhizal formation (Theodorou and Bowen, 1971).

As already indicated, the best known environmental effect on mycorrhizal formation is that of nutrient availability. Mycorrhizal formation can readily be inhibited by applying large amounts of nitrogen to the rooting medium in the laboratory (Slankis, 1973). But some EM fungi such as *Laccaria laccata* (Scop. ex Fr.) Cooke can colonise root systems equally efficiently over a range of nutrient availability (Molina and Chamard, 1983). Similarly some AM fungi such as *Acaulospora scrobiculata* Trappe, *A. morrowiae* Spain & Schenck, *A. longula* Spain & Schenck, *A. spinosa* Walker & Trappe, *A. myriocarpa* Spain, Sieverding & Schenck, *Glomus aggregatum* Schenck & Smith emend. Koske, *G. versiforme* (Karsten) Berch and *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders were identified in a wide range of pH (3-8-8.0) and at diverse chemical fertilities (Sieverding, 1991).

Early work on mycorrhizal colonisation showed that low light availability inhibits colonisation (Björkman, 1942), and this result has been repeated for both AM (Hayman, 1974; Daft and El Giahmi, 1978; Son and Smith, 1988; Olsen *et al.*, 1999) and EM (Reid *et al.*, 1983; Ekwebelam and Reid, 1983; Eltrop and Marschner,

1996) associations. Elevated CO_2 in the atmosphere has similarly been found to increase colonisation (O'Neill *et al.*, 1987; Lovelock *et al.*, 1996). This is most probably due to the effects of light and CO_2 on photosynthetic activity and hence carbon availability to the fungus.

Temperatures lower than 17-18°C and higher than 40-45°C were found to be problematic for development of AM and their effectiveness (Sieverding, 1991). Theodorou and Bowen (1971) found that growth and mycorrhizal development by various fungi on *Pinus radiata* (D.) Don. was remarkably reduced by a decrease of temperature from 25°C to 20°C to 16°C. On the other hand, high temperatures inhibit mycorrhizal formation as well, different fungi having different temperature optima: *P. tinctorius* formed mycorrhizas on *P. taeda* best at 34°C, whereas *Thelephora terrestris* Ehrenb. ex Fr. formed hardly any at this temperature (Marx *et al.*, 1970).

EM fungi are highly aerobic compared to AM fungi (Smith and Read, 1997). Boyd (1987) found fewer *Pisolithus* mycorrhizas on *Betula* in peat kept at field capacity than in 70% or 30% moisture content of field capacity. Boyd (1987) also found that mycorrhizal colonisation increased the transpiration rates and water potentials, and hence the soil-plant conductance to water in the *Betula* plants exposed to moderate water stress. As the plants were of comparable size, and their shoot phosphorus concentrations were not different, Boyd maintained that the increased conductance was caused either by the fungal mycelium extending the potential volume of soil exploited by a mycorrhizal plant, and also, it may have improved the contact between the peaty substance and root. In this experiment, the plants were grown in different water regimes for a relatively long time, 42 or 63 days. Moyersoen and Fitter (1999) found that a dual mycorrhizal species in Cameroon rainforests tended to have more AM associations in the habitats with higher moisture regimes while it maintained more EM associations in habitats of lower moisture regimes.

1.9 Mycorrhizal Associations of *Eucalyptus* Species

The genus *Eucalyptus* contains the most important timber producing forest species in Australia (Malajczuk *et al.*, 1982) and has been introduced into various regions of the world because of its growth potential under adverse soil conditions (Penfold and Willis, 1961). In Australia, the tree genus is found to develop EM (Chilvers and Pryor, 1965). It has been observed that *Eucalyptus* can also naturally form EM outside its geographic origin, for example, in India, *Eucalyptus* forms ectomycorrhizas with *Scleroderma verrucosum* Persoon (Bakshi, 1966). There are several 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 characterisation (Chilvers, 1968; Seviour *et al.*, 1978 and Rose *et al.*, 1981), synthesis of ectomycorhizas 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 ectomycorrhizas (Chilvers and Gust, 1982a and Massicote *et al.*, 1987). There have been reports of growth stimulation of *Eucalyptus* species inoculated with ectomycorrhizal fungi in nurseries, plantations and glasshouse experiments (Garbaye *et al.*, 1988; Grove *et al.*, 1991; Burgess *et al.*, 1993, 1994). In most studies *P. tinctorius* has been used as an inoculant ectomycorrhizal fungus. The mycorrhizal fungal species *Hydnangium carneum* Wallr. in Dietr., a basidiomycete that is thought to be specific to *Eucalyptus* species, has been only briefly described in association with this genus (Malajczuk *et al.*, 1982, 1984). Malajczuk *et al.* (1984) carried out ultrastructural studies on ectomycorrhiza formation between *E. marginata* Donn ex Sm. and *E. diversicolor* F. Muell. with *H. carneum*. Moore *et al.* (1989) carried out a similar study between *E. pilularis* Sm. and *H. carneum*. They found this association was characterised by the greatly enlarged inner mantle hyphae, the distinct layering of the mantle and abundant deposits in the mantle hyphae.

Some *Eucalyptus* species form both AM and EM in the same root system or even on the same root (Chilvers *et al.*, 1987; Boudarga *et al.*, 1990). However, studies on AM are fairly recent, with the first controlled synthesis by Malajczuk *et al.* in 1981 and the first ultrastructural study by Boudarga and Dexheimer in 1988 (Adjoud *et al.*, 1996). Lapeyrie and Chilvers (1985) suggested that arbuscular mycorrhizas of *E. dumosa* A. Cunn. ex Schau. contributed largely to tolerance of calcareous soil and stimulated growth; they were the dominant type of mycorrhiza on roots of 2-month-old plants. Schoeneberger (1984) found that *Gigaspora margarita* Becker & Hall was able to stimulate the growth of 4-month-old *E. regnans* F. Muell. In contrast, none of the 30 AM isolates tested was able to stimulate growth of eight different *Eucalyptus* species up to three months after inoculation (Gomez *et al.*, 1987). More recently, AM inoculation was found to have no effect on *E. grandis* growth, and even to have a depressive effect on plant growth when inoculum was added to EM plants, as compared with exclusively EM plants (Amorim and Muchovej, cited by Lapeyrie *et al.*, 1992; Muchovej and Amorim, 1990). Most recently Adjoud *et al.* (1996) studied the response in growth of 11 *Eucalyptus* species by inoculating them with the AM fungi and found that most species tested showed a significant growth stimulation compared to control plants. Therefore it seems that the dependency of *Eucalyptus* species on AM is still debatable.

1.9.1 *Mycorrhizal Associations of Eucalyptus camaldulensis*

Eucalyptus camaldulensis is particularly important for the production of domestic products, such as poles, posts and timber, but above all, and increasingly, fuelwood. Because of its economic importance and because it is planted as an exotic, inoculation of this species with mycorrhizal fungi may be required depending on the availability of inocula. The effect of mycorrhizal inoculation on growth of this species is therefore of potential importance. Studies on this particular species are very few. Malajczuk and Hartney (1986) compared EM (by using *P. tinctorius, H. carneum, S. verrucosum* and *L. laccata*) formation on micropropagated plantlets and seedlings of *E. camaldulensis* and observed much more uniform mycorrhizal formation on micropropagated plantlets than seedlings. 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 *T. terrestris*. According to Jamaluddin and Chandra (1997), *E. camaldulensis* in undisturbed plantations in India exhibited root colonisation of up to 59% by AM fungi. They have also reported that in *E. camaldulensis*, the initial colonisation takes place by AM which is later replaced by various EM fungal species. Improvement of soil properties along with higher biomass accumulation and N uptake were observed in a long term (two year period) study where 5-6 month old *E. camaldulensis* seedlings co-inoculated with *Azotobacter chroococcum* (SM3) and *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske were planted on a waste/barren land in India using farmyard manure and chemical fertilisers (Meshram *et al.*, 1997). Vishwakarma and Singh (1996a) reported a significantly higher height growth and total biomass attainment in *E. camaldulensis* seedlings inoculated with seven AM fungi as compared to the non-inoculated seedlings. In another study, Vishwakarma and Singh (1996b) found that a dual inoculation of *P. tinctorius* and AM fungi of *E. camaldulensis* and *E. tereticornis* resulted in increased biomass with 137.8 and 164.85% respectively, compared with the uninoculated controls. However, they did not quantify the nutrient regimes used in their experiments. In none of these studies was there a systematic attempt to assess the developmental aspects of AM and EM in *E. camaldulensis*. Also these studies did not properly address the host nutrition aspect which may be critical for dual mycorrhizal development in *E. camaldulensis*.

1.10 The Present Study

The general objectives of this project are to look at the effects of various AM and EM fungal inoculation, and of possible dual inoculation (involving both AM and EM fungi) on mycorrhiza formation and growth performance of seedlings of *E. camaldulensis* under different nutrient regimes. The background of the project is based on the growth performance of *E. camaldulensis* in plantations in Bangladesh. There was no attempt to include any program of mycorrhizal inoculation when the first systematic introduction of this species began in early 1978 in Bangladesh (White, 1979; Kamaluddin, personal communication). Because of its fast growth this species is very popular among rural people for fuelwood, poles and houseposts. The Forest Department in Bangladesh (Rahman, pers. comm.) recently found that this species was not performing well despite its initial (first 4-5 years' time period) good growth on degraded hill forest areas. At the outset of this study, it was hypothesised that the poor growth performance of *E. camaldulensis* in Bangladesh was the result of inadequate mycorrhizal colonisation, or due to presence of inappropriate fungal symbionts or absence of inoculum. Soil samples were, therefore, brought from sites of *E. camaldulensis* plantations in Bangladesh to assess them for existence of any mycorrhizal fungal flora, and to use them as a source of inoculum.

The aim of the project was to identify suitable mycorrhizal associations for *E. camaldulensis* in Bangladesh so that the species can be encouraged to grow well in degraded forest areas by mycorrhizal inoculation. The objectives of the present study were (1) to identify the most appropriate growth medium and nutrient availabilities for laboratory experimentation concerning mycorrhizal colonisation and growth of *E. camaldulensis*, (2) to screen the species with a wide range of fungi to assess which fungi are able to form mycorrhiza with it, (3) to investigate interactions between fungi and nutrient availability on growth and nutrient uptake in *E. camaldulensis*, and (4) to investigate the structures produced by AM and EM to see if they indicate compatible/effective mycorrhizal associations.

It was hypothesised that:

(1) *E. camaldulensis* seedlings can form effective AM associations and that they have a positive impact on growth and nutrient uptake;
(2) *E. camaldulensis* seedlings can form effective EM associations in the early period of growth and that they have a positive impact on growth and nutrient uptake;

(3) *E. camaldulensis* seedlings can form both AM and EM in the same root system and that they have a positive impact on growth and nutrient uptake;

(4) there is a relationship between plant growth and nutrient uptake, and mycorrhizal colonisation, with respect to variation in nutrient supply; and

(5) the AM and EM structures indicate a compatible and effective association.

CHAPTER 2

General Materials and Methods

CHAPTER 2

General Materials and Methods

2.0 Introduction

The experiments were carried out between May 1996 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 day/night thermoperiod of 16/8 h. Natural light was supplemented with high pressure mercury vapour lamps to ensure a day length of 16 h. Ventilation was provided by one paddle fan and two automated vents at the apex of the glasshouse.

However, the environmental conditions (for example, temperature, light) varied during the course of the experiments. Day temperatures sometimes could be as high as 35°C during hot summer days. Light at pot height varied between 400-800 μmol photons m^{-2} s^{-1} (expressed as photosynthetic photon flux density, PPFD). For some periods of cloudy weather the supplementary light provided did not fully compensate for the low availability of natural light (see Plate 2.1).

2.1 Experimental Tree Species

Seeds of *E. camaldulensis* Dehnh. of SILVERTON (UMBER.CK) provenance were obtained from Australian Tree Seed Centre, Commonwealth Scientific and Industrial Research Organisation(CSIRO), Canberra, Australia. The seedlot no. was 15195 which was collected from New South Wales, Australia (located at 31° 53′ S latitude and 141° 13′ E longitude). The same seedlot was used throughout the study.

Plate 2.1: **(a)** Layout of preliminary, first-year experiments in one litre pots, **(b)** subsequent, second- and third-year experiments in two litre pots, **(c)** seedlings growing under good natural light conditions and **(d)** seedlings growing under low light conditions supplemented by mercury vapour lamps.

2.2 Inocula

The experiments involved a range of arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi. The isolates which were cultured for inoculation purposes are listed in Tables 2.2.1 and 2.2.2.

Table 2.2.1: Origin of AM inocula.

Inoculum	Isolate code	Country of origin	Place of origin	Date of collection	Plant of origin
Glomus intraradices Schenck and Smith.	UT143-2*	USA	Not known	Not known	Not known
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	*E. 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).

Table 2.2.2: Origin of EM inocula.

Inoculant fungus	Isolate code	Country of origin	Place of Origin	Date of collection	Plant of origin
Pisolithus tinctorius (Pers.) Coker and Couch.	PTE * (IMI368132)	Philippines	Not known	1990	*E. camaldulensis*
do	Vietnam	Vietnam	Binh Son	1997	*E. camaldulensis*
do	K55 * (IMI368154)	Portugal	Obidos	1993	*E. globulus*
do	PT1	India	Madras	1989	*E. tereticornis*
do	PT3	Tasmania	Murdunna	1990	*E. globulus*
do	PT7*	Scotland	Glasshouse experiment	1993	*E. globulus*
do	PT8*	Scotland	Glasshouse experiment	1993	*E. globulus*
Hydnangium carneum Wallr. in Dietr.	UAMH6196*	USA	Oregon	1978	*E. globulus*

* 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. Isolates PTE and K55 were supplied by the University of Kent, Kent, UK which were deposited under IMI (International Mycological Institute, Surrey, UK) collection. Isolate UAMH6196 was supplied by the University of Alberta, Edmonton, Canada.

Pisolithus tinctorius (Pers.) Coker and Couch. is synonymous with *Pisolithus arhizus* (Pers.) Rausch. Although *P. arhizus* is now accepted as the correct name for the fungus, *P. tinctorius* has been used in this thesis as this is the more extensively quoted name in mycorrhizal literature and the name given to the sporocarp collections from which these isolates were made.

2.3 AM Inoculum Preparation

Loam (top soil from a Scottish field), sand (river-washed sand called 'Silvasand', Silvaperl) and grit ('Tarmac Quartzag' grit) supplied by Scottish Agricultural Industries (SAI) Horticulture Limited, Auchterarder, Perthshire were mixed in the proportions 2:2:1 by volume and sterilised for one hour in an autoclave at a temperature of 121°C and 1.06 kg cm^{-2} pressure. The pH of the mixture after autoclaving was 6.5. The proportions of N, P and K in the loam were 150:10:85. The concentrations of N, P and K were in mg kg^{-1} mixture. 250 ml plastic pots were filled with the mixture. 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 filled pots. 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 roots of cowpea and millet were sub-sampled for assessment of mycorrhizal colonisation (details of assessment in section 2.10). Pots where root samples were found to be ≥60% mycorrhizal (≥60% of the total root length being colonised) were used for collecting roots and/or soil as inoculum. In some experiments, washed mycorrhizal roots were used as inoculum but mostly a soil-root mixture was used for inoculation.

2.4 EM Inoculum Preparation

EM fungal isolates 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 either vermiculite-peat soaked in 180 ml MMN or simply 180 ml MMN solution depending on the species of the fungus being cultured. For preparation of flasks and composition of MMN see Mason (1980). Flasks were maintained at room temperature for four months prior to use as inocula.

2.5 Substrate Preparation

Two kinds of substrate were used in the preliminary experiments (experiments 1 and 2): sand-perlite (SP) and vermiculite-peat (VP). For the subsequent experiments only VP was used as it was found suitable for both mycorrhiza formation and growth of *E. camaldulensis* seedlings.

For SP, sand was collected from Kittleyknowe quarry in the Pentland Hills (located between $3^{\circ}\ 19^{\prime}$ W and $55^{\circ}\ 47.5^{\prime}$ N), southern Scotland, and washed with water over a sieve (mesh size 0.25 mm). The sand was thoroughly washed in water to free it from mineral nutrients so that it became virtually inert (although no chemical analysis was carried out to assess any remaining mineral elements). Perlite (Silvaperl, William Sinclair Horticulture Limited, Firth Road, Lincoln, UK) was washed with water in the same manner. Washed sand and perlite were then mixed in the proportions 3:1 by volume. Perlite was mixed with the sand to enhance its water holding capacity.

For VP, vermiculite (Silvaperl, William Sinclair Horticulture Limited, Firth Road, Lincoln, UK) was mixed with *Sphagnum* peat (Bordnamone, Newbridge, Republic of Ireland) which had been dried, shredded and sieved (using a sieve of mesh size 2 mm). Vermiculite, peat and tap water were mixed in the proportions 45:10:175 by mass.

Both SP and VP mixtures were autoclaved at 121°C and 1.06 kg cm^{-2} for one hour. For all experiments, plastic pots (of one or two litre capacity) were used, which were new or were disinfected by soaking in diluted (7 ml per litre water) Jeyes Fluid disinfectant (21% Tar Acids and Methanol) for 48 hours, and thoroughly rinsed before being filled with either of the mixtures.

2.6 Seed Germination

Seeds of *E. camaldulensis* were pre-soaked in 0.1% Thiram (a dithiocarbamate fungicide) for 24 h, dried and sown into sterile trays containing sterilised 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}). Germination started one week after sowing and at the end of three weeks, the tray with the germinated seedlings was transferred to the glasshouse bench for hardening off in natural light for a further week.

2.7 Planting and Fungal Inoculation

One seedling was transplanted from the tray into each pot. For AM experiments, inoculation was effected by adding 2 g portions of washed root inoculum, or 5 g portions of soil-root mixture inoculum to the planting hole. For EM experiments, inoculation was effected by adding 2 or 4 g portions of mycelial VP (or pure mycelium from the liquid cultures) to the planting hole. Control seedlings received autoclaved portions of the same inoculum. For the first two experiments, pots were covered with sterilised grit so as to prevent development of algae on the surface of the substrates. When no algal development was observed on the spare pots without grit maintained alongside those experiments, no further use of sterilised grit for the subsequent experiments was made.

2.8 Nutrient Solution and Nutrient Treatment

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. The composition of the solution is given in the Appendix A.

It is well established that mycorrhizas help plants in the uptake of nutrients, particularly the immobile ones such as P (Smith and Read, 1997). Many mycorrhizal experiments have been carried out where improved P uptake in mycorrhizal plants has been found to be a main beneficial effect of inoculation. In these kinds of experiments with mineral nutrition (for example, experiments done by Bougher *et al.*, 1990; Burgess *et al.*, 1994; Jones *et al.*, 1998; Mason *et al.*, 1999b), N, K and other elements are usually kept constant while varying P; P is either added as a powdered solid, or in solution. Some authors have carried out experiments where liquid nutrient solutions have been used in different concentrations keeping all elements in proportion (for example, Abouelkhair *et al.*, 1986; Schier *et al.*, 1996). In these kinds of experiments, one can see how a proportional reduction or increase in the supply of all nutrient elements affects mycorrhizal formation and growth responses in seedlings. Tropical infertile soils (where a beneficial effect from mycorrhizal inoculation of seedlings is expected) often present combined deficiencies of P, N and K (Sieverding, 1991). It is well established that mycorrhizal effects are usually greater under low nutrient availability (Gerdemann, 1975), so it is important to see how the variation in N, P, K and micro-nutrients could affect mycorrhiza formation and growth responses in plants. It is claimed that *E. camaldulensis* can grow on a wide variety of site types (Florence, 1996) and it is, therefore, important to see how mycorrhizal inoculation of this species affect its growth and survival under various concentrations of nutrient elements. Therefore, in this series of experiments, the approach was to use various concentrations of nutrients keeping all elements in the same proportions.

Nutrient solutions were applied as exponentially increased dosages over the experimental period. Exponentially increasing fertiliser additions are reported to have stimulated mycorrhizal development compared with conventional constant-rate fertiliser addition (Ingestad *et al.*, 1986; Quoreshi and Timmer, 1998). Exponential fertiliser delivery keeps the concentration in the growth medium at a minimum and maintains a stable pH and conductivity. It also allows mycorrhizas to form and develop even at optimum nutrition (Kähr and Arveby, 1986).

2.9 Harvesting

Shoots were cut at soil level and separated into stem and leaves. For each seedling, stem diameter above the root-collar, shoot height (from the soil surface to the base of apical bud) and leaf number (all leaves) were measured. Total leaf area was determined for each seedling using a Delta -T area meter (Delta-T Devices, Cambridge, England). Stems and leaves of each seedling were also dried at 80°C for 3-4 days (Burgess *et al.*, 1994) and the dry mass recorded. Shoot dry mass and total dry mass were calculated.

2.10 Arbuscular Mycorrhizal Assessment

Pots were soaked overnight in water and roots from each pot were washed free (over a 2 mm sieve with a 0.25 mm sieve underneath to collect any root fragments that became detached) from 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 respectively from 1-2, 4-5, and 7-8 cm on the grid (Figure 2.2). These sections were sampled in order to correspond with the top, middle and bottom parts of the root system in a one litre pot. For a two litre pot, sub-samples from 1-2, 7-8, and 13-14 cm on the grid corresponded with top, middle and bottom of the pot. Each sub-sample and the remaining sample were then uniformly blotted on filter paper, and their fresh mass determined.

The remaining root fractions of each root system were dried at 80°C and dry mass recorded so that total root dry mass could be estimated from the proportions of fresh mass. Sampled roots were stained using the method of Phillips and Hayman (1970) with modifications given by Koske and Gemma (1989). The modified syringe method of Claassen and Zasoski (1992) was used for handling the samples during staining. 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. If doubt existed, the root fragments were mounted on glass slides and squashed under a cover slip for examination under a compound microscope to confirm or discount presence of mycorrhizal structures. After assessment sub-samples were preserved in 2% glutaraldehyde for study of mycorrhizal structures (see Chapter 5).

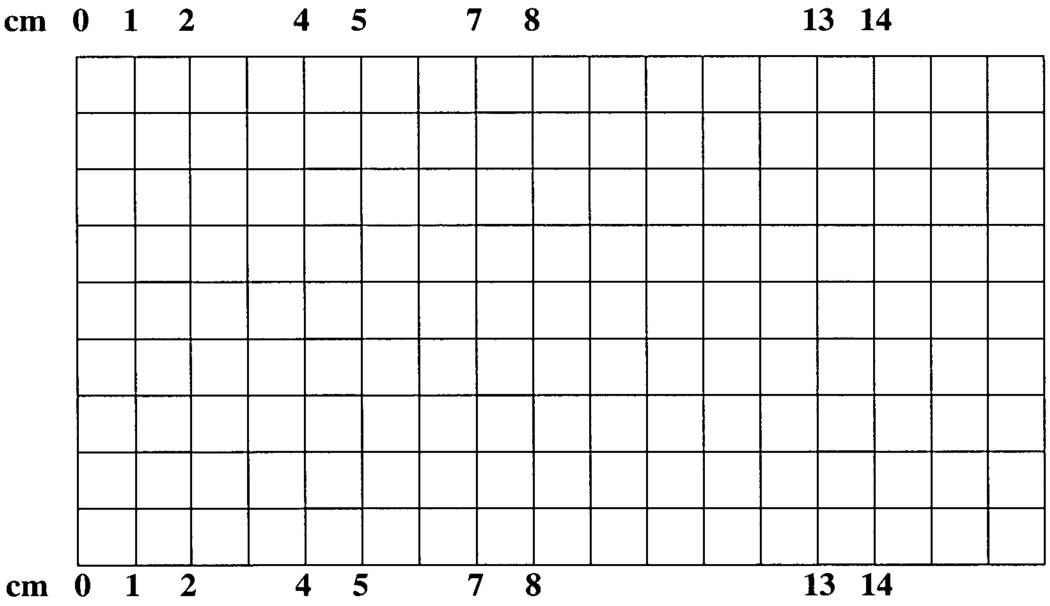

Fig. 2.1: Schematic presentation of a graduated glass plate used for sub-sampling a root system for mycorrhizal assessment.

2.11 Ectomycorrhizal Assessment

After washing and sub-sampling (Fig. 2.2), samples were placed in Petri dishes in water for examination of the tips of all short roots under a dissecting microscope. The tips were categorised according to whether they were mycorrhizal or not. An ectomycorrhizal root tip was defined as a short root with a mycelial mantle, however thin. If closer examination was necessary to confirm the mycorrhizal status or the fungal species, short roots were squashed under a cover slip with 1% cotton blue in 10% lactophenol and their mantle and hyphal characteristics were inspected under a compound microscope (Ingleby *et al.*, 1990). When no ectomycorrhizal colonisation was observed in the sub-samples, the remaining roots were checked for mycorrhizal root tips. After assessment, sub-samples were preserved in 2% glutaraldehyde for study of mycorrhizal structures (see Chapter 5).

Recording of dry masses were done following the same procedure as described in section 2.10.

2.12 Relative Growth Analysis

There is evidence that mycorrhizas increase growth of plants by altering their biomass allocation pattern (Baas *et al.*, 1989; Tinker *et al.*, 1994, Lovelock *et al.*, 1996). In most mycorrhizal studies, there were attempts to interpret biomass allocation pattern in terms of root:shoot ratio only (for example, studies done by Bethlenfalvay *et al.*, 1982; Bougher *et al.*, 1990; Burgess *et al.*, 1993; Vaast *et al.*, 1996; Reddy and Satyanarayana, 1998). But there is a range of other variables such as Relative Growth Rate (RGR), Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Specific Leaf Area (SLA), Leaf Mass Ratio (LMR) and Root Mass Ratio (RMR) which can be used to interpret results from any mycorrhizal experiments (Lovelock *et al.*, 1996). Calculating these 'relative growth' variables, therefore, helped comparison of biomass allocation pattern between seedlings inoculated with different mycorrhizal fungi and the non-mycorrhizal seedlings.

The relative growth variables are defined as according to Hunt (1978):

RGR represents the efficiency of the plant as a producer of new material. NAR represents the net gain in mass per unit of leaf area. While RGR treats all of the mass (for root, stem and leaf), NAR treats only leaf mass giving an estimate of the carbon-assimilatory capacity of the leaves. LAR represents the ratio of photosynthesising to respiring material within the plant. RGR depends on both NAR and LAR. SLA represents the leaf density or relative thickness (mean area of leaf displayed per unit of leaf mass), while LMR represents the proportion of leaf mass relative to the plant mass. LAR depends on both SLA and LMR. RMR represents the proportion of root mass relative to the plant mass, and RSR represents relative proportions of plant root and shoot mass.

Different variables of relative growth were calculated as the following equations:

1) average Relative Growth Rate, RGR= $(\ln W_2 - \ln W_1)/(t_2 - t_1)$,
where W_2 and W_1 are the dry masses at the end and the beginning of the experiment respectively, and $t_2 - t_1$ is the duration of the experiment in days;

2) Net Assimilation Rate, NAR= $[(W_2 - W_1)/(t_2 - t_1)]/[(LA_2 - LA_1)/(\ln LA_2 - \ln LA_1)]$,
where LA_2 and LA_1 are the leaf area at the end and the beginning of the experiment respectively; for other terms see Equation 1;

3) Leaf Area Ratio, LAR= LA_2/W_2; for terms see Equations 1 and 2;

4) Specific Leaf Area, SLA= LA_2/LW_2,
where LW_2 is the leaf dry mass at the end of the experiment; for LA_2, see Equation 2;

5) Leaf Mass Ratio, LMR= LW_2/W_2; for terms see Equations 1 and 4;

6) Root Mass Ratio, RMR= RW_2/W_2,

where RW_2 is the root dry mass at the end of the experiment; for W_2, see Equation 1; and

7) Root Shoot Ratio, RSR= RW_2/SW_2,

where SW_2 is the shoot dry mass at the end of the experiment; for RW_2, see Equation 6.

2.13 Foliar Nitrogen, Phosphorus and Potassium Analysis

Leaf samples for nutrient analysis were oven dried at 70°C for at least 24 h and kept in a desiccator prior to analysis. Dried leaf samples were ground to a fine powder with a ball-mill grinder (Glen Creston Ball-Mill, Nottinghamshire, England). Analyses of nitrogen (N), phosphorus (P) and potassium (K) concentrations were carried out on the ground leaf samples according to the method described by Allen (1989), with some modifications.

100 (±10) mg samples were weighed into test tubes. An acid oxidation process prior to analysis is necessary for oxidation of organic matter. Before the elemental analysis was carried out, 2 cm^3 of concentrated H_2SO_4 were carefully added to the sample followed by 100 vol H_2O_2 ($2x0.75\ cm^3$). The sample tubes were then heated at 320°C for 6 h; cooled and made up to 50 cm^3.

N concentration was determined by a gas diffusion method using a flow injection analyser (Perstorp Flow Solution 3000, Oregon, USA). P concentration was determined by the molybdenum blue method using a flow injection analyser as described in the application note ASN 60-04/83 (Perstorp Flow Solution 3000, Oregon, USA). K concentration was determined by atomic absorption spectrometry (919 Atomic Absorption Spectrometer, Unicam, Cambridge, UK).

Based on 100 mg of dried sample made up to a fixed volume of 50 cm^3, the concentration of N, P and K was calculated as follows:

$$c\ element\ (\%) = [c \times V_{sol}]/[10^4 \times \text{mass}_{sample}]$$

where:

c ($mg\ dm^{-3}$): Concentration of the solution;

V_{sol} (cm^3): Volume of the solution (50 cm^3); and

mass $_{sample}$ (g): Original mass of the sample (0.10 g).

2.14 Statistical Analysis

Data were analysed by Analysis of Variance (ANOVA). When data were found not to have a normal distribution, necessary transformations (for example, square-root transformation for ratios, arcsine transformation for mycorrhizal percentages and log transformation for all other variables) were carried out to normalise distributions and enable statistical comparisons of means. Normality and Heterogeneity of variances for data were tested using the Kolmogorov-Smirnov test and the Bartlett's test respectively in Minitab version 12.1 (Minitab Inc., USA, 1998). Means were compared by Fisher's least significant difference test when the results of Fisher's *F*-test from ANOVA were significant at $P \leq 0.05$. While treatment means from untransformed data were presented in graphs and tables, significant differences between the means were shown, where necessary, by using results obtained from *F*-test after the ANOVA was undertaken on the transformed data.

Throughout the study, GENSTAT version 5.3 (Lawes Agricultural Trust, Rothamsted, Harpenden, Hertfordshire, UK) was used for statistical analysis and Sigmaplot version 4 (SPSS Inc., Chicago, USA, 1997) for graphics.

CHAPTER 3

Effects of Rooting Medium and Nutrient Concentration on Arbuscular and Ectomycorrhizal Colonisation and Growth of *Eucalyptus camaldulensis* Seedlings

CHAPTER 3

Effects of Rooting Medium and Nutrient Concentration on Arbuscular and Ectomycorrhizal Colonisation and Growth of *Eucalyptus camaldulensis* Seedlings.

3.0 Introduction

Mycorrhizal studies aimed at establishing the relationship between nutrient availability and mycorrhizal formation may either require the use of a virtually inert growth medium or field soils analysed for their nutrient contents so that experiments can be done in controlled environments using known concentrations of mineral nutrients. Inert media are chosen when there is a need in the experiments to minimise interactions between soil P and applied P, and to focus on host-fungus growth relationships at applied P only (Bethlenfalvay *et al.*, 1982). In Australia, mycorrhizal experiments with *Eucalyptus* involving mineral nutrition used a yellow sand (pH 5.5, Bray extractable P <2 mg P kg $^{-1}$) (Bougher *et al*, 1990; Burgess *et al.*, 1993; Burgess *et al.*, 1994). Field soils (with 0.21-0.67 mg kg $^{-1}$ soil available P) are also reported to have been used in this kind of experiments with *Eucalyptus* (Aggangan *et al.*, 1996; Reddy and Satyanarayana, 1998) where nutrient analysis was undertaken before administering additional nutrient regimes. Sand-perlite mixture has been used as a growth medium for mycorrhizal studies of soybean (Bethlenfalvay *et al.*, 1982). A vermiculite-peat mixture has also been used in mycorrhizal experiments with *Eucalyptus* (Ingleby and Mason, 1996; Mason *et al.*, 1999a,b,c).

In general, a low (<20 mg kg^{-1}) to medium (20-100 mg kg^{-1} P) concentration of soluble P in growth medium is reported to be favourable for AM formation in some tropical tree species (Siqueira, 1986; Hurtado and Sieverding, 1986; as cited in Sieverding, 1991). In those studies, both obligate and facultative plants were reported to have taken advantage of mycorrhiza for their enhanced growth. However, growth responses to AM inoculation have been noted up to 148 mg kg^{-1} P (Lamar and

Davey, 1988) in *Fraxinus pennsylvanica* Marsh. Adjoud *et al.* (1996) used a 41 mg l^{-1} P nutrient solution in an AM experiment where they found successful mycorrhiza formation in 11 species of *Eucalyptus*, where most of them had a positive growth response to inoculation. EM formation occurs mostly at low P concentrations (Smith and Read, 1997). A concentration of 10 mg l^{-1} P was favourable for EM formation by most of the 10 fungi used in mycorrhizal experiments of *Eucalyptus globulus* Labill. by Mason *et al.* (1999b). However, P concentrations as high as 36 mg kg^{-1} P have resulted in EM colonisation in *Eucalyptus diversicolor* F. Muell. seedlings, but soil P additions ranging from only 2 to 12 mg kg^{-1} P resulted in a growth response to mycorrhizal inoculation (Bougher *et al.*, 1990). While in some of these experiments, P was added as powdered form, in others it was added in soluble form. In all the studies except that of Adjoud *et al.* (1996) cited above all other nutrients were kept constant while varying P only. Growth responses in mycorrhizal inoculated seedlings have been observed irrespective of the use of solid or liquid sources of nutrients (for example, Bougher *et al.*, (1990) used solid P and Adjoud *et al.* (1996) used liquid P, and both groups of workers observed positive growth responses in *Eucalyptus* seedlings). Therefore, comparison of concentrations of P and other nutrients used in these experiments is difficult. Also it is difficult to determine how much P or other nutrients will be available as actual amounts in soil irrespective of the sources of applied nutrients- insoluble or soluble. Nutrient elements including P, in particular, are more freely available in a nutrient solution but less freely available in a soil which may influence mycorrhizal formation and growth responses in seedlings. As nutrient elements are freely available in solution, it is relatively easy to control their concentrations in soil/growth media so as to maximise mycorrhiza formation. However, the great advantage of mycorrhizas in accessing relatively immobile ions such as P might be lost when they are in solution, and mycorrhizal effects on plant growth may therefore be less likely to be expressed.

The initial objective of the present study was to find the appropriate experimental conditions for AM and EM formation in *E. camaldulensis* seedlings. As an adjunct to the main objective, growth responses in the seedlings resulting from mycorrhizal inoculation were also measured. However, relative growth analysis was not carried out except that root:shoot ratio was calculated which gives a picture of change in biomass allocation pattern between the above-ground and below-ground parts of the seedling. Experiments were set up to compare effects of various substrates or growth media, e.g., sand-perlite (SP) and vermiculite-peat (VP) and two different nutrient regimes on both AM and EM formation on seedlings of *E. camaldulensis* using only one species of AM and EM fungus of each type. The nutrient regimes, 10 mg l^{-1} P and 30 mg l^{-1} P were chosen to correspond to those given by other authors as mentioned above for both the AM and the EM experiments but other nutrient elements were proportionally varied. Experiments of this nature were necessary to define the kinds and limitations of various treatments before embarking on more complicated experiments involving a range of AM and EM inoculant fungi and different nutrient regimes. Although it is difficult to arrive at any conclusion about the appropriate experimental conditions for mycorrhizal formation in plants using only one species of AM or EM fungus, the experiments were set up to identify an initial set of conditions which could be modified in later experiments in light of different requirements of different fungi.

The hypotheses tested were:

1) VP is better than SP as a growth medium for the AM fungus, *Glomus intraradices* UT143-2 or the EM fungus *Pisolithus tinctorius* isolate PTE in association with *E. camaldulensis* seedlings;

2) there is a significant difference in AM or EM formation and efficiency in *E. camaldulensis* seedlings at different nutrient concentrations with two substrates.

3.1. Effects of Rooting Medium and Nutrient Concentration on Arbuscular Mycorrhizal Colonisation and Growth of *Eucalyptus camaldulensis* Seedlings (Experiment 1).

3.1.1. Materials and Methods

3.1.1.1. *Experimental design*

The experiment contained a factorial combination of two inoculation treatments (one fungal and the other control), two substrates and two nutrient treatments; eight treatments in all. Ten randomised blocks each containing one pot per treatment were set up, with a single seedling per pot. Five of these blocks were harvested at each time. Growth conditions were as described in section 2.0 (Chapter 2).

3.1.1.2 *Fungal materials*

Glomus intraradices Schenck and Smith isolate UT143-2 (Nutralink- a commercial inoculum of Native Plants Inc., Salt Lake City, USA) was used as an AM inoculant fungus.

3.1.1.3 *Inoculum preparation*

The source inoculum of the AM fungus *G. intraradices* UT143-2 was a mixture of colonised roots and soil. Inoculum preparation was carried out as detailed in section 2.3 (Chapter 2).

3.1.1.4 *Substrate preparation*

The two substrate treatments were SP and VP mixtures. Preparation of the substrates were carried out as described in section 2.5 (Chapter 2). Plastic pots (130 mm diameter) of one litre capacity were used in this experiment.

3.1.1.5 *Seed germination, planting and fungal inoculation*

Seeds of *E. camaldulensis* were germinated and seedlings hardened off before transplanting as described in section 2.6 (Chapter 2). On May 29, 1996, one seedling was transplanted from the tray into each pot. Inoculation was effected by adding 2 g portions of washed root inoculum to the planting hole. Control seedlings received autoclaved portions of the same mycorrhizal roots.

3.1.1.6 *Nutrient treatment*

Two concentrations of nutrient solution containing 10 mg l^{-1}- a low P, and 30 mg l^{-1}- a high P were used (all other nutrient elements were proportionally varied between these concentrations). For the ease of describing, they are denoted in terms of phosphorus. Plants were supplied thrice a week with the solution with a gradually increased dosage every three weeks so that a total of 10 mg and 30 mg phosphorus had been added to low P and high P pots respectively by the end of the experiment. The proportions of N, P and K (100:16:55) were chosen to correspond to those used in mycorrhizal experiments at ITE, Edinburgh, involving other species of *Eucalyptus* than *E. camaldulensis*, that is, *E. globulus* by Mason *et al.* (1999a,b,c). In some of their experiments, however, only P varied between different nutrient concentrations while other nutrient elements were kept constant.

3.1.1.7 *Non-destructive assessments and maintenance of seedlings*

Seedling height was measured (from the root-collar to the tip) every week after two weeks of transplanting and watering (with deionised water) was done whenever necessary.

3.1.1.8 *Harvesting and mycorrhizal analysis*

Seedlings were harvested twice, at six weeks and at 12 weeks after transplanting. Harvesting procedure, measurements and method of root washing for mycorrhizal analysis was as described in sections 2.9 and 2.10 respectively (Chapter 2).

Each root system was about nine cm long. The complete root-system was laid out on top of a graduated glass-plate with the root collar at 0 cm. Three sub-samples, each one cm long, were taken respectively from 1-2, 4-5, and 7-8 cm on the grid (Fig. 2.1). Samples were stained and mycorrhizal assessment were done following the procedure as described in section 2.10 (Chapter 2).

Remaining root fractions of each root system were dried and total root dry mass estimated from the proportions of fresh mass.

3.1.1.9 *Statistical analysis*

Three-way analysis of variance was conducted using the factors- substrate, nutrient and inoculation. Comparison of means of different variables was done according to the procedure described in section 2.14 (Chapter 2).

3.1.2 Results

3.1.2.1 *Mycorrhizal formation*

At the end of six weeks of growth, colonisation of *E. camaldulensis* by the AM fungus was observed in a very few inoculated seedlings and only in the low P nutrient regime with both VP and SP. The control seedlings remained uncolonised. At 12 weeks, colonisation was again observed in a few low P mycorrhizal seedlings but still many inoculated seedlings had no mycorrhizal colonisation at all. No colonisation was found in high P mycorrhizal seedlings. Only the inoculated seedlings became colonised and the control seedlings remained uncolonised. The overall level of colonisation was very low (up to 6% of total root length).

3.1.2.2 *Non-destructive height growth*

After an initial lag phase of 2-3 weeks, seedlings in each treatment grew almost linearly in height, but at different rates (Fig. 3.1.1). The difference between height of mycorrhizal and non-mycorrhizal seedlings at both the nutrient treatments in SP or VP was not significant.

3.1.2.3 *Growth at the 6-week harvest*

At six weeks, there were significant substrate-inoculation interactive effects on stem diameter, height, leaf number, shoot dry mass and total dry mass (Table 3.1.1). In VP, average stem diameter, height and shoot dry mass of seedlings were significantly ($P \leq 0.05$) lower in the mycorrhizal treatment than in the non-mycorrhizal treatment, but in SP there were no significant difference between mycorrhizal and non-mycorrhizal seedlings in terms of these variables (Table 3.1.2). Non-mycorrhizal seedlings in VP had significantly higher total dry mass as compared to either mycorrhizal or non-mycorrhizal seedlings in SP. However total dry mass of mycorrhizal and non-mycorrhizal seedlings in VP did not differ significantly.

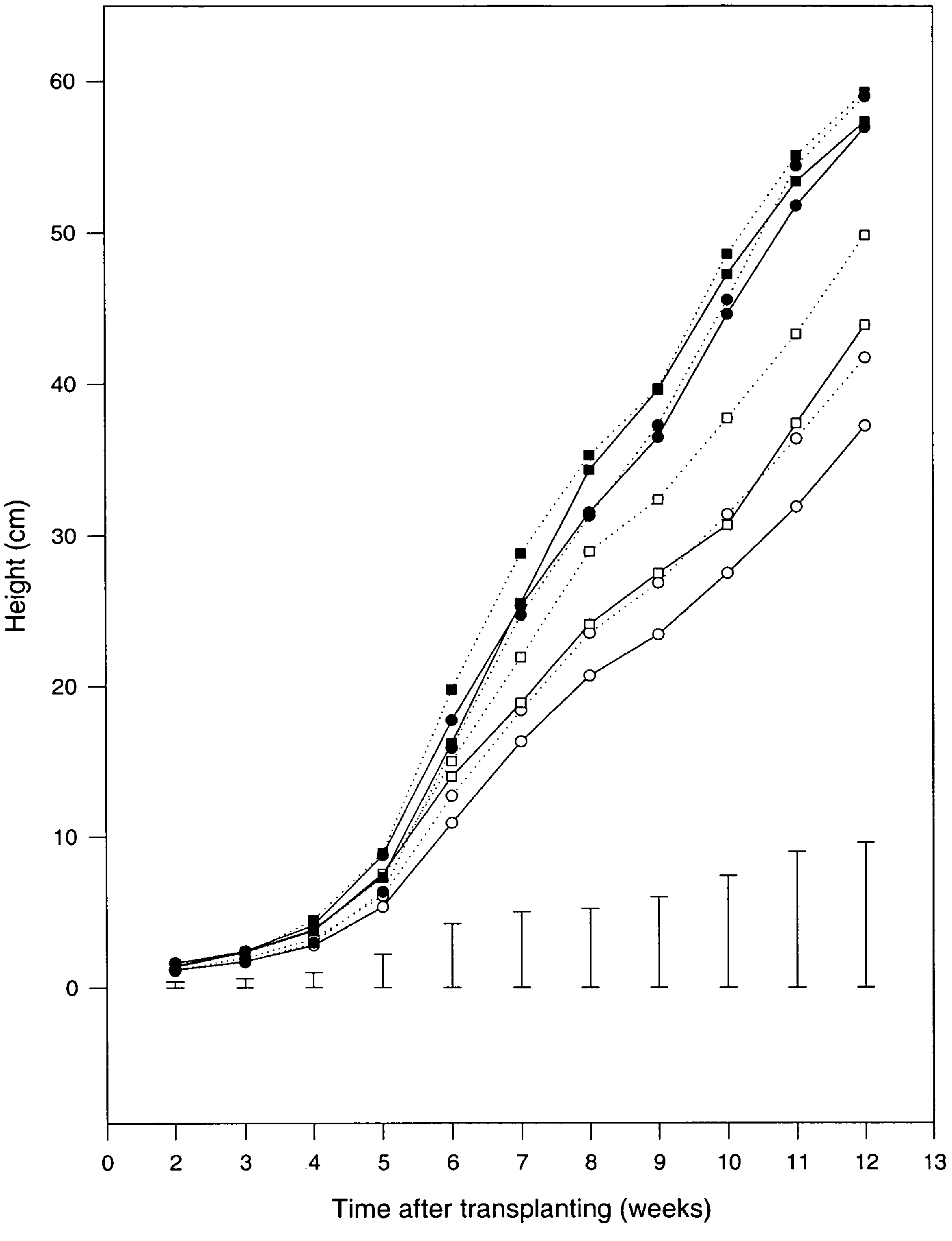

Fig. 3.1.1: Height assessment of *E. camaldulensis* seedlings grown in two substrates, two nutrient regimes and inoculated or uninoculated with the AM fungus *G. intraradices* UT143-2 in experiment 1(bars indicate SED between eight treatment means; open symbols, low P; closed symbols, high P; circles, SP; squares, VP; continuous lines, +AM inoculated and dotted lines, uninoculated control).

Table 3.1.1: Results of ANOVA of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), average leaf area (ALFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g) and root shoot ratio (RSR) of *E. camaldulensis* seedlings at the 6-week harvest of experiment 1. Factors are- substrate (Sub), nutrient (Nut) and inoculation (Inoc). Figures in bold indicate where effects were significant at *P* ≤0.05 (ANOVA).

Variable	Sub	Nut	Inoc	Sub*Nut	Sub*Inoc	Nut*Inoc	Inoc*Sub*Nut
DIAM	0.825	**<0.001**	0.053	0.601	**0.015**	0.076	0.329
HT	0.302	**<0.001**	0.235	0.082	**0.043**	0.995	0.210
LFNO	**0.004**	**<0.001**	**0.002**	0.087	**0.031**	0.622	0.599
LFAR	**0.032**	**<0.001**	0.054	0.419	0.080	0.669	0.700
ALFAR	0.788	**0.001**	0.747	0.693	0.967	0.886	0.811
SDM	0.094	**<0.001**	0.107	0.561	**0.047**	0.703	0.619
RDM	0.866	**0.031**	0.187	0.640	0.438	0.317	0.904
TDM	**0.003**	**<0.001**	0.764	0.405	**0.041**	0.287	0.580
RSR	0.392	0.413	0.092	0.827	0.282	0.589	0.886

Table 3.1.2: Substrate-inoculation interactions on stem diameter (DIAM), height (HT), leaf number (LFNO) shoot dry mass (SDM) and total dry mass (TDM) of *E. camaldulensis* seedlings at the 6-week harvest of experiment 1. Sub= substrate, SP= sand-perlite; VP= vermiculite-peat and MYCO= mycorrhizal; CON= the uninoculated control. Values for each variable followed by different letters differ significantly at *P* ≤0.05 (ANOVA).

	DIAM (mm)		HT (cm)		LFNO		SDM (g)		TDM (g)	
Sub	MYCO	CON	MYCO	CON	MYCO	CON	MYCO	CON	MYCO	CON
SP	2.8*ab*	2.7*ab*	27.06*ab*	25.50*a*	18.70*a*	21.20*a*	1.274*a*	1.211*a*	1.722*ab*	1.514*a*
VP	2.5*a*	3.0*b*	25.22*a*	30.96*b*	20.00*a*	29.80*b*	1.382*a*	1.895*b*	1.966*bc*	2.258*c*

Overall, the AM fungus had significantly negative growth effects on leaf number with the AM inoculated seedlings having one fifth fewer leaves compared to the uninoculated control, though the extent of colonisation was very low at the end of six weeks (up to 3% of total root length). The 6-week harvest showed that leaf area, leaf number and total dry mass of *E. camaldulensis* seedlings were significantly higher in VP as compared to SP (Table 3.1.3). There were main effects of nutrient on all measured variables except RSR, seedlings receiving 30 mg l^{-1} P having higher values than those receiving 10 mg l^{-1} P.

Table 3.1.3: Means of leaf number (LFNO), leaf area (LFAR), and total dry mass (TDM) of *E. camaldulensis* seedlings grown in two substrates, SP, sand-perlite; and VP, vermiculite-peat at the 6-week harvest of experiment 1. Means for each variable with different letters are significantly different at *P* ≤0.05 (ANOVA).

Substrate	LFNO	LFAR (cm^2)	TDM (g)
SP	19.95*a*	145.2*a*	1.618*a*
VP	24.90*b*	188.5*b*	2.112*b*

3.1.2.4 *Growth at the 12-week harvest*

By 12 weeks (Table 3.1.4), the situation had changed. Substrate-inoculation interaction had disappeared, but there were nutrient-inoculation interactions on root dry mass and root shoot ratio. At low P, root dry mass of mycorrhizal seedlings was significantly higher than that of the control seedlings; but at high P, control seedlings had a significantly (*P* ≤0.05) higher root dry mass than that of the mycorrhizal seedlings (Table 3.1.5). Low P mycorrhizal seedlings had a significantly higher root:shoot ratio as compared to high P mycorrhizal seedlings (Table 3.1.5), while root:shoot ratio of the control seedlings at low and high P did not differ significantly. Shoot dry mass was found to vary significantly between the substrates and nutrient regimes (Appendix C).

Table 3.1.4: Results of ANOVA of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), average leaf area (ALFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g) and root shoot ratio (RSR) of *E. camaldulensis* seedlings at the 12-week harvest of experiment 1. Factors are- substrate (Sub), nutrient (Nut) and inoculation (Inoc). Figures in bold indicate where effects were significant at *P* ≤0.05 (ANOVA).

Variable	Sub	Nut	Inoc	Sub*Nut	Sub*Inoc	Nut*Inoc	Inoc*Sub*Nut
DIAM	**<0.001**	**<0.001**	**0.031**	0.333	0.820	0.141	0.107
HT	0.072	**<0.001**	0.147	0.230	1.000	0.574	0.751
LFNO	**0.033**	**<0.001**	0.519	0.222	0.247	0.064	0.691
LFAR	**<0.001**	**<0.001**	0.152	0.860	0.278	0.751	0.499
ALFAR	0.591	**0.029**	0.613	0.473	0.466	0.086	0.472
SDM	**<0.001**	**<0.001**	**0.021**	**0.012**	0.350	0.994	0.706
RDM	**0.008**	**<0.001**	**0.035**	0.245	0.408	**0.022**	0.439
TDM	**<0.001**	**<0.001**	**0.002**	0.804	0.326	0.654	0.611
RSR	**0.047**	**0.014**	0.840	0.494	0.925	**0.038**	0.505

Table 3.1.5: Nutrient-inoculation interactions on root dry mass (RDM) and root:shoot ratio (RSR) of *E. camaldulensis* seedlings at the 12-week harvest of experiment 1. MYCO= mycorrhizal; CON= the uninoculated control. Values for each variable followed by different letters differ significantly at *P* ≤0.05 (ANOVA).

	RDM (g)		RSR	
Nutrient	MYCO	CON	MYCO	CON
low P	1.409*b*	1.380*a*	0.4688*b*	0.3976*ab*
high P	2.142*c*	2.767*d*	0.3255*a*	0.3845*ab*

At the 12-week harvest, stem diameter, leaf number, leaf area and total dry mass (Table 3.1.6) were still significantly higher in VP. Nutrient treatment effects were more pronounced than the other treatment effects (Appendix C). Overall, the AM fungus had a slight but significantly negative effect on stem diameter, shoot dry mass, root dry mass and total dry mass (Fig. 3.1.2).

Table 3.1.6: Means of stem diameter (DIAM), leaf number (LFNO), leaf area (LFAR), and total dry mass (TDM) of *E. camaldulensis* seedlings grown in two substrates, SP, sand-perlite; and VP, vermiculite-peat at the 12-week harvest of experiment 1. Means for each variable with different letters are significantly different at *P* ≤0.05 (ANOVA).

Substrate	DIAM (mm)	LFNO	LFAR (cm^2)	TDM (g)
SP	4.102*a*	32.5*a*	413.0*a*	6.04*a*
VP	4.649*b*	39.8*b*	549.4*b*	8.18*b*

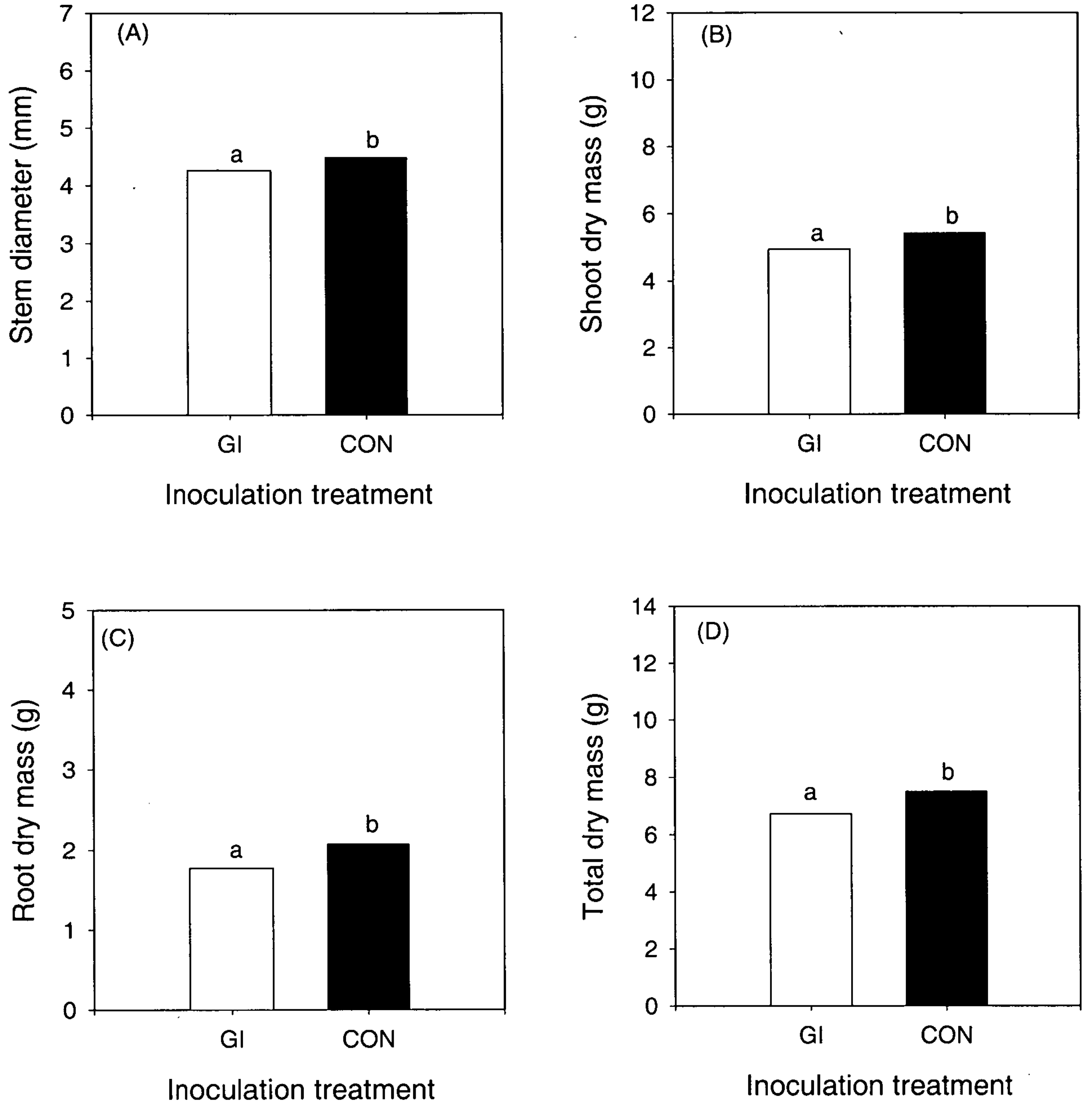

Fig. 3.1.2: Means of (A) stem diameter, (B) shoot dry mass, (C) root dry mass and (D) total dry mass of *E. camaldulensis* seedlings at the 12-week harvest of experiment 1 under two inoculation treatments; GI, *G. intraradices* UT143-2, CON, the uninoculated control. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

3.1.3 Discussion

The main feature of this experiment was the low AM colonisation. There was no difference between SP and VP in terms of AM colonisation, with only very few inoculated seedlings in each medium found to be colonised. The rate of colonisation at six weeks was found to be very low (1-3 % total root length being colonised). At 12 weeks, the rate of colonisation increased only slightly (up to 6 % of total root length being colonised). A number of reasons may account for this. Experimental conditions such as a very high or low temperature, a low quantity of available light, or excessive nutrient application may induce very little colonisation by the AM fungi (see below). However, low colonisation may also result from inoculation with an unsuitable fungus.

Sieverding (1991) has observed that temperatures between 40-45°C or less than 17°C can be problematic for AM fungal colonisation. In this case, average glasshouse day/night temperatures ranged between 25-30/15-17°C which seems to be suitable for AM formation. AM growth reductions associated with low light intensities (<260 μmol m^{-2} s^{-1}) have been commonly observed (Hayman, 1974; Daft and El Giahmi, 1978; Son and Smith, 1988). In this case, light availability was in the range of 500-600 μmol m^{-2} s^{-1} with a photoperiod of 16 h which was found suitable for AM fungal growth in similar experiments (Peng *et al.*, 1993; Lovelock *et al.*, 1996).

High availability of P is known to be inhibitory to the development of AM (Mosse, 1973; Menge *et al.*, 1978; Douds and Schenck, 1990; Bruce *et al.*, 1994). The concentration of P (10 mg l^{-1} P and 30 mg l^{-1} P) were not very high in this experiment relative to those of other studies. P availability more than 200 mg kg^{-1} soil (where Hoagland's nutrient solution was added) found to induce colonisation in *Belischiemidia pendula* (Sw.) Hemsl., a tropical tree species, by the AM fungal inoculation in the experiment of Lovelock *et al.* (1996). Other nutrients such as N or K were also not very high (62.5 mg l^{-1} and 34.4 mg l^{-1} respectively) at least for 10 mg l^{-1} P nutrient regime as compared to those of the AM study of *Eucalyptus* by Adjoud *et al.* (1996) which were 42 mg l^{-1} and 39.0 mg l^{-1} respectively for similar P concentration.

Different methods of assessment of AM colonisation could give different results (Giovanetti and Mosse, 1980). Also different approaches in the same method such as different length of time for clearing of samples in potassium hydroxide (KOH) prior to trypan blue staining could give different result due to variability of roots of various plant species in taking alkali. However, the same assessment method (grid line intersect method) and the staining method (trypan blue method) as used in this experiment was used in other AM experiments in this thesis where higher colonisation rates were observed. Thus it is unlikely that the extent of colonisation was underestimated or misjudged at least relative to subsequent experiments.

Low availability of inoculum could also result in low colonisation in seedlings. The inoculum was 60% mycorrhizal. The inoculum was handled carefully during preparation so it was unlikely that it had lost viability. However, the quantity of inoculum put to each planting hole could have been too low (2 g).

Another possibility is that the isolate used in the experiment was not an efficient coloniser of *E. camaldulensis* seedlings. Interestingly, even at such a low level of colonisation, the AM inoculation had a negative effect on seedling growth. Adjoud *et al.* (1996) studied responses of 11 *Eucalyptus* species to inoculation with three AM fungi and found that seedling growth stimulation was not related to the extent of root colonisation. In their study, *E. urophylla* S. T. Blake and *E. gomphocephala* D. C. did not show any significant growth stimulation despite high root colonisation by *G. intraradices* isolate CP103/CP (80% of root length); conversely, only 7% of *E. dives* Schauer root length was colonised by *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerd. isolate CP105/VF, but its stem dry mass was increased by 49%. Similarly, Jones *et al.* (1998) found no correlation between the proportion of the root system colonised by the different AM or EM fungi, and the growth response of the

Eucalyptus coccifera Hook. seedlings. Low colonisation has been reported to have significant growth responses in pigeonpea (*Cajanus cajan* L. (Millsp.) cv ICPL 86009), cowpea (*Vigna unguiculata* L. cv Kurumame) and groundnut (*Arachis hypogaea* cv Nakateyutaka) (Ahiabor and Hirata, 1994). However, none of these authors found any situation where a very low level of colonisation produced a negative growth response in terms of seedling dry mass as in the studies mentioned above.

This absence of positive correlation between mycorrhizal colonisation and plant growth responses has been observed with various AM associations involving other plant hosts than *Eucalyptus* (Lambert *et al.*, 1980; Powell, 1980; Saif, 1987). The nutrient effects were pronounced, with seedlings under high nutrition demonstrating significantly higher growth rate as compared to those under low nutrition. Colonisation was found to be significantly higher in low P plant treatment samples which also relates to other findings that mycorrhizal formation occurs at low nutrition availability (Smith and Read, 1997).

In this experiment, VP was found to be the best medium for growth of seedlings of *E. camaldulensis*. Seedlings in VP had significantly higher leaf area and total dry mass compared to those in SP. Although the water holding capacity of this medium was not measured in the experiment, it is obvious that its texture gives it an advantage over sand-perlite (which has a more porous texture) and thus it can hold water better than the other.

3.2 Effects of Rooting Medium and Nutrient Concentration on Ectomycorrhizal Colonisation and Growth of *Eucalyptus camaldulensis* Seedlings (Experiment 2).

3.2.1 Materials and Methods

3.2.1.1 *Experimental design*

The experimental design and replication were the same as in the preceding experiment (section 3.1.1.1).

3.2.1.2 *Fungal materials*

Pisolithus tinctorius (Pers.) Coker and Couch syn. *P. arhizus* (Pers.) Rausch isolate PTE was used as an EM fungal inoculant.

3.2.1.3 *Inoculum preparation*

Inoculum preparation was carried out following the procedure described in section 2.4 (Chapter 2).

3.2.1.4 *Substrate preparation*

Substrate preparation was carried out as described in section 2.5 (Chapter 2).

3.2.1.5 *Seed germination, planting and fungal inoculation*

Germination of seeds of *E. camaldulensis* and transferring of seedlings to pots were done in the same way as described earlier for the preceding experiment.

Three weeks after sowing, on July 17, 1996 one seedling was transplanted from the tray into each pot.

Mycelial plugs contained in vermiculite-peat were used as inoculum. Control pots received autoclaved inoculum.

3.2.1.6 *Nutrient treatment*

Initially the same two nutrient concentrations as described in section 3.1.1.6 were used until the first harvest. However, as little mycorrhizal colonisation was observed, it was thought that may be high nutrient concentrations were responsible for this. Therefore, after the first harvest, the nutrient concentrations were reduced to 5 and 15 mg l^{-1} P respectively. Until the first harvest, plants were supplied thrice a week with the solution with a gradually increased dosage every three weeks. After the first harvest, plants were supplied twice every week with the solution at adjusted nutrient concentrations so that the final amounts of P added were 10 mg and 30 mg to the low P and high P nutrient pots respectively (the same as in the previous experiment, but given over a longer period).

3.2.1.7 *Non-destructive assessment and maintenance*

Weekly height measurement and maintenance of seedlings were carried out as described in section 3.1.1.7.

3.2.1.8 *Harvesting and mycorrhizal analysis*

Seedlings were harvested twice, once at eight weeks and again at 16 weeks. Post harvest measurements were carried out as described in section 2.9 (Chapter2).

Mycorrhizal assessment was carried out and dry mass recorded as described in section 2.11 (Chapter 2).

3.2.1.9 *Statistical analysis*

Statistical analysis was carried out as described in section 3.1.1.9.

3.2.2 Results

3.2.2.1 *Mycorrhizal formation*

At the end of eight weeks of growth, colonisation of *E. camaldulensis* by the EM fungus was observed in a very few inoculated seedlings and only in the low P nutrient regime with both VP and SP (<1% of the root tips). The control seedlings remained uncolonised. At 16 weeks, colonisation was again observed in a very few low P mycorrhizal seedlings but still most inoculated seedlings had no mycorrhizal colonisation at all. No colonisation was found in high P mycorrhizal seedlings. Only the inoculated seedlings became colonised and the control seedlings remained not to be colonised. The overall level of colonisation was very low (<5% of total root length) at the end of 16 weeks of growth.

3.2.2.2 *Non-destructive height growth*

After an initial lag phase of three weeks, seedlings in each treatment grew almost linearly in height up to the eighth week. However, when nutrient regimes were altered at the ninth week, lines for different treatments tended to show a sigmoidal pattern. Seedlings grew at different rates in height (Fig. 3.2.1). The difference between height of mycorrhizal and non-mycorrhizal seedlings at both the nutrient treatments in SP or VP was not significant (Fig. 3.2.1).

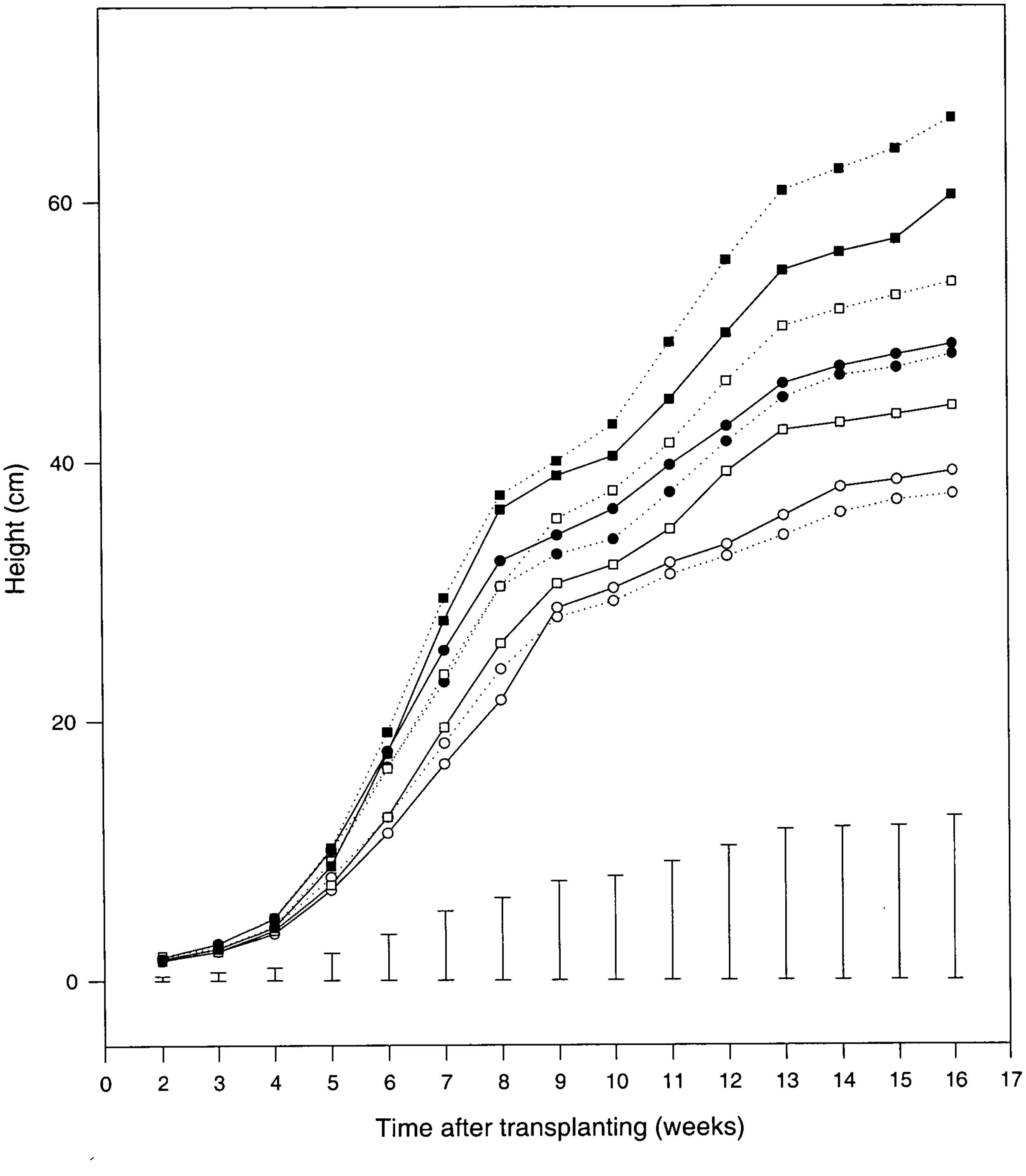

Fig. 3.2.1: Height assessment of *E. camaldulensis* seedlings grown in two substrates, two nutrient regimes and inoculated or uninoculated with the EM fungus *P. tinctorius* (isolate PTE) in experiment 2 (bars indicate SED for eight treatment means; open symbols, low P; closed symbols, high P; circles, SP; squares, VP; continuous lines, +EM inoculated and dotted lines, the uninoculated control). Note the difference in height growth from the eighth week when nutrient concentrations were reduced.

3.2.2.3 *Growth at the 8-week harvest*

At eight weeks, there was a significant substrate-nutrient-inoculation interactive effects effect on stem diameter (Table 3.2.1). At low P, stem diameter of SP seedlings was significantly (*P* ≤0.05) lower in mycorrhizal treatment than that in the non-mycorrhizal treatment, but with VP there was no significant difference in stem diameter of mycorrhizal and non-mycorrhizal seedlings at either low P or high P (Table 3.2.2).

Table 3.2.1: Results of ANOVA of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), average leaf area (ALFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g) and root shoot ratio (RSR) of *E. camaldulensis* seedlings at the 8-week harvest of experiment 2. Factors are- substrate (Sub), nutrient (Nut) and inoculation (Inoc). Figures in bold indicate where effects were significant at *P* ≤0.05 (ANOVA).

Variable	Sub	Nut	Inoc	Sub*Nut	Sub*Inoc	Nut*Inoc	Inoc*Sub*Nut
DIAM	**<0.001**	**<0.001**	0.749	0.917	0.190	0.075	**0.021**
HT	0.540	**0.006**	0.248	0.442	0.482	0.898	0.851
LFNO	**0.009**	0.076	0.296	0.649	0.527	0.338	0.963
LFAR	**<0.001**	**<0.001**	0.711	0.931	0.548	0.695	0.337
ALFAR	0.133	**<0.001**	0.438	0.533	0.757	0.458	0.429
SDM	**<0.001**	**<0.001**	0.554	0.184	0.501	0.802	0.369
RDM	0.245	**0.014**	0.452	0.218	0.698	0.747	0.187
TDM	**<0.001**	**<0.001**	0.909	0.605	0.668	0.761	0.221
RSR	**0.021**	**0.001**	0.133	0.666	0.202	0.510	0.166

Table 3.2.2: Substrate-nutrient-inoculation interaction on stem diameter of *E. camaldulensis* seedlings at the 8-week harvest of experiment 2. MYCO, mycorrhizal; and CON, the uninoculated control. Values followed by different letters differ significantly (*P* ≤0.05, ANOVA).

Substrate	Nutrient			
	low P		high P	
	MYCO	CON	MYCO	CON
SP	2.154*a*	2.498*b*	2.852*cd*	2.676*bc*
VP	2.688*bc*	2.594*bc*	3.134*d*	3.112*d*

The 8-week harvest showed that stem diameter, leaf number, leaf area, shoot dry mass, root dry mass and total dry mass of *E. camaldulensis* seedlings were significantly higher in VP as compared to SP (Tables 3.2.1 and 3.2.3). These results are similar to those from the first experiment and indicate that VP is preferable to SP as a growth medium for *E. camaldulensis* in terms of growth. There were main effects of nutrient on almost all variables measured, seedlings receiving 30 mg l^{-1} P having higher values than those receiving 10 mg l^{-1} P. There were no main effects of inoculation on any variables of seedling growth.

Table 3.2.3: Means of stem diameter (DIAM), leaf number (LFNO), leaf area (LFAR), and shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *E. camaldulensis* seedlings grown in two substrates, SP, sand-perlite; and VP, vermiculite-peat at the 8-week harvest of experiment 2. Means for each variable with different letters are significantly different (*P* ≤0.05, ANOVA).

Substrate	DIAM (mm)	LFNO	LFAR (cm^2)	SDM (g)	RDM (g)	TDM (g)
SP	2.545*a*	18.65*a*	191.8*a*	1.321*a*	1.478*a*	4.380*a*
VP	2.882*b*	22.50*b*	254.2*b*	1.715*b*	1.783*b*	6.580*b*

3.2.2.3 *Growth at the 16-week harvest*

There was a significant substrate-nutrient interaction effect on leaf area at 16 weeks (Table 3.2.4). At both the nutrient concentrations, leaf area for VP was significantly higher than that for SP (Appendix E).

Table 3.2.4: Results of ANOVA of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g) and root shoot ratio (RSR) of *E. camaldulensis* seedlings at the 16-week harvest of experiment 2. Factors are-substrate (Sub), nutrient (Nut) and inoculation (Inoc). Figures in bold indicate where effects were significant at *P* ≤0.05 (ANOVA).

Variable	Sub	Nut	Inoc	Sub*Nut	Sub*Inoc	Nut*Inoc	Inoc*Sub*Nut
DIAM	**<0.001**	**<0.001**	0.736	0.071	0.952	0.207	0.257
HT	**<0.001**	**<0.001**	0.376	0.594	0.090	0.620	0.664
LFNO	**<0.001**	**0.022**	0.410	0.179	0.772	0.934	0.901
LFAR	**<0.001**	**<0.001**	0.129	**0.005**	0.507	0.422	0.986
ALFAR	**0.045**	**0.001**	0.458	0.398	0.566	0.766	0.743
SDM	**<0.001**	**<0.001**	0.749	0.507	0.107	0.923	0.976
RDM	**0.009**	**0.004**	0.821	0.730	0.943	0.388	0.490
TDM	**<0.001**	**<0.001**	0.691	0.117	0.264	0.731	0.568
RSR	**<0.001**	**<0.001**	0.948	0.694	0.172	0.258	0.259

At the 16-week harvest, stem diameter, height, leaf number, leaf area, average leaf area and total dry mass were significantly higher in VP as compared to SP (Tables 3.2.4 and 32.5). Nutrient treatment effects were again found to be more pronounced than any other treatment effect with high P nutrient having a significantly higher positive impact on different variables of growth (Appendix E). Overall effects of inoculation on different variables of growth were not significant.

Table 3.2.5: Means of stem diameter (DIAM), height (HT), leaf number (LFNO), leaf area (LFAR), average leaf area (ALFAR) and total dry mass (TDM) of *E. camaldulensis* seedlings grown in two substrates, SP, sand-perlite; and VP, vermiculite-peat at the 16-week harvest of experiment 2. Means for each variable with different letters are significantly different (*P* ≤0.05, ANOVA).

Substrate	DIAM (mm)	HT (cm)	LFNO	LFAR (cm^2)	ALFAR (cm^2)	TDM (g)
SP	3.408*a*	45.3*a*	20.00*a*	297.3*a*	15.0*a*	4.380*a*
VP	3.963*b*	59.3*b*	28.60*b*	475.3*b*	17.6*b*	6.580*b*

3.2.3 Discussion

In this EM experiment, substrate and nutrient had similar effects on *E. camaldulensis* seedlings as were observed in the AM experiment. Again, the extent of colonisation was very low which appeared to be not adequate in forming active mycorrhiza. Successful EM colonisation was obtained in similar experiments involving *E. globulus* under similar environmental conditions except light which was maintained at 1800 μmol m^{-2} s^{-1} (Mason *et al.*, 1999a,b,c). In this experiment light availability was in the range of 500-600 μ mol m^{-2} s^{-1}. However, even a lesser availability of light (that is, 400 μmol m^{-2} s^{-1}) was found to be suitable for EM formation on *Eucalyptus* in a growth cabinet study of Jones *et al.* (1998).

The fungal isolate used for inoculation in this experiment, grew well in agar medium as well as in vermiculite-peat carrier soaked in MMN. However, Marx (1981) found no consistent relationship between the rate of growth of isolates in pure culture and the capacity of isolates to form EM. Recently, Thomson *et al.* (1993) observed similar trends in their experiment with *E. globulus* involving a number of selected isolates of EM fungi. In their study, rates of colonisation by three isolates of *Hebeloma westraliense* Bough., Tomm. & Mal. on *E. globulus* did not correspond with their growth rates on agar. The amount of inoculum put to planting hole was 2 g which could have been too low to encourage extensive mycorrhiza formation. However, use of inoculum in similar amounts has been reported to have resulted in successful EM formation on *E. globulus* in ITE experiments (Mason, personal communication). Also it has been reported that inoculum in a vermiculite-peat carrier does not help early mantle formation in glasshouse conditions (Ba *et al.*, 1994). In their experiment with *Pisolithus* sp. (ORS.X004) and *Afzelia africana* Sm., Ba *et al.* (1994) found that fungal mantle formation and Hartig net development were only conspicuous after 180 days of inoculation. A similar process may have occurred in this experiment. But the workers at ITE did not experience this in their EM experiments with *E. globulus* (Mason *et al.*, 1999b,c; Mason, personal communication).

High P availability can be the cause for a low EM colonisation (Smith and Read, 1997). However, in this experiment the low P treatment was 10 mg l^{-1} in concentration; a similar concentration of P was found to be favourable for EM formation with the *E. globulus-P. tinctorius* association in the study of Mason *et al.* (1999b). In the study of Bougher and Malajczuk (1990), EM colonisation of *E. diversicolor* by *Pisolithus* was found at a P concentration of as high as 24 mg kg^{-1}, where, of course, P was supplied as a powdered form [$Ca(H_2PO4)_2.H_2O$].

An alternative possibility is that the fungal isolate (*P. tinctorius* PTE, which was collected from under a *E. camaldulensis* plantation in the Philippines in 1990) used in the experiment may have lost the potential to colonise roots of *E. camaldulensis*. Similarly, a few *Pisolithus* isolates failed to form any mycorrhiza and some resulted in a very low colonisation (<2% of the root tips colonised) in a study with seedlings and micropropagated plantlets of *E. camaldulensis* (Malajczuk and Hartney, 1986). Similar reports regarding failure of some *Pisolithus* isolates in initiating colonisation (see Burgess *et al.*, 1994), and regarding ineffectiveness of some isolates in *Eucalyptus* can also be found (see Lei *et al.*, 1990; Tonkin *et al.*, 1989; Aggangan *et al.*, 1996).

Although VP and SP did not differ in terms of both AM (in the preceding experiment) and EM formation (in this experiment), VP was found to be superior to SP for growth of *E. camaldulensis* seedlings in both the experiments. In the next experiment(s) VP will be used as growth medium. As there was a very limited AM and EM formation from the use of the AM fungus *G. intraradices* UT143-2 and the EM fungus *P. tinctorius* PTE in the experiments done so far, a variety of different isolates of AM and EM fungi was used in the following experiment to select some of them in terms of their efficiency in mycorrhiza formation. Although the nutrient regimes adopted in the experiments were chosen on the basis that they were suitable for mycorrhiza formation in the studies conducted by other people, they might not have been appropriate for the mycorrhizal experiments of *E. camaldulensis* undertaken here. It could have been possible that a lower nutrient regime may have induced better mycorrhiza (both AM and EM) formation. Therefore, a lower nutrient regime was chosen for the subsequent experiment.

3.3 Comparison of AM and EM Fungal Inoculants and Their Types with Respect to Their Ability to Colonise Roots of *E. camaldulensis* Seedlings (Experiment 3).

3.3.0 Introduction

In the previous experiments, emphasis was given to three factors, namely- substrate, nutrient and inoculation. There was a limitation in observing colonisation potential of mycorrhizal fungi because only one isolate of each AM and EM fungi was used. As a very low colonisation rate was found in both the AM and the EM experiments, there was a need to include a number of both AM and EM fungi (fungal isolates) in a following experiment to determine whether some fungi may result in considerable colonisation, or at least higher colonisation compared to those in the previous experiments. As it was thought that the fungi already used in the experiments could have different effects on mycorrhiza formation when experimental conditions (for example, a change in nutrient regime) were varied, they were also used along with new fungal isolates at a lower nutrient regime. This experiment was, therefore, designed to enable the suitable fungi/fungal isolates to be identified for use in subsequent experiments. A lower nutrient regime, 5.0 mg l^{-1} P, was chosen to see if that was more suitable for mycorrhiza formation as compared to the earlier 10 mg l^{-1} P or 30 mg l^{-1} P.

The hypotheses tested were:

i) various fungal species and isolates (both AM and EM) differ in their effects on colonisation and growth of *E. camaldulensis* seedlings;

ii) the same isolate of fungus as different kinds of inoculum vary in their effects on colonisation and growth of *E. camaldulensis* seedlings.

3.3.1 Materials and Methods

3.3.1.1 *Experimental design*

The experiment contained 12 inoculation treatments (11 fungal and the other control) under one nutrient regime. Six randomised blocks each containing one pot per treatment were set up, with a single seedling per pot. Growth conditions were as described in section 2.0 (Chapter 2).

3.3.1.2 *Fungal materials*

Five isolates of *Pisolithus tinctorius* (PTE, K55, PT1, PT3 and PT7) and one isolate of *Hydnangium carneum* (UAMH6196) were used as EM inoculant fungi, and *Glomus intraradices* UT143-2, as both washed roots and unwashed roots and soil), *Gigaspora rosea* FL105-5, *Glomus clarum* BR148-1 were used as AM inoculant fungi.

3.3.1.3 *Substrate preparation*

Only VP was used in this experiment. Substrate preparation was carried out as described in section 2.5 (Chapter 2).

3.3.1.4 *Inoculum preparation*

AM and EM inoculum preparation were carried out as described in sections 2.3 and 2.4 respectively (Chapter 2). Inoculum containing *Hydnangium carneum* was also prepared in full strength MMN solution without using VP.

3.3.1.5 *Seed germination*

Germination of seeds of *E. camaldulensis* and transferring of seedlings to pots were done in the same way as described earlier for the preceding experiments.

3.3.1.6 *Planting and fungal inoculation*

Two weeks after sowing, on September 14, 1996, one seedling was transplanted from the tray into each pot.

Washed mycorrhizal roots (with about 60% colonised root length) or colonised root (unwashed) and soil mixture were used for AM inoculation. EM inoculation was effected by adding 2 g portions of mycelial VP (or pure mycelium from the liquid cultures) to the planting hole. Non-inoculated seedlings received autoclaved mycorrhizal roots.

3.3.1.7 *Nutrient treatment*

One nutrient concentration (5.0 mg l^{-1} P) was used. Plants were supplied twice a week with the solution with a gradually increased dosage every three weeks so that a total of 5.0 mg phosphorus had been added to individual pots by the end of the experiment.

3.3.1.8 *Maintenance of seedlings*

The maintenance of pots were done as described for the preceding experiments.

3.3.1.9 *Harvesting and mycorrhizal analysis*

Seedlings were harvested at twelve weeks after transplanting. Harvesting procedure, measurements, and method of recording dry masses were carried out following the procedures as described in sections 2.9 and 2.10 (Chapter 2). Methods for mycorrhizal assessment were as described in sections 2.10 and 2.11 for AM and EM respectively (Chapter 2).

3.3.1.10 *Statistical analysis*

A one-way ANOVA was carried out. Comparison of means were carried out following the same procedure as described in section 2.14 (Chapter 2).

3.3.2 Results

Only two AM fungi, notably *Gigaspora rosea* FL105-5 and *Glomus clarum* BR148-1 were found to produce some colonisation (<20% of the total root length), the other three produced very little colonisation <1% of the total root length). None of the EM fungi produced any colonisation at all. The EM fungi virtually died at the end of the experiment as no traces of live mycelium could be found attached to the root systems. Mycorrhizal fungal inoculation did not have any significant effect on growth of seedlings *of E. camaldulensis* (Tables 3.3.1 and 3.3.2).

Table 3.3.1: Results of ANOVA of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), and total dry mass (TDM, g) of *E. camaldulensis* seedlings under 11 fungal inoculation treatments and the uninoculated control in experiment 3. Figures in bold indicate where effects were significant at $P \leq 0.05$ (ANOVA).

Inoculation	Variable				
	DIAM	HT	LFNO	LFAR	TDM
	0.809	0.550	0.320	0.502	0.087

Table 3.3.2: Means of stem diameter (DIAM), height (HT), leaf number (LFNO), leaf area (LFAR), and total dry mass (TDM) in *E. camaldulensis seedlings* in experiment 3 under various inoculation treatments- PTE, K55, PT1, PT3 and PT7 are different isolates of *P. tinctorius*; HCVP and HCSS are the same isolate of *H. carneum* UAMH6196 in vermiculite-peat and MMN solution carriers respectively; GIWR and GISR are the same isolate of *G. intraradices* UT143-2 as washed root inoculum and soil and colonised root inoculum respectively; GR, *G. rosea* FL105-5; GC, *G. clarum* BR148-1; and CON, the uninoculated control. Means with different letters are significantly different ($P \leq 0.05$, ANOVA).

Inoculation treatment	DIAM (mm)	HT (cm)	LFNO	LFAR (cm^2)	TDM (g)
PTE	2.205*a*	26.70*a*	17.67*a*	150.0*a*	1.530*a*
K55	2.143*a*	26.13*a*	18.67*a*	145.4*a*	1.330*a*
PT3	2.160*a*	28.00*a*	20.00*a*	165.3*a*	1.570*a*
PT7	2.143*a*	25.93*a*	17.17*a*	139.6*a*	1.519*a*
PTcal	2.182*a*	29.92*a*	19.00*a*	150.9*a*	1.458*a*
HCVP	2.193*a*	28.73*a*	18.00*a*	150.3*a*	1.526*a*
HCSS	2.142*a*	26.78*a*	18.50*a*	149.5*a*	1.445*a*
GIWR	2.252*a*	22.90*a*	17.67*a*	156.0*a*	1.450*a*
GISR	2.148*a*	27.10*a*	17.33*a*	148.5*a*	1.365*a*
GR	2.097*a*	25.82*a*	19.17*a*	154.2*a*	1.347*a*
GC	2.032*a*	25.20*a*	15.00*a*	150.3*a*	1.379*a*
CON	2.147*a*	25.63*a*	19.00*a*	161.9*a*	1.462*a*

3.3.3 Discussion

The experiment helped to identify two suitable AM fungi for subsequent inoculation experiments which were *G. rosea* FL105-5 and *G. clarum* BR148-1. They resulted in <20% colonisation of the total root length.

No EM fungi survived at the end of this experiment. It is to be noted here that one of the EM fungi (*P. tinctorius* isolate PTE) used in this experiment had been able to initiate some colonisation in the second (EM) experiment as described earlier in this chapter. A general deficit in the availability of light (<400 µmol photon m^{-2} s^{-1}) was probably responsible for this. The time of the year when this experiment was carried out was from early autumn to early winter when availability of light was, in general, very low and there was a gradual decrease in day length. The temperature was unlikely to have been a factor because during daytime 25°C was maintained by heated pipes, but occasionally, at night, temperature could be as low as <10°C. The general health of the seedlings showed symptoms such as yellowing of leaves etc. which indicated that conditions other than mycorrhizal association was probably responsible for poor growth performance of seedlings.

3.4 Assessment of Soil Samples from Bangladesh for Mycorrhizal Fungal Spore Types

The objectives for the collection of soil samples from Bangladesh were to assess them for existence of any mycorrhizal fungal flora, and to obtain some indigenous fungi for inoculation purposes in the glasshouse studies.

Soil samples were collected during March, 1996 from forest plantation sites of two species, *E. camaldulensis* and *Albizia procera* (Roxb.) Benth. at Cox's Bazar located between 20°51′ and 21°47′ N and between 91°54′ and 92°19′ E (Chaudhury, 1969) in Bangladesh. No sporocarps of any EM fungi were found at either of the two sites. Trap cultures were established out of the soil samples using either cowpea (*V.*

unguiculata) and millet (*P. typhoides*) seeds or *E. camaldulensis* seedlings as hosts, and maintained in glasshouse light and temperature with regular watering (with deionized water, up to four months) for about nine months beginning from May, 1996. After nine months, soils from the trap cultures were subjected to AM spore extraction (for any available spores). From each trap culture, two tablespoonfuls (equivalent to 12 g) of soil were brought under spore assessment. The associated fungal spore types with approximate population density (number of total spores per g soil) are mentioned here with respect to different soil sources and hosts:

1) soil from *E. camaldulensis* plantation used with *E. camaldulensis* seedlings as host showed spores belonging to mostly *Acaulospora mellea* Spain and Schenck and some *Glomus occultum* Walker with approximate population density of <9 spores g^{-1} soil;
2) soil from *A. procera* plantation used with *E. camaldulensis* seedlings as host showed spores belonging to mostly undescribed *Acaulospora spp.*(very small, 50 micron, no Melzers reaction) and some *A. spinosa* and some *G. occultum* with approximate population density of <17 spores g^{-1} soil;
3) soil from *E. camaldulensis* plantation used with cowpea (*V. unguiculata*) and millet (*P. typhoides*) as host showed spores belonging to *A. mellea, Glomus manihotis* Howeler, Sieverding & Schenck, *G. occultum* and very few *Scutellospora sp.* with an approximate population density of <170 spores g^{-1} soil;
4) soil from *A. procera* plantation used with cowpea (*V. unguiculata*) and millet (*P. typhoides*) as host showed spores belonging to mostly undescribed *Acaulospora spp.* (very small, 50 micron, no Melzers reaction) and some *G. occultum* with an approximate population density of <90 spores g^{-1} soil.

A. procera soil was found to possess a different fungal flora from *E. camaldulensis* soil with both having a common species *G. occultum.* Later pot cultures were established using the trap culture consisting of soil from the *E. camaldulensis* plantation at Cox's Bazar in Bangladesh with cowpea (*V. unguiculata*) and millet (*P. typhoides*) as host. This trap culture was chosen because it gave the highest measure of spore population density including a variety of AM fungi namely *A. mellea, G. manihotis, G. occultum* and *Scutellospora sp.*

3.5 General Summary of the Findings From the Three Experiments And the Trap Culture Analysis of Bangladesh Soils.

Although VP and SP did not differ in terms of both AM and EM formation in the first two experiments, VP was found superior to SP for growth of *E. camaldulensis* seedlings in both the experiments. In the following experiments VP was therefore used as growth medium.

In the third experiment, two AM inoculant fungi resulted in a significant colonisation (<20% root length) of *E. camaldulensis* seedlings. They were *G. clarum* UT148-1 and *G. rosea* FL105.5. These two fungi were, therefore, found to be suitable for use in the subsequent experiments. Besides these two AM fungi, a mixed AM culture from Bangladesh was also considered to be suitable for subsequent use because this culture produced sufficient spores of a number of identifiable fungi. As no colonisation was observed by the EM fungi used in the third experiment it was decided that the number of EM fungal isolates to be used for the subsequent experiment should be increased. In the third experiment, one isolate of *Hydnangium carneum* (UAMH6196) and five isolates of *P. tinctorius* (PTE, K55, PT1, PT3 and PT7) were used. Previously *P. tinctorius* has been widely used in the inoculation experiments of *Eucalyptus* with successful results (Smith and Read, 1997). In the subsequent EM experiments, emphasis would only be given to *P. tinctorius*, and different isolates of this fungus would be used.

As it still could not be ascertained whether the nutrient regimes used so far were suitable or not for both AM and EM formation, a range of nutrient regimes would be considered for next experiments. It was decided that a further lower nutrient regime than 5 mg l^{-1} P, that is, a 2.5 mg l^{-1} P would be used alongside 5 mg l^{-1} P, 10 mg l^{-1} P and 20 mg l^{-1} P.

CHAPTER 4

Effects of Various Arbuscular and Ectomycorrhizal Inoculant Fungi on Mycorrhiza Formation, Growth and Foliar Nutrient Uptake in *E. camaldulensis* Seedlings Under Different Nutrient Regimes

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Effects of Various Arbuscular and Ectomycorrhizal Inoculant Fungi on Mycorrhiza Formation, Growth and Foliar Nutrient Uptake in *E. camaldulensis* Seedlings Under Different Nutrient Regimes.

4.0 Introduction

Eucalypts predominantly form EM associations in native forests (Chilvers, 1973; Malajczuk and Hington, 1981) and plantations (Chu-Chou and Grace, 1982; Brundrett *et al.*, 1996c). As noted in section 1.8 (Chapter 1), 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 *Eucalyptus 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. As noted in section 1.8.1 (Chapter1), 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.*

Reports of the AM associations of *Eucalyptus* are, however, relatively few. There are reports on the AM associations of *Eucalyptus* species in their native Australia (Malajczuk *et al.*, 1981; Brundrett and Abbott, 1995; Brundrett *et al.*, 1996a; Brundrett *et al.*, 1996b), and in exotic sites or plantations (Bakshi, 1966; Bala *et al.*, 1989; Coelho *et al.*, 1997a; Coelho *et al.*, 1997b; Gong, *et al.*, 1997; Jamaluddin and Chandra, 1997; Oliveira *et al.*, 1997). Jamaluddin and Chandra (1997) reported that *E. camaldulensis* in an undisturbed plantation in India exhibited root colonisation of up to 59.74% by AM fungi. Coelho *et al.* (1997b) found spores of *Acaulospora longula* Spain & Schenck, *Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders and *Glomus microcarpum* Tulasne & Tulasne, *G. invermaium* Hall, *G. macrocarpum* Tulasne & Tulasne, *Acaulospora denticulata* Sieverding & Toro, *A. mellea* Spain & Schenck and *Scutellospora heterogama* (Nicol. & Gerd.) Walker & Sanders with a greater abundance of the first three AM fungi in 1-8 year old plantations of *E. camaldulensis* in Minas Gerais in Brazil. Growth promotion of *Eucalyptus* seedlings by AM fungi in nurseries (Zambolim *et al.*, 1982; Lima *et al.*, 1994) and in pot experiments (Liang and Gong, 1995; Mehrotra, 1996; Vishwakarma and Singh, 1996a) have been reported in many studies. Improvement of soil properties along with higher biomass accumulation and N uptake were observed in a long term (two year period) study where 5-6 month old *E. camaldulensis* seedlings co-inoculated with *Azotobacter chroococcum* (SM3) and *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske were planted on a waste/barren land in India using farmyard manure and chemical fertilisers (Meshram *et al.*, 1997). Vishwakarma and Singh (1996a) reported a significantly higher height growth and total biomass attainment in *E. camaldulensis* seedlings inoculated with seven AM fungi as compared to the non-inoculated seedlings.

In a study of AM inoculation of *E. grandis* W. Hill. ex Maiden, *E. pellita* F. Muell., *E. saligna* Sm., *E. urophylla* S. T. Blake and *E. cloeziana* F. Muell., shoot dry mass of the seedlings was found to have decreased by 9.2% as compared to the controls where average AM colonisation was 45% of the total root length (Oliveira *et al.*, 1995). In the same study, shoot dry mass of the *P. tinctorius* inoculated seedlings increased by 26.5% where the EM colonisation rate was 71% of the total root tips. In a comparative study of AM and EM inoculation on *E. coccifera* Hook., Jones *et al.* (1998) found that growth promotion was largest in the EM inoculated seedlings. In their study, P inflow rates of EM and AM seedlings were 3.8 times, and 2.0-2.7 times those of non-mycorrhizal seedlings, and P acquisition efficiencies were similar for the two mycorrhizal types.

In spite of its association with AM fungi in the field, there are very few studies in the glasshouse or controlled environments regarding AM experiments of the genus *Eucalyptus*, and the species, *E. camaldulensis* in particular. One of the objectives of this project was to study the effect of a dual inoculation treatment on *E. camaldulensis* seedlings. Keeping that objective in mind, it was first necessary to know how a number of both AM and EM fungal isolates responded to the same range of nutrient concentrations so that the suitable concentration(s) could be identified for later use in a dual inoculation experiment. It has been confirmed that AM fungi need low to medium while EM fungi need very low to low P concentrations for mycorrhiza formation (Alexander, 1989). Although this is the case in general, individual tree and fungal species may vary in their responses to different nutrient concentrations. There is therefore a need to identify appropriate nutrient regimes so that both AM and EM can colonise the same root system simultaneously.

The overall objective of the experiments described here was to assess the development of both types of mycorrhizas separately in the root systems of *E.*

camaldulensis at various nutrient concentrations. The fourth experiment involved two inoculant fungi which were associated with a significant colonisation of *E. camaldulensis* seedlings in the third experiment, and a mixed AM culture from Bangladesh. As no colonisation was observed by the EM fungi in the third experiment, the number of EM fungal isolates to be used for the fifth experiment was increased. In the third experiment, one isolate of *H. carneum* and five isolates of *P. tinctorius* were used. Previously *P. tinctorius* has been widely used in the inoculation experiments of *Eucalyptus* with many successful results (Smith and Read, 1997). In the fifth experiment, emphasis was, therefore, only given to *P. tinctorius*, and five isolates of this fungus were used. The results of the experiments would help in designing a dual inoculation experiment where development of two different types of mycorrhizas in the same root system will be studied.

The hypotheses tested were:

1) there are effects of interaction between nutrient availability and mycorrhizal colonisation (both AM and EM) on growth and nutrient uptake in *E. camaldulensis* seedlings;
2) the availability of nutrient influences the colonising ability of mycorrhizal (both AM and EM) fungi; and
3) there is a relationship between the extent of colonisation and plant dry mass.

4.1 Effects of Various Inoculant Arbuscular Fungi on Mycorrhiza Formation, Growth and Foliar Nutrient Uptake in *E. camaldulensis* Seedlings Under Different Nutrient Regimes (Experiment 4).

4.1.1 Materials and Methods

4.1.1.1 *Experimental design*

The experiment contained a factorial combination of four inoculation treatments (three fungal and the other control) and four nutrient treatments; 16 treatments in all. Five randomised blocks each containing one pot per treatment were set up, with a single seedling per pot. Growth conditions were as described in section 2.0 (Chapter 2).

4.1.1.2 *Fungal isolates*

G*lomus clarum* Nicolson & Schenck. BR148-1, *Gigaspora rosea* Nicolson & Schenck. FL105-5 and a mixed AM culture from Bangladesh soil consisting mainly of *Glomus occultum* Walker and a few *Acaulospora* spp. were used as AM inoculant fungi.

4.1.1.3 *Substrate preparation*

VP was used as growth medium. Preparation of the growth medium was carried out as described in section 2.5. Pots of 160 cm diameter (2 litre capacity) were filled with the mixture.

4.1.1.4 *Seed germination, planting and fungal inoculation*

Seeds of *E. camaldulensis* were germinated according to the procedure described in section 2.6 (Chapter2).

On April 23, 1997, one seedling was transferred from the germination tray to each pot. Inoculation was effected by putting 5 g portions of soil-root mixture into the planting hole. Non-inoculated seedlings received autoclaved mycorrhizal soil-root mixture.

4.1.1.5 ***Nutrient treatment***

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 (see section 2.8 of Chapter 2). Plants were supplied twice a week with the solution with a gradually increased dosage every three weeks so that a total of 2 mg, 4 mg, 8 mg and 16 mg phosphorus had been added to various nutrient treatment pots respectively by the end of the experiment.

4.1.1.6 ***Harvesting and mycorrhizal analysis***

An initial harvest of 10 seedlings in the germination tray was carried out immediately before the experiment was established. The objective of this harvest was to provide initial measurements of leaf area and total dry mass measurements so that Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) could be calculated after the final harvest. Plant leaf area and dry mass at the beginning of the experiment were 0.255± 0.1272 cm^2 and 0.0023±0.0007 g respectively (n= 10 plants). The experiment was terminated at 14 weeks. RGR, NAR and other relative growth variables, for example, LAR, SLA, LMR, RMR and RSR were calculated using the formulas mentioned in section 2.12 (Chapter 2). Harvesting procedure, measurements and method of root washing for mycorrhizal analysis was as described in sections 2.9 and 2.10 respectively (Chapter 2).

As the pot size was larger for this experiment, each root-system was about 15 cm long from the root collar to the bottom. Therefore, three sub-samples, each one cm long, were taken respectively from 1-2, 7-8 and 13-14 cm on the grid (Figure 2.1). Mycorrhizal assessment was done and dry masses were taken following the same method as detailed in section 2.10 (Chapter 2).

4.1.1.7 *Foliar N, P and K analysis*

N, P and K contents in dried leaf samples were carried out as detailed in section 2.13 (Chapter 2).

4.1.1.8 *Statistical analysis*

A two-way analysis of variance (ANOVA) was conducted where the factors were nutrient regime and inoculation treatment. Statistical comparisons of means were done according to the method described in section 2.14 (Chapter2).

4.1.2 Results

4.1.2.1 *Influence of inoculation and nutrient regime on mycorrhizal colonisation*

In this experiment, inoculation with AM fungi was successful in terms of mycorrhiza formation. Casual observations of seedlings (from some extra-experimental seedlings inoculated at the same time in the same way as the experimental seedlings, and maintained alongside the experiment) indicated that AM colonisation started to spread (>10% of total root) by 6 weeks after inoculation. At the end of 14 weeks, extent of colonisation by the three AM fungi varied between 30-50% (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 (Table 4.1.1). Colonisation by *Glomus clarum* isolate BR148-1 or the Bangladesh culture did not vary significantly between 2.5 mg l^{-1} P and 20 mg l^{-1} P or between 5.0 mg l^{-1} P and 10 mg l^{-1} P. The extent of colonisation was highest at 10 mg l^{-1} P, which varied significantly from those at the former two. Colonisation by *Gigaspora rosea* isolate FL105-5 was not significantly different among the four nutrient treatments (Fig. 4.1.1).

Table 4.1.1: Results of ANOVA of extent of colonisation (EXC), stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g), relative growth rate (RGR, g g^{-1}day^{-1}), net assimilation rate (NAR, mg cm^{-2} day^{-1}), leaf area ratio (LAR, cm^2 g^{-1}), specific leaf area (SLA, cm^2 g^{-1}), leaf mass ratio (LMR), root mass ratio (RMR) and root shoot ratio (RSR) of *E. camaldulensis* seedlings in experiment 4 (the AM experiment). Factors are- nutrient and fungus. Figures in bold indicate where effects were significant at *P* ≤0.05 (ANOVA).

Variable	Nutrient	Fungus	Nutrient*Fungus
EXC	**0.004**	**<0.001**	**0.028-**
DIAM	**<0.001**	**0.004**	0.654
HT	**<0.001**	0.486	0.147
LFNO	**<0.001**	0.571	0.857
LFAR	**<0.001**	**<0.001**	0.074
SDM	**<0.001**	**<0.001**	**0.007**
RDM	**<0.001**	**0.012**	0.514
TDM	**<0.001**	**<0.001**	**0.003**
RGR	**<0.001**	**<0.001**	**0.003**
NAR	**<0.001**	**0.003**	0.659
LAR	**0.038**	**0.007**	0.717
SLA	0.513	**0.018**	0.129
LMR	0.456	0.936	0.822
RMR	0.471	0.931	0.827
RSR	0.397	0.913	0.881

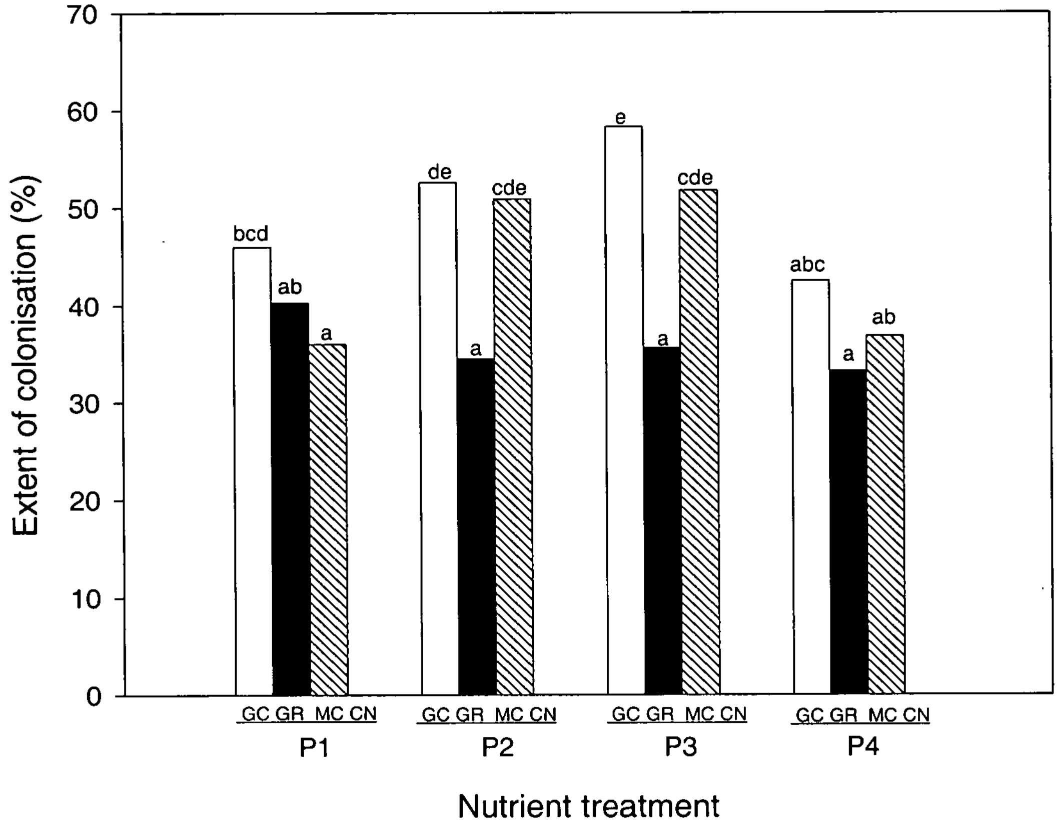

Fig. 4.1.1: Mean extent of colonisation (% root length colonised) of *E. camaldulensis* seedlings in the AM experiment (experiment 4) under four nutrient regimes; P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P and four inoculation treatments: GC, *G. clarum* BR148-1; GR,*G. rosea* FL105-5, MC, a mixed AM culture from Bangladesh and CN, the uninoculated control. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

On an overall basis, the three inoculant fungi had significantly different colonisation potential with *G. clarum* BR148-1having the highest (up to 50% of the total root length), followed by the Bangladesh culture (44%) and *G. rosea* FL105-5 (36%) respectively (Fig. 4.1.2).

Considering all the fungi together, highest colonisation was found to have occurred in 10 mg l^{-1} P, which was significantly higher compared to 2.5 mg l^{-1} P or 20 mg l^{-1} P. Colonisation at 10 mg l^{-1} P was not significantly different from that at 5.0 mg l^{-1} P. Colonisation at 20 mg l^{-1} P was the lowest although it did not differ significantly from that at 2.5 mg l^{-1} P (Fig. 4.1.2). There was no significant regression between extent of colonisation and nutrient concentration (r^2 =0.0009, $F_{1,57}$=0.03, P =0.903).

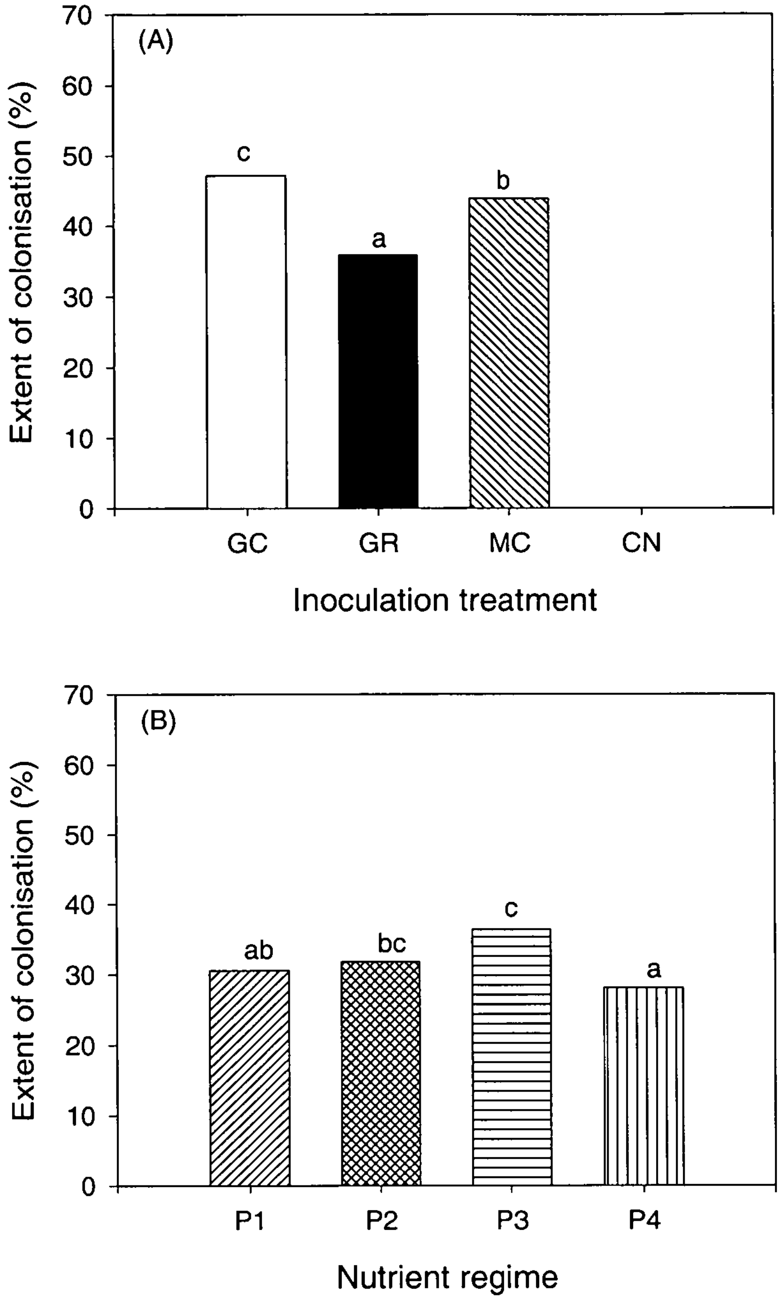

Fig. 4.1.2: Mean extent of colonisation of (% root length colonised) roots of *E. camaldulensis* seedlings (A) in different inoculation treatments: GC, *G. clarum* BR148-1; GR, *G. rosea* FL105-5; MC, a mixed AM culture from Bangladesh, and (B) under various nutrient regimes: P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P in the AM experiment (experiment 4). Values are treatment means and means with different letters are significantly different at $P < 0.05$ (ANOVA).

4.1.2.2 *Interaction of inoculation and nutrient regime on growth*

There were significant interactive effects of inoculation and nutrient treatments on shoot dry mass and total dry mass (Table 4.1.1). The three fungi did not vary significantly in their effects on both shoot dry mass and total dry mass of the seedlings grown at 2.5 mg l^{-1} P and 5.0 mg l^{-1} P but each of them was significantly different from the uninoculated control at these nutrient regimes (Fig. 4.1.3). 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 20 mg l^{-1} P, the effects of four inoculation treatments on dry mass of seedlings were not significantly different.

RGR of the seedlings inoculated by the three fungi were not significantly different at either 2.5 mg l^{-1} P or 5.0 mg l^{-1} P but they were significantly lower compared to the uninoculated control at each of these nutrient treatments. At 10 mg l^{-1} P, *G. clarum* BR148-1 was associated with significantly lower RGR of seedlings as compared to those of the other two fungi of which none was significantly different from the uninoculated control. At 20 mg l^{-1} P, effects of the four inoculation treatments on RGR of seedlings were not significantly different (Fig. 4.1.4).

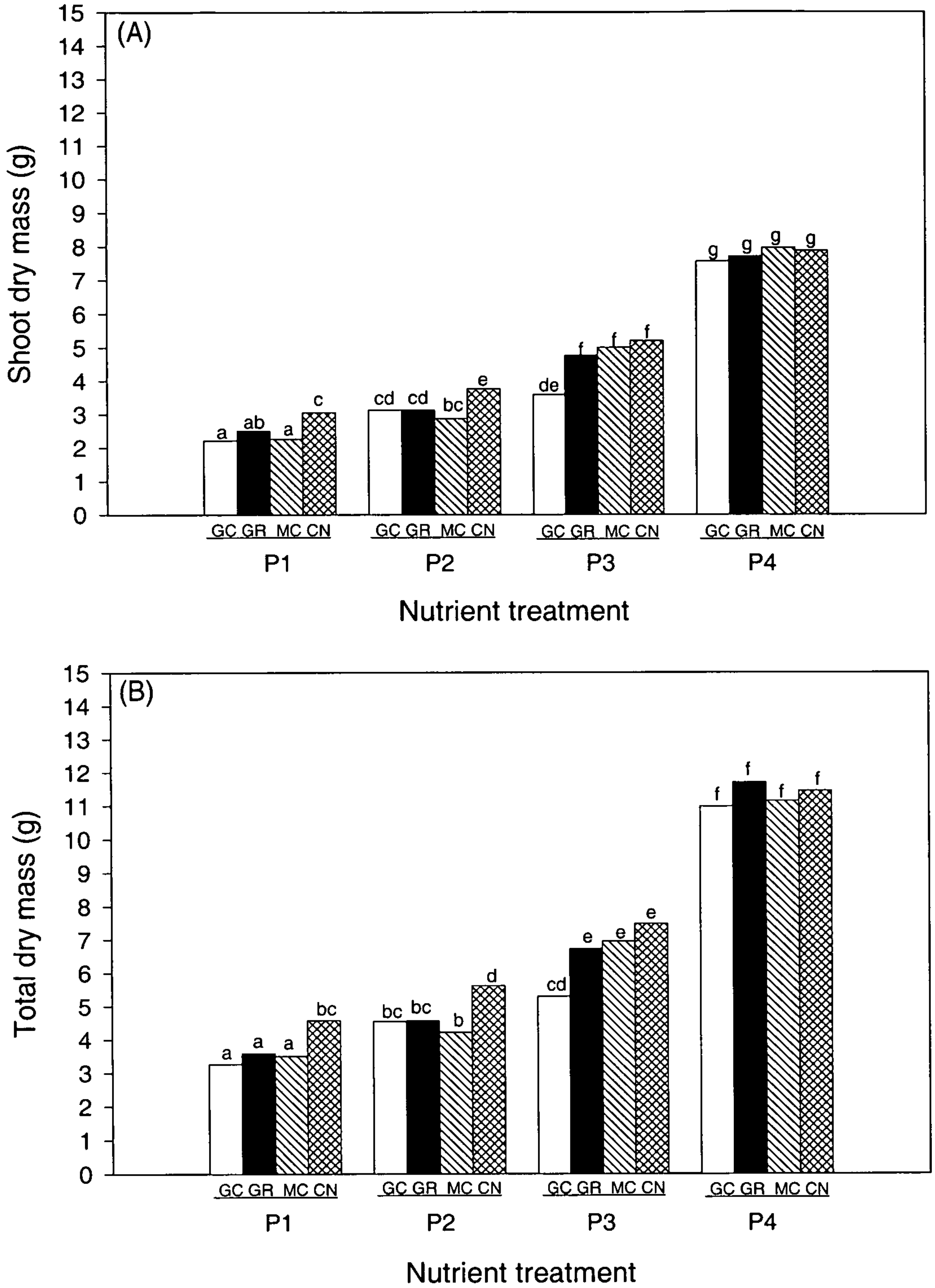

Fig. 4.1.3: Means of (A) shoot dry mass, and (B) total dry mass of *E. camaldulensis* seedlings in the AM experiment (experiment 4) under four inoculation treatments: GC, *G. clarum* BR148-1; GR, *G. rosea* FL105-5, MC, a mixed AM culture from Bangladesh, and CN, the uninoculated control and four nutrient regimes: P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P. Means with different letters are significantly different at P <0.05 (ANOVA).

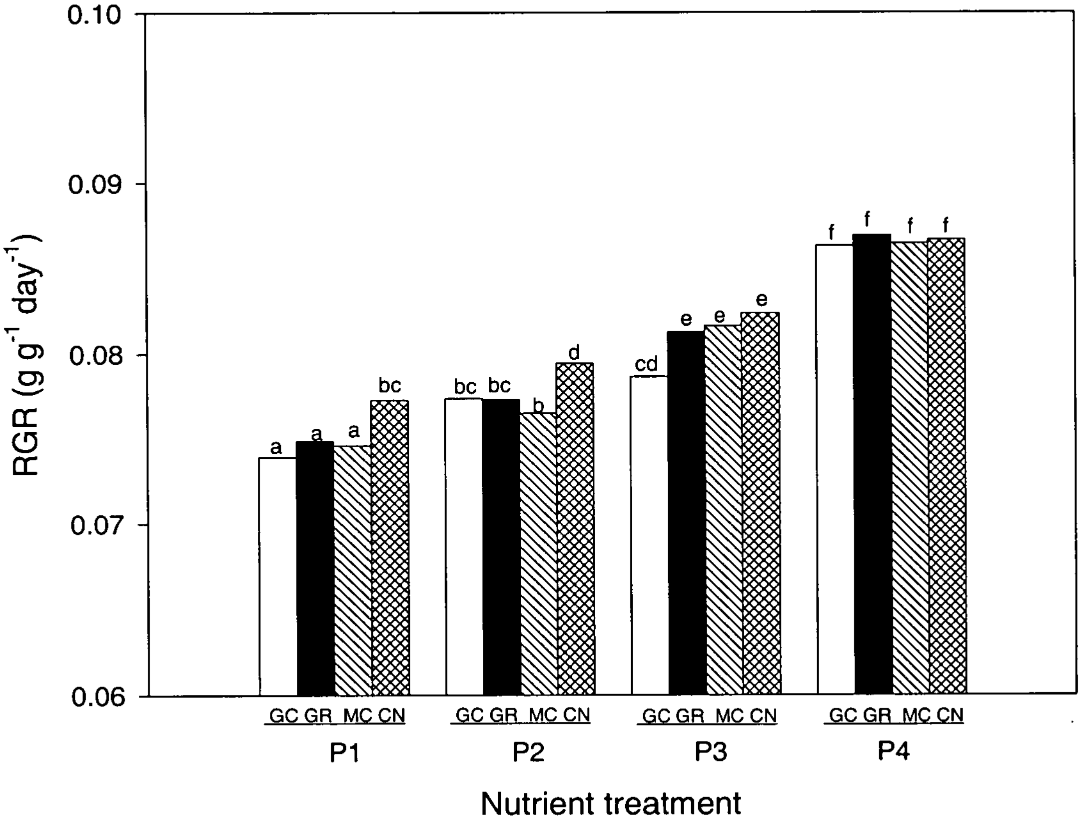

Fig. 4.1.4: Mean RGR of *E. camaldulensis* seedlings in the AM experiment (experiment 4) under four nutrient regimes; P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P and four inoculation treatments: GC, *G. clarum* BR148-1; GR, *G. rosea* FL105-5, MC, a mixed AM culture from Bangladesh and CN, the uninoculated control. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

4.1.2.3 *Influence of mycorrhizal inoculation on growth*

Mycorrhizal colonisation resulted in negative growth responses in terms of stem diameter, leaf area, shoot dry mass, root dry mass and total dry mass (Table 4.1.2). The *G. clarum* BR148-1 treatment was associated with the most striking depressive effects on stem diameter growth followed by the mixed culture of AM fungi from Bangladesh and *G. rosea* FL105-5 (Table 4.1.2). No difference between the three inoculant fungi was recorded in terms of their effect on leaf area and root dry mass (Table 4.1.2). 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 4.1.2). There was no significant regression between total dry mass and extent of colonisation by the three inoculant fungi (r^2 =0.057; $F_{1,57}$= 0.345; P =0.068). Regression between root dry mass and extent of colonisation was statistically significant (Fig. 4.1.5); root dry mass decreased with an increase in the extent of colonisation.

Table 4.1.2: Means of stem diameter (DIAM), leaf area (LFAR), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of *E. camaldulensis* seedlings in experiment 4 (the AM experiment) under four inoculation treatments: GC, *G. clarum* isolate BR148-1; GR, *G. 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* ≤0.05 (ANOVA).

Inoculation treatment	DIAM (mm)	LFAR (cm^2)	SDM (g)	RDM (g)	TDM (g)
GC	43.16*a*	329.6*a*	4.122*a*	1.897*a*	6.019*a*
GR	45.50*bc*	329.9*a*	4.523*b*	2.116*a*	6.639*b*
MC	46.08*ab*	338.4*a*	4.521*b*	1.928*a*	6.450*b*
CON	45.54*c*	383.4*b*	4.967*c*	2.308*b*	7.275*c*

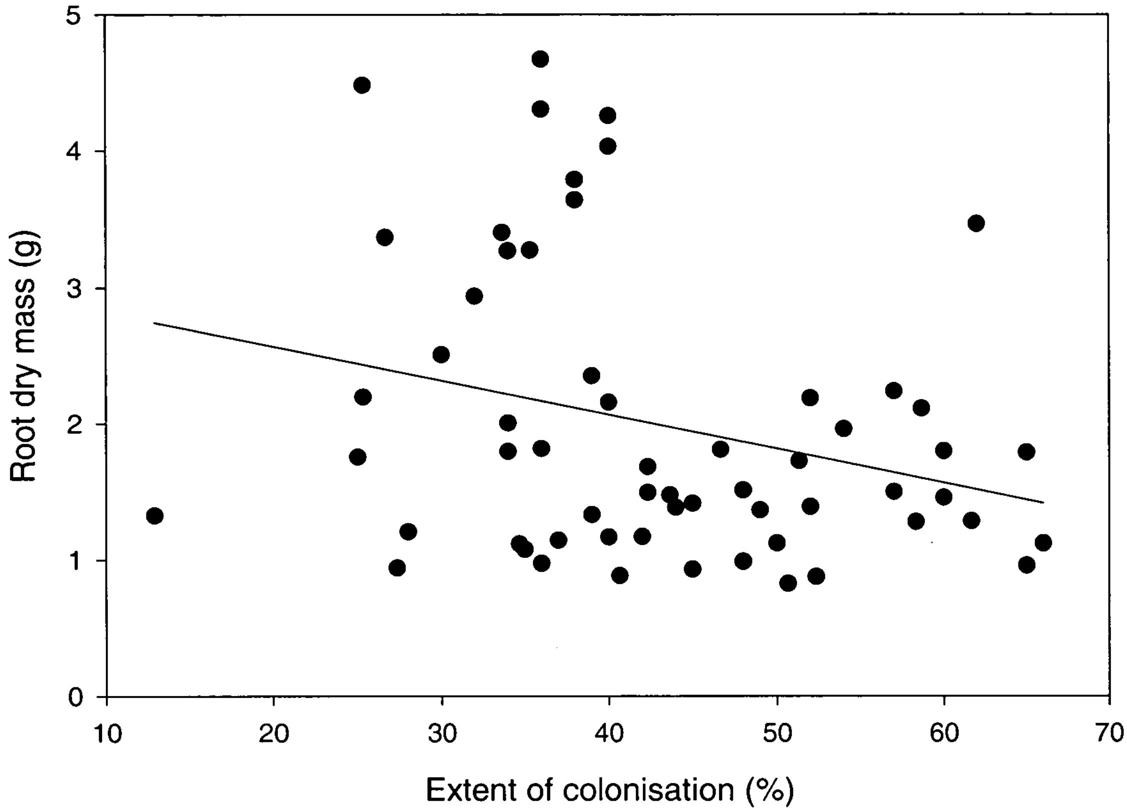

Fig. 4.1.5: Root dry mass (RDM) of *E. camaldulensis* seedlings as a function of extent of colonisation (EXC) by the three AM fungi in the AM experiment (experiment 4). Regression equation is: RDM =-0.025+3.071xEXC (r^2 =0.078; $F_{1,57}$ =4.85; P =0.032).

RGR of the seedlings colonised by all three inoculant fungi was significantly reduced as compared to the uninoculated control, with *G. clarum* BR148-1 contributing to the lowest RGR. *G. clarum* BR148-1 contributed to a significantly reduced NAR compared to either *G. rosea* FL105-5 or the uninoculated control. SLA for *G. clarum* BR148-1 was significantly higher than that for either *G. rosea* FL105-5 or the Bangladesh culture, but SLA for any individual inoculation treatment was not significantly different from the uninoculated control. LAR for *G. clarum* BR148-1 was significantly higher than that for *G. rosea* FL105-5, but LAR for any individual inoculation treatment was not significantly different from the uninoculated control (Table 4.1.3).

Table 4.1.3: Means of Relative Growth Rate (RGR, g g^{-1} day^{-1}), Net Assimilation Rate (NAR, mg cm^{-2} day^{-1}), Specific Leaf Area (SLA, cm^2 g^{-1}), Leaf Area Ratio (LAR, cm^2 g^{-1}), Leaf Mass Ratio (LMR), Root Mass Ratio (RMR) and Root Shoot Ratio (RSR) of *E. camaldulensis* seedlings in experiment 4 (the AM experiment) under four inoculation treatments: GC, *G. clarum* isolate BR148-1; GR, *G. rosea* isolate FL105-5; MC, a mixed culture from Bangladesh; and CON, the uninoculated control. Means with different letters are significantly different at $P \leq 0.05$ (ANOVA).

Variable	Inoculation treatment			
	GC	GR	MC	CON
RGR	0.079*a*	0.080*b*	0.080*b*	0.081*c*
NAR	1.310*a*	1.456*c*	1.377*ab*	1.411*bc*
SLA	107.9*b*	96.90*a*	100.9*a*	103.7*ab*
LAR	55.87*b*	50.02*a*	52.83*ab*	53.08*ab*
LMR	0.520*a*	0.519*a*	0.527*a*	0.516*a*
RMR	0.316*a*	0.311*a*	0.308*a*	0.319*a*
RSR	0.470*a*	0.460*a*	0.456*a*	0.482*a*

4.1.2.4 *Influence of nutrient regime on growth*

The nutrient treatments had a positive influence on growth. The four nutrient regimes had significantly different effects on stem diameter, height, leaf number, leaf area, shoot dry mass, root dry mass and total dry mass (Table 4.1.4) but there was no significant difference between the effects of 2.5 mg l^{-1} P and 5.0 mg l^{-1} P nutrient treatments on height, leaf number and root dry mass although other nutrient treatments had significantly different effects on them.

Table 4.1.4: Means of stem diameter, height (HT, cm), leaf number, leaf area (cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g) and total dry mass (TDM, g) of *E. camaldulensis* seedlings in experiment 4 (the AM experiment) under four nutrient treatments: P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P; and P4, 20 mg l^{-1} P. Means with different letters are significantly different at *P* ≤0.05 (ANOVA).

Variable	Nutrient treatment			
	P1	P2	P3	P4
SDM	3.269*a*	3.542*b*	3.988*c*	4.565*d*
HT	35.24*a*	37.37*a*	46.80*b*	60.88*c*
LFNO	20.00*a*	21.93*a*	28.10*b*	32.95*c*
LFAR	196.0*a*	250.5*b*	362.2*c*	572.5*d*
SDM	2.509*a*	3.225*b*	4.636*c*	7.764*d*
RDM	1.220*a*	1.506*b*	1.974*c*	3.550*d*
TDM	3.728*a*	4.730*b*	6.610*c*	11.32*d*

RGR varied significantly between nutrient treatments, with lower RGR for the lower P treatment and higher RGR for the higher P treatment. NAR for 2.5 mg l^{-1} P, 5.0 mg l^{-1} P and 10 mg l^{-1} P were significantly lower than that for the 20 mg l^{-1} P treatment but were not significantly different from each other. LAR for 20 mg l^{-1} P was significantly lower than that for the 10 mg l^{-1} P treatment while none of these was significantly different from 2.5 mg l^{-1} P and 5.0 mg l^{-1} P. SLA, LMR, RMR and RSR were not affected by different nutrient treatments (Table 4.1.5).

Table 4.1.5: Means of Relative Growth Rate (RGR, g g^{-1} day $^{-1}$), Net Assimilation Rate (NAR, mg cm^{-2} day^{-1}), Specific Leaf Area (SLA, cm^2 g^{-1}), Leaf Area Ratio (LAR, cm^2 g^{-1}), Leaf Mass Ratio (LMR), Root Mass Ratio (RMR) and Root Shoot Ratio (RSR) of *E. camaldulensis* seedlings in experiment 4 (the AM experiment) under four nutrient treatments: P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P; and P4, 20 mg l^{-1} P. Means with different letters are significantly different at $P \leq 0.05$ (ANOVA).

Variable	Nutrient treatment			
	P1	P2	P3	P4
RGR	0.075*a*	0.078*b*	0.081*c*	0.087*d*
NAR	1.310*a*	1.334*a*	1.351*a*	1.569*b*
SLA	103.5*a*	100.9*a*	104.8*a*	100.3*a*
LAR	52.65*ab*	53.10*ab*	55.38*b*	50.66*a*
LMR	0.513*a*	0.531*a*	0.531*a*	0.508*a*
RMR	0.327*a*	0.318*a*	0.298*a*	0.314*a*
RSR	0.497*a*	0.475*a*	0.429*a*	0.468*a*

4.1.2.5 *Influence of inoculation and nutrient regime on foliar N, P and K uptake*

There were no interactive effects of inoculation and nutrient treatments on leaf N, P and K concentrations (Table 4.1.6). On an overall basis, seedlings inoculated with *G. clarum* BR148-1 had significantly higher foliar concentration of N and P. There was no significant difference between the uninoculated control and the other two fungal inoculation treatments in the foliar concentration of these two nutrient elements by the seedlings. Also there was no significant difference among the four inoculation treatments in the foliar concentration of K by the seedlings (Fig. 4.1.6). When calculated on the basis of total leaf dry mass per individual seedling, there was no significant difference between the three inocula and the uninoculated control in terms of foliar N or P contents but leaves of seedlings inoculated with *G. clarum* BR148-1

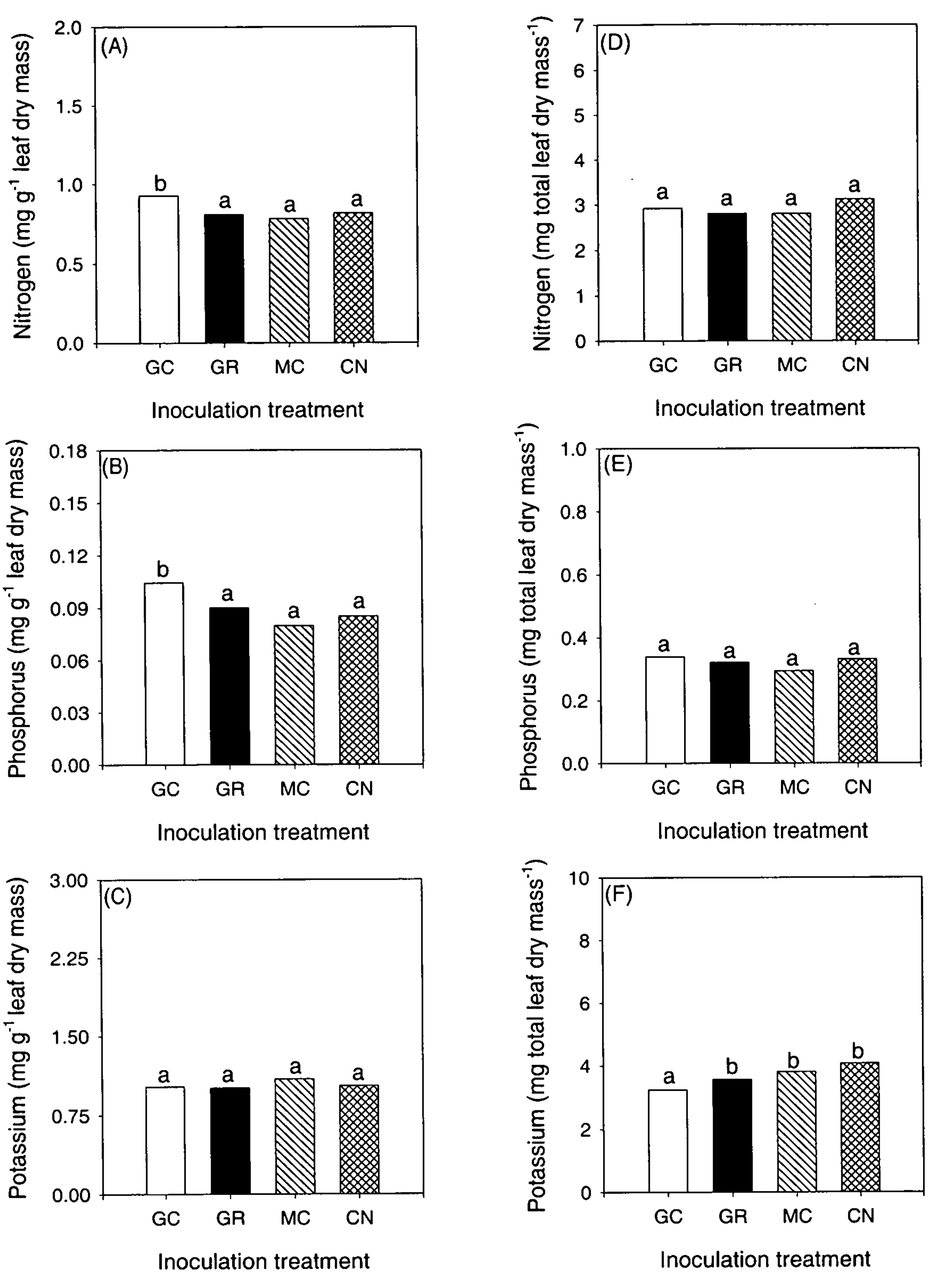

Fig. 4.1.6: Means of foliar (A) nitrogen, (B) phosphorus and (C) potassium concentrations, and (D) nitrogen, (E) phosphorus and (F) potassium contents of *E. camaldulensis* seedlings in the AM experiment (experiment 4) under four inoculation treatments. GC, *G. clarum* BR148-GR, *G. rosea* FL105-5; MC, a mixed AM culture from Bangladesh; and CN, the uninoculated control. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

had a significantly (*P* =0.027) lower K content compared to the uninoculated control (Fig. 4.1.6). There was no significant relationship between leaf nutrient concentration and mycorrhizal colonisation.

Table 4.1.6: Results of ANOVA of foliar nitrogen (N), phosphorus (P) and potassium (K) uptake of *E. camaldulensis* seedlings in experiment 4 (the AM experiment). Factors are- nutrient and fungus. Figures in bold indicate where effects were significant at $P \leq 0.05$ (ANOVA).

Treatment	N		P		K	
	mg $gldm^{-1}$	mg $tldm^{-1}$	mg $gldm^{-1}$	mg $tldm^{-1}$	mg $gldm^{-1}$	mg $tldm^{-1}$
Nutrient	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Fungus	**0.003**	0.578	**<0.001**	0.226	0.700	**0.027**
Nutrient*Fungus	0.876	0.898	0.965	0.740	0.270	0.613

Note: mg $gldm^{-1}$ =mg nutrient per gm leaf dry mass and mg $tldm^{-1}$ =mg nutrient per total leaf dry mass.

Seedlings grown in the 20 mg l^{-1} P nutrient regime had significantly higher foliar concentration of N and P compared to those in other nutrient regimes. There was no significant difference between the foliar concentration of these two nutrient elements in seedlings grown in 5.0 mg l^{-1} P and 10 mg l^{-1} P while seedlings grown in 2.5 mg l^{-1} P had the lowest concentration (Fig. 4.1.7). Foliar concentration of K by the seedlings grown in the 20 mg l^{-1} P was significantly higher than those grown in 2.5 mg l^{-1} P or 5.0 mg l^{-1} P, but there was no significant difference either between seedlings grown in 2.5 mg l^{-1} P and 5.0 mg l^{-1} P or between those grown in 5.0 mg l^{-1} P and 10 mg l^{-1} P.

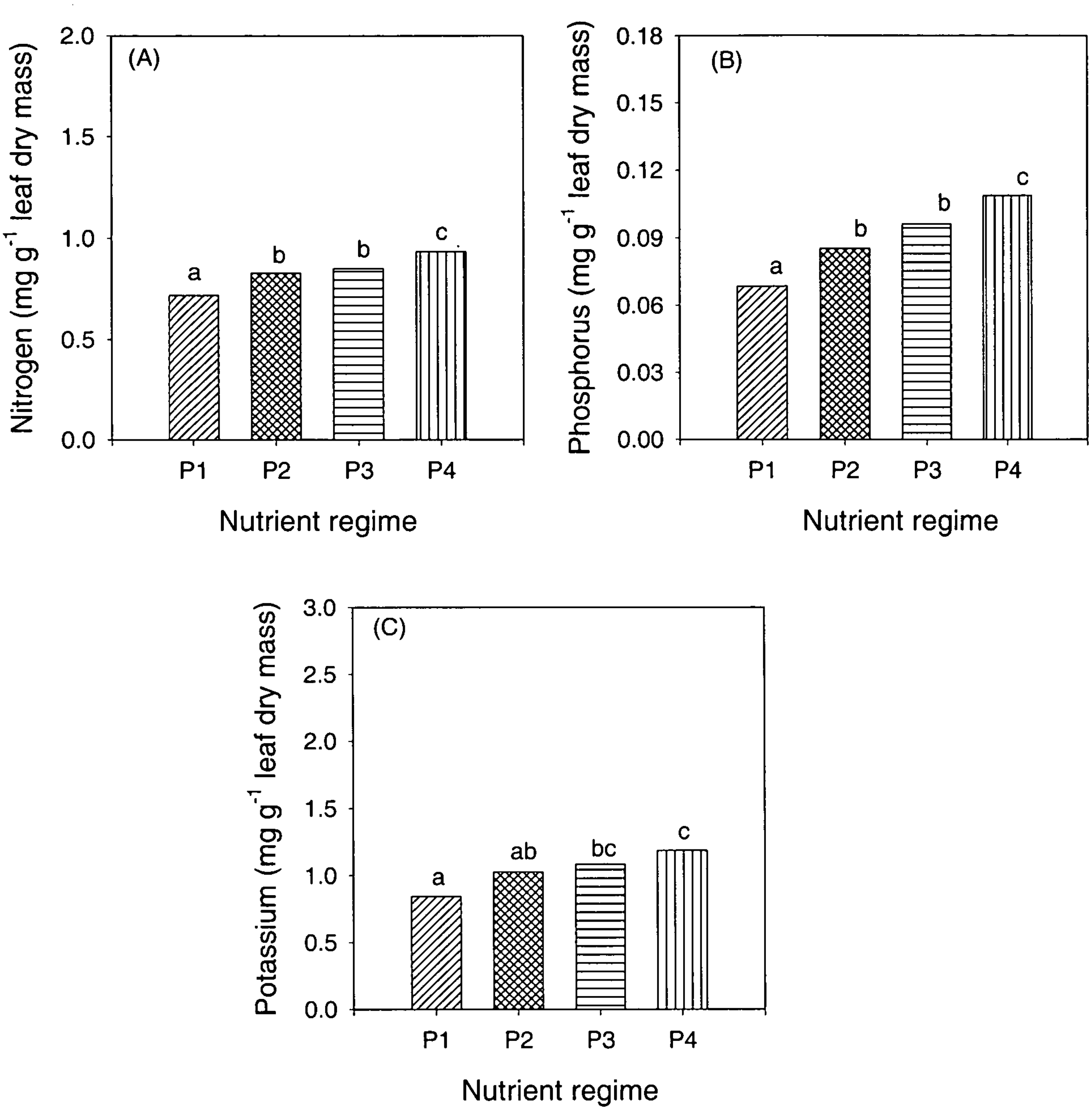

Fig. 4.1.7: Means of foliar (A) nitrogen, (B) phosphorus, and (C) potassium concentrations of *E. camaldulensis* seedlings in the AM experiment (experiment 4) under four nutrient regimes. P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

4.1.3 Discussion

Although variation among different fungi in terms of their potential in colonising *E. camaldulensis* roots at different nutrient (P) regimes was observed, there was no significant relationship between extent of colonisation and nutrient (expressed in terms of P) concentration. Extent of colonisation by any mycorrhizal fungus decreases with an increasing availability of soluble P in the growth medium, a trend which has been observed in many mycorrhizal experiments (Smith and Read, 1997). In general, colonisation by AM fungi at P concentrations ranging from >20 to 100 ppm were reported to have positive growth responses in tropical forest tree seedlings (Sieverding, 1991), and P concentrations higher than the upper limit of this range may therefore not be favourable for effective mycorrhiza formation. In this experiment, the range of nutrient regimes (from 2.5 to 20 mg l^{-1} P) was probably too narrow to reflect such a trend. Therefore, one interesting thing was observed: except for *G. rosea* FL105-5, the extent of colonisation by the other two inoculant fungi were at their maximum at 5 and 10 mg l^{-1} P nutrient regimes, but decreased significantly both at the lower nutrient (2.5 mg l^{-1} P) and at the higher nutrient regime (20 mg l^{-1} P) indicating that the range of nutrient concentrations applied spanned the optimum for the two fungi. Colonisation by *G. rosea* FL105-5 did not differ between the nutrient treatments. This indicated that different AM fungi may have different responses to different nutrient availabilities.

The decrease in mycorrhizal colonisation in response to phosphate application arises from the resulting increase in tissue P concentration (Sanders, 1975; Menge *et al.*, 1978). The nutrient concentration of host seedlings at 20 mg l^{-1} P may, therefore, have affected quantity or quality of root metabolites or root exudates (Twaraya *et al.*, 1996) which resulted low colonisation in those seedlings. AM plants are reported to allocate 7-10% more of their photosynthate to the root system than non-mycorrhizal plants (Lambers, 1987). Therefore, as suggested by Colpaert and Van Tichelen, (1996), another explanation is that high nutrient availability may have reduced the carbon allocation to the roots and resulted in C deficiency for the fungus.

In general, there was no significant relationship between the extent of colonisation and total dry mass. The AM colonisation led to a negative growth response mainly in the lower P nutrient regimes, that is, at 2.5 mg l^{-1} and 5.0 mg l^{-1} P (Fig. 4.1.3) where each of the three inoculant fungi had significantly lower shoot and total dry mass than the uninoculated control. The two nutrient regimes in this experiment involved very low concentrations of P, and seedlings of *E. camaldulensis* were growing relatively slowly under these nutrient regimes. At 10 mg l^{-1} P, 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 which could have caused a higher carbon drain on the seedlings. 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. The large spores of AM fungi, in general, act as energy reserves (Janos, 1996) thus storing C in them.

In this case, *G. clarum* BR148-1 was an aggressive fungus having the highest colonisation rate among the inoculant fungi (around 50%), and most of the colonisation occurred at 10 mg l^{-1} P (60%). At 20 mg l^{-1} P, none of the inoculant fungi produced any significant growth depression. This has probably happened because of the lowest colonisation by the AM fungi at that nutrient regime. Growth reductions in the host plant generally occur at soil fertilities high enough to render the mycorrhiza superfluous, but not so high as to inhibit colonisation (Bowen, 1978).

In the experiment of Adjoud *et al.* (1996), positive growth response in most of the 11 *Eucalyptus* species was observed with a 41 mg l^{-1} P nutrient solution where *G. intraradices* Schenck & Smith (isolate CP103/CP), *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (isolate CP101/VF) and *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd. (isolate CP105/VF) were used as inoculant fungi. However, different AM fungi are reported to have different effects on their hosts. *G. rosea* Nicol. & Schenck (isolate BEG111) contributed to a negative growth response in *Pueraria phaseoloides* L. plants while *Acaulospora tuberculata* Janos & Trappe (BEG41) or *Glomus manihotis* Howeler, Sieverding & Schenck (BEG112) resulted in a positive growth response in the plants of the same species in experiments done in three levels of localised phosphate source (Boddington and Dodd, 1998). However, in a Brazilian study *G. clarum* has been reported to have promoted the highest growth rate and most efficient use of nutrients in seedlings of *E. grandis* and *E. tereticornis* after 75 days of inoculation compared to five other AM fungi used in the experiment (Zambolim *et al.*, 1982). Bethlenfalvay *et al.* (1982) observed growth depression in soybean inoculated with *Glomus fasciculatus* (Thaxt. emend. Gerd.) Gerd. & Trappe for up to 15 weeks after inoculation when soil P concentration was higher than 10 mg kg^{-1} soil but growth promotion started to appear from the 16th week when P concentration decreased to 10 mg kg^{-1} soil. 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) found that, increased root density in mycorrhizal pot-grown barley (*Hordeum vulgare* L.) resulted in its yield depression, but higher P concentration in grain and straw was recorded. Also in this study, growth depression in *G. clarum* BR148-1 inoculated *E. camaldulensis* seedlings concurrently occurred with higher foliar P (and also N) concentration.

The AM colonisation contributed to a negative RGR mainly in the lower P nutrient regimes, that is, at 2.5 mg l^{-1} and 5.0 mg l^{-1} P (Fig. 4.1.4) where each of the three inoculant fungi had significantly lower RGR than the uninoculated control. But at 10 mg l^{-1} P, only *G. clarum* BR148-1 contributed to a significantly reduced RGR of seedlings, the two other fungi were not significantly different from the uninoculated control. *Glomus* spp which colonise plant roots aggressively have been reported to reduce RGR of seedlings (Graham *et al.*, 1996) which is consistent with an increase in below-ground C expenditure (Eissenstat *et al.*, 1993). Here at 20 mg l^{-1} P, there was no significant difference between the inoculation treatments. This variation in RGR according to different treatments conforms to the variation in total dry mass as described above. Only *G. clarum* BR148-1 had a significantly reduced NAR compared to the uninoculated control. Decreases in NAR are generally associated with reduced resource availability (Chiariello, Mooney and Williams, 1989) and also stress (e.g. Ball and Pidsley, 1995). This is probably not the case in this experiment because plants were well-watered and supplied with nutrients. *G. clarum* BR148-1 inoculated seedlings had generally higher leaf P (and N) concentrations than the non-mycorrhizal seedlings, and seedlings inoculated with other AM fungi had similar P concentrations as the non-mycorrhizal seedlings. Lower NAR in *G. clarum* BR148-1 mycorrhiza probably represents the carbon cost associated with AM. Lovelock *et al.* (1996) obtained a similar result in their experiment with AM associations of *Beilschmiedia pendula* (Sw.) Hemsl., and offered this explanation. In the study of Baas *et al.* (1989), decreases in NAR were found to be partially correlated with increased root respiration in mycorrhizal plants. RGR is a function of NAR and LAR. Compensation for reduced NAR in mycorrhizal plants occur largely through an increase in LAR (Lovelock *et al.*, 1996; Baas *et al.*, 1989; Snellgrove *et al.*, 1982; Harris, Pacovsky and Paul, 1985). Although LAR in *G. clarum* BR148-1 treated seedlings was significantly higher than the non-mycorrhizal seedlings, this could not help to compensate for the carbon cost of the symbiosis because RGR for this mycorrhizal treatment still remained significantly lower compared to the non-mycorrhizal treatment (Table 4.1.3).

NAR and LAR for the seedlings inoculated with the other two fungi were not significantly different from the non-mycorrhizal treatment but their RGR was significantly lower than the uninoculated control. This again confirms the suggestion of a higher carbon cost for the symbiosis on the part of *E. camaldulensis* seedlings in the three fungal inoculation treatments. The *G. clarum* BR148-1 treatment resulted in a significantly higher SLA in seedlings compared to *G. rosea* FL105-5 or the Bangladesh culture but the total dry mass for that treatment was significantly lower. Increased specific leaf area without increase in dry mass for an inoculation treatment suggests greater fluxes of carbon through the mycorrhizal fungus involved in that treatment as compared to other inoculation treatments in the experiment (Lewis and

Strain, 1996). This again indicated that the *G. clarum* BR148-1 treatment has resulted in carbon drain.

Increased RGR with an increasing nutrient concentration indicated that different nutrient treatments had a pronounced effect on seedling growth (Table 4.1.5). However, NAR and LAR respectively for 2.5 mg l^{-1}, 5.0 mg l^{-1} and 10 mg l^{-1} P were not significantly different. This indicated that even though NAR and LAR in different nutrient treatments are not significantly different among themselves, their functional product, RGR in different nutrient treatments can be significantly different.

As *G. clarum* BR148-1 caused the most pronounced depressive effect on growth of seedlings, foliar N and P concentration were significantly higher in that inoculation treatment compared to the other two. However, total leaf N and P contents for *G. clarum* BR148-1 treatment did not significantly vary from the uninoculated control. These suggest that although *G. clarum* BR148-1 colonisation resulted in reduction in growth rate, nutrient uptake was not affected. Also the occurrence of a relatively high SLA and LAR of seedlings in the *G. clarum* BR148-1 treatment supports this (Table 4.1.3). Despite a higher SLA and LAR, NAR was significantly reduced in that treatment indicating that a major part of the photosynthate was drawn below ground by the fungus (which resulted in a reduced NAR), and was used for its growth and maintenance. This evidence probably consolidates the fact that there was a net carbon drain on the seedlings by *G. clarum* BR148-1. Although Sieverding (1991) argues that there is hardly any evidence of a greater consumption of other nutrient elements than carbon by the AM fungi for their growth and maintenance, Colpaert *et al.* (1996) maintains that the diversion of nitrogen for fungal growth might have consequences for seedling growth. Therefore, nitrogen can be a growth limiting factor for mycorrhizal plants (see Attiwill and Adams, 1993). In this experiment, most of the colonisation occurred at 10 mg l^{-1} P treatment where the N concentration was 62.5 mg l^{-1}. In the experiment of Adjoud *et al.* (1996), N concentration was even lower (43 mg l^{-1}) for a similar P concentration, but they found a positive growth response in most *Eucalyptus* species as a result of AM inoculation. However, growth responses in *E. camaldulensis* seedlings from AM inoculation have been reported in a study done by Vishwakarma and Singh (1996a) but no account on the nutrient regimes used in that experiment was reported in their work. One of the other differences between their study and this study is that they used different AM fungal inoculants.

4.2. Effects of Various Isolates of Ectomycorrhizal Inoculant Fungus *Pisolithus tinctorius* on Mycorrhiza Formation, Growth and Foliar Nutrient Uptake in *E. camaldulensis* Seedlings at Different Nutrient Regimes (Experiment 5).

4.2.1 Materials and Methods

4.2.1.1 *Experimental design*

The experiment contained a factorial combination of six inoculation treatments (five fungal and the other control) and four nutrient treatments; 24 treatments in all. Five randomised blocks each containing one pot per treatment were set up, with a single seedling per pot. Growth conditions were as described in section 2.0 (Chapter 2).

4.2.1.2 *Fungal isolates*

Five isolates of *Pisolithus tinctorius* (K55, PTE, PT3, PT7 and PT8) were used as EM inoculant fungi.

4.2.1.3 *Substrate preparation*

Preparation of VP as growth medium was carried out as described in section 2.5 (Chapter 2).

4.2.1.4 *Seed germination, planting and fungal inoculation*

Germination of seeds was carried out as described in section 2.6 (Chapter 2).

On June 8, 1997, one seedling from the germination tray was transferred to each pot. Fungal cultures were prepared and inoculation was carried out as described in sections 2.4 and 2.7 respectively of Chapter 2 except that amount of inoculum put into each planting hole was 4 g.

4.2.1.5 *Nutrient treatment*

Details of preparation of nutrient solution and application of different nutrient treatments followed as described in sections 2.8 (Chapter 2) and 4.1.1.5 respectively. As the seedlings were raised over a longer period (18 weeks), each individual pot received 3, 6, 12, or 24 mg of phosphorus at the end of the experimental period.

4.2.1.6 *Harvesting and mycorrhizal analysis*

An initial harvest of 10 seedlings was carried out of the seedlings in the germination tray before transplanting which was as described in section 4.1.1.6. Plant leaf area and dry mass at the beginning of the experiment were 0.245±0.1202 cm^2 and 0.0017±0.0002 g respectively (n= 10 plants). Seedlings were harvested at 18 weeks. RGR, NAR and other relative growth variables, for example, LAR, SLA, LMR, RMR and RSR were calculated using the formulas mentioned in section 2.12 (Chapter 2). Harvesting procedure, measurements and method of root washing for mycorrhizal analysis was as detailed in sections 2.9 and 2.10 (Chapter 2).

4.2.1.7 *Foliar N, P and K analysis*

N, P and K contents in dried leaf samples were carried out as detailed in section 2.13 of Chapter 2.

4.2.1.8 *Statistical analysis*

A two-way analysis of variance (ANOVA) was conducted where the factors were nutrient regime and inoculation treatment. Statistical comparisons of means was done according to the method described in section 2.14 (Chapter 2).

4.2.2 Results

4.2.2.1 *Influence of inoculation and nutrient regime on mycorrhizal colonisation*

Out of the five fungal inoculants, only *P. tinctorius* isolate K55 resulted in considerable colonisation. Casual observations of seedlings (from extra-experimental seedlings inoculated at the same time in the same way as the experimental seedlings and maintained alongside the experiment) indicated that EM colonisation started to spread (>10% of total root) by 16 weeks after inoculation. At the end of 18 weeks, *P. tinctorius* isolate K55 resulted in significant colonisation (up to 27% of the root tips mycorrhizal). The other inoculants, although remaining alive (as live mycelia were found with the washed roots) at harvest, did not result in any considerable colonisation (only <1% of the root tips mycorrhizal).

Most colonisation was found to have occurred at 2.5 mg l^{-1} P which was significantly (*P* =0.007; see Table 1 of Appendix H) higher than that at either 10 mg l^{-1} P or 20 mg l^{-1} P. 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. 4.2.1). Regression between the extent of colonisation by the isolate K55 and nutrient concentration was statistically significant (Fig. 4.2.2); colonisation decreased with an increase in nutrient concentration.

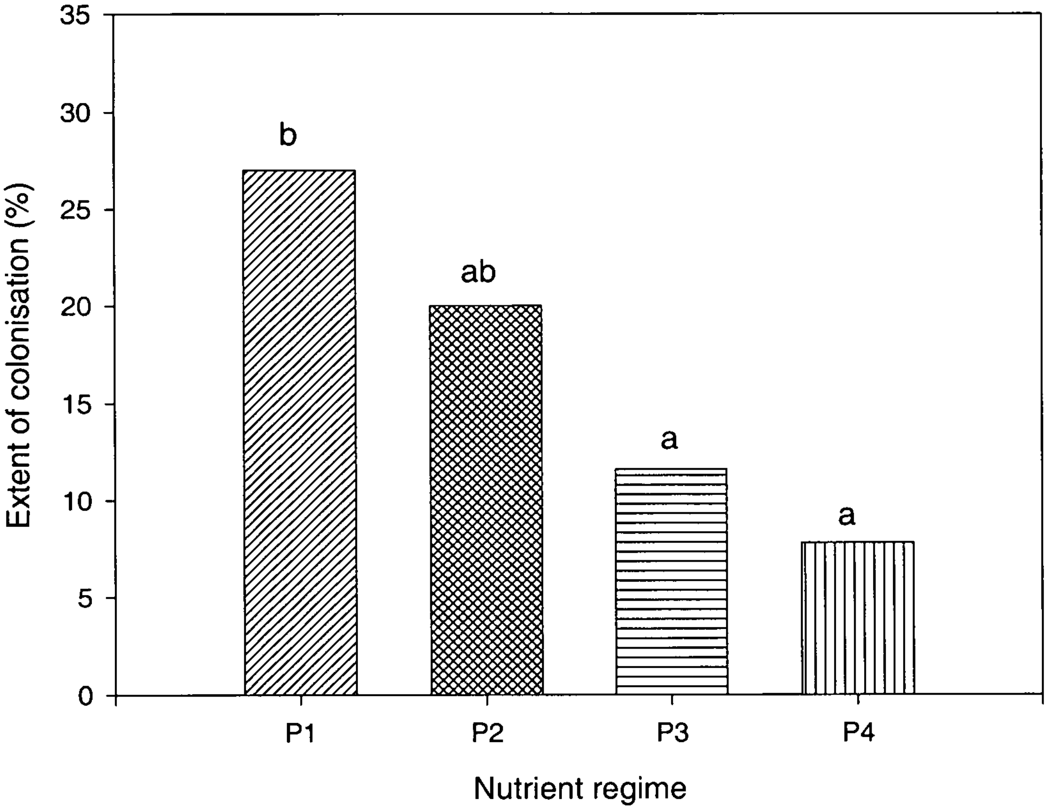

Fig. 4.2.1: Mean extent of colonisation of the roots (% root tips mycorrhizal) of *E. camaldulensis* seedlings by the EM fungus *P. tinctorius* K55 under four nutrient regimes. P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P in the EM experiment (experment 5). Values are treatment means and means with different letters are significantly different at $P < 0.05$ (ANOVA).

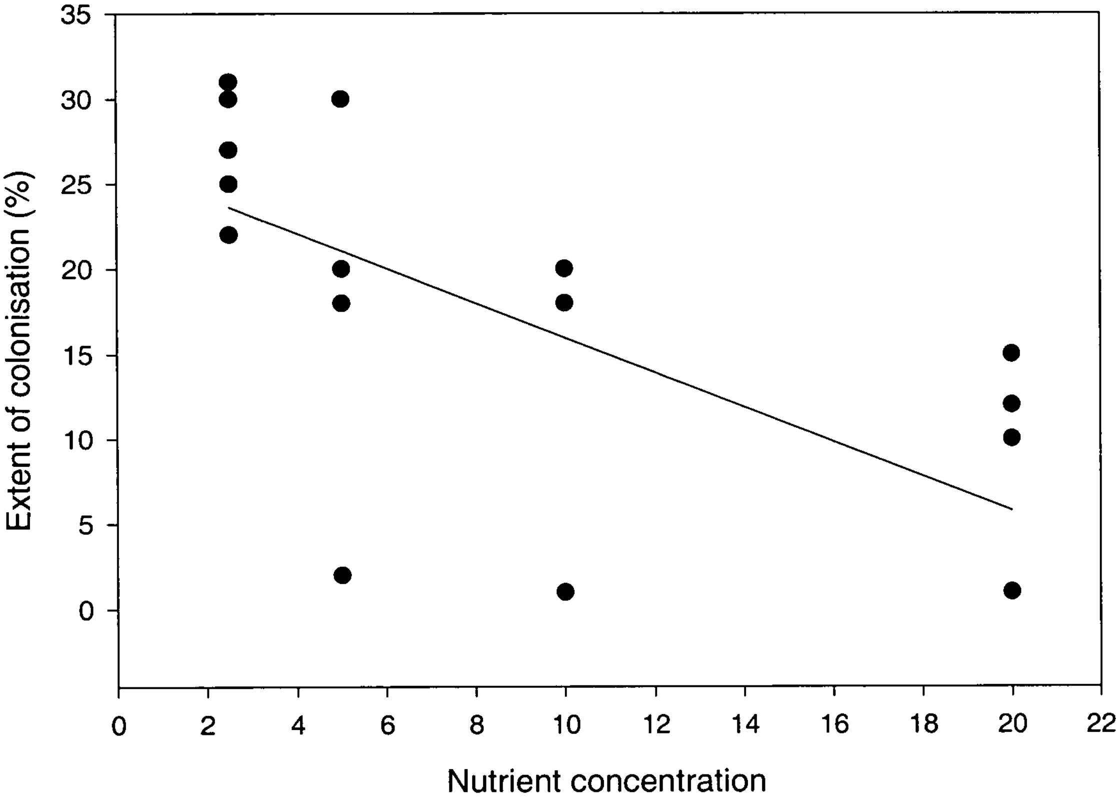

Fig. 4.2.2: Extent of colonisation (EXC) in *E. camaldulensis* seedlings as a function of applied nutrient concentration (PCN) with respect to the *P. tinctorius* K55 treatment in the EM experiment (experiment 5). Regression equation is: EXC =-1.108+26.148xPCN (r^2 =0.417; $F_{1,18}$ =12.85; P =0.002). Note: nutrient concentration is illustrated in terms of P.

4.2.2.2 *Interaction of inoculation and nutrient regime on growth*

There were significant interactive effects of nutrient and fungus on shoot dry mass, root mass ratio and root shoot ratio of seedlings (Table 4.2.1). 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. 4.2.3). 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.

Table 4.2.1: Results of ANOVA of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g), relative growth rate (RGR, $g\ g^{-1}day^{-1}$), net assimilation rate (NAR, $mg\ cm^{-2}\ day^{-1}$), leaf area ratio (LAR, $cm^2\ g^{-1}$), specific leaf area (SLA, $cm^2\ g^{-1}$), leaf mass ratio (LMR), root mass ratio (RMR) and root shoot ratio (RSR) of *E. camaldulensis* seedlings in experiment 5 (the EM experiment). Factors are- nutrient and fungus. Figures in bold indicate where effects were significant at $P \leq 0.05$ (ANOVA).

Variable	Nutrient	Fungus	Nutrient*Fungus
DIAM	**<0.001**	**0.023**	0.759
HT	**<0.001**	0.796	0.461
LFNO	**<0.001**	0.713	0.303
LFAR	**<0.001**	0.626	0.192
SDM	**<0.001**	0.144	**0.043**
RDM	**<0.001**	0.085	0.159
TDM	**<0.001**	0.335	0.187
RGR	**<0.001**	0.335	0.187
NAR	**<0.001**	0.929	0.467
LAR	**0.010**	0.951	0.392
SLA	**0.004**	0.055	0.154
LMR	0.062	0.083	0.077
RMR	0.758	**0.015**	**0.018**
RSR	0.733	**0.015**	**0.019**

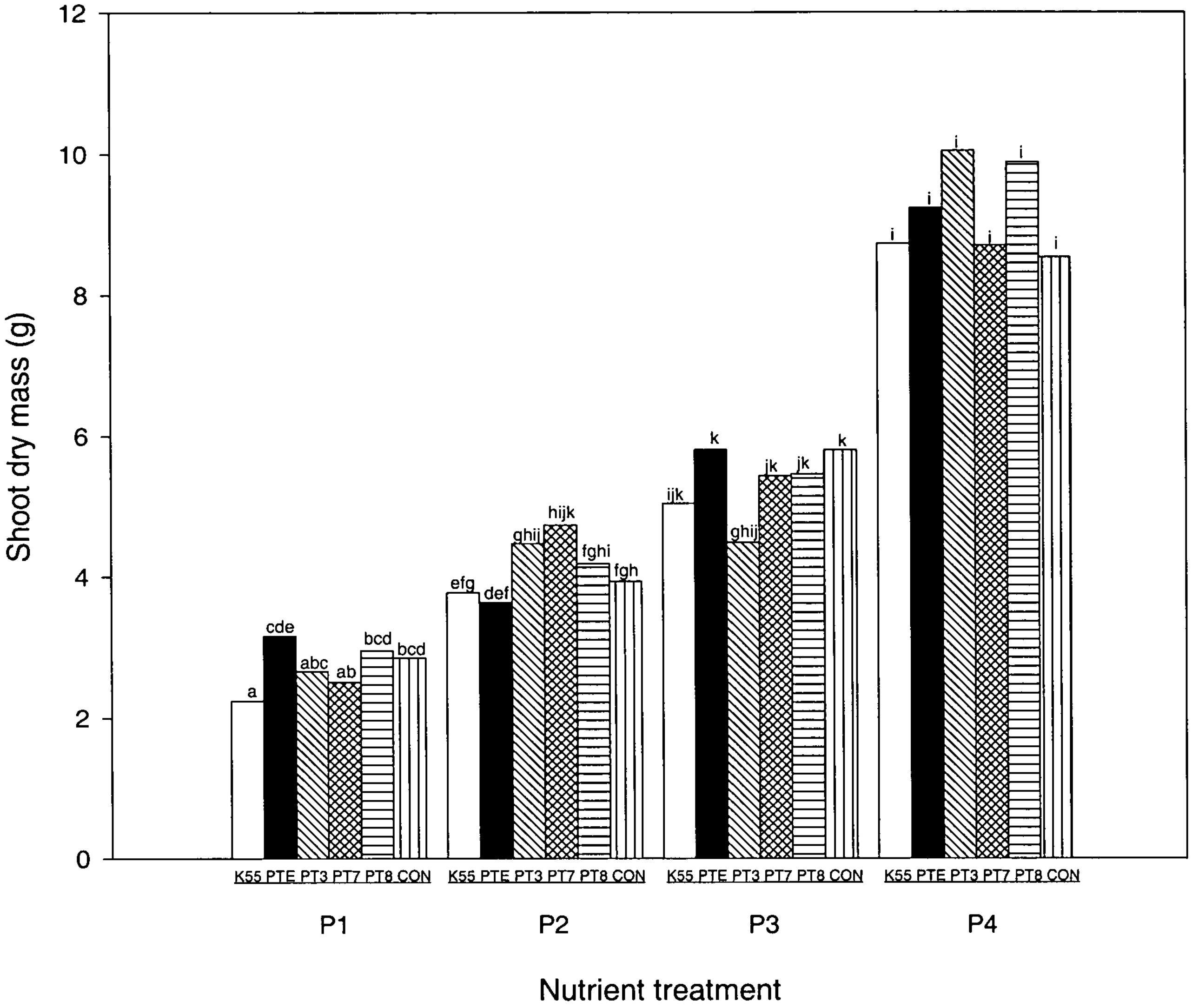

Fig. 4.2.3: Mean shoot dry mass of *E. camaldulensis* seedlings in the EM experiment (experiment 5) under four nutrient regimes; P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P and six inoculation treatments: five different isolates of *P. tinctorius*: K55, PTE, PT3, PT7, PT8 and CON, the uninoculated control. Means with different letters are significantly different at P <0.05 (ANOVA).

4.2.2.3 *Influence of mycorrhizal inoculation on growth*

Mycorrhizal colonisation resulted in negative growth responses in terms of stem diameter only (Fig. 4.2.4). 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 (Table 4.2.1). Stem diameter in the *P. tinctorius* isolate K55 treatment which resulted in most of the colonisation 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 (Fig. 4.2.4). Regressions between total dry mass and the extent of colonisation (Fig. 4.2.5) and between root dry mass and the extent of colonisation (Fig. 4.2.6) by the isolate K55 were statistically significant ($P < 0.05$); both decreased with an increasing extent of colonisation by the isolate K55.

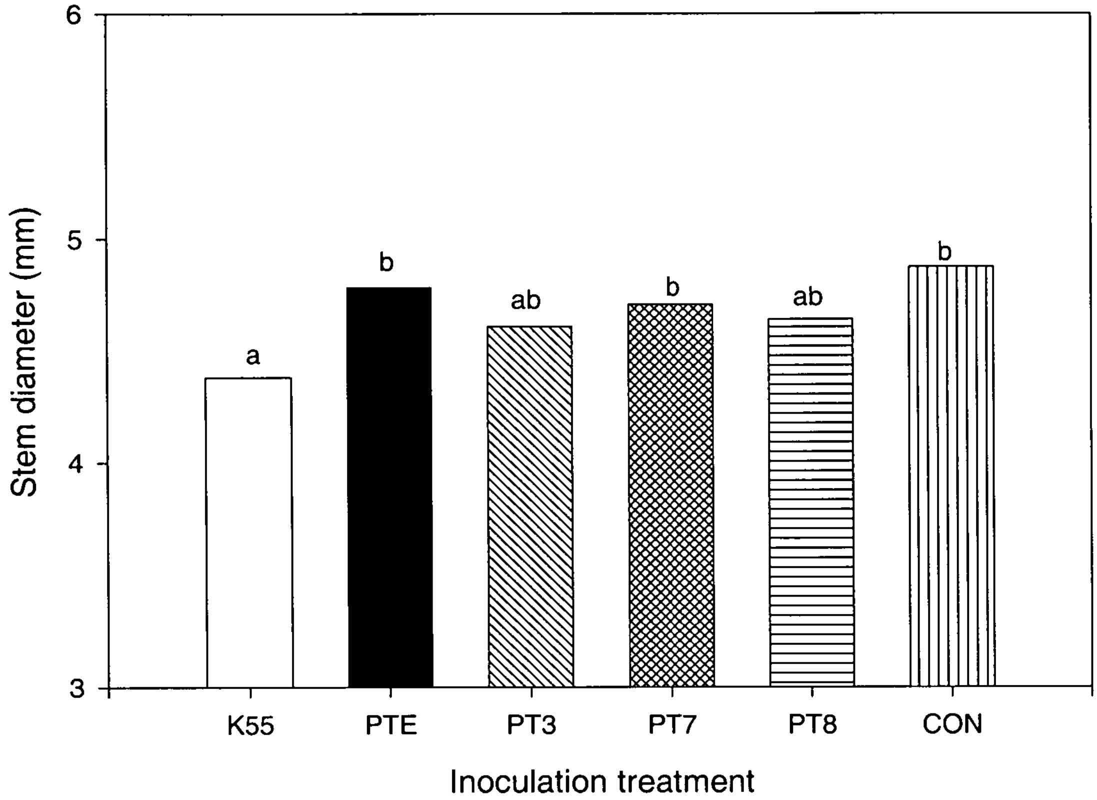

Fig. 4.2.4: Mean stem diameter of *E. camaldulensis* seedlings in the EM experiment (experiment 5) under six inoculation treatments; five isolates of *P. tinctorius*: K55, PTE, PT3, PT7, and PT8, and CON, the uninoculated control. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

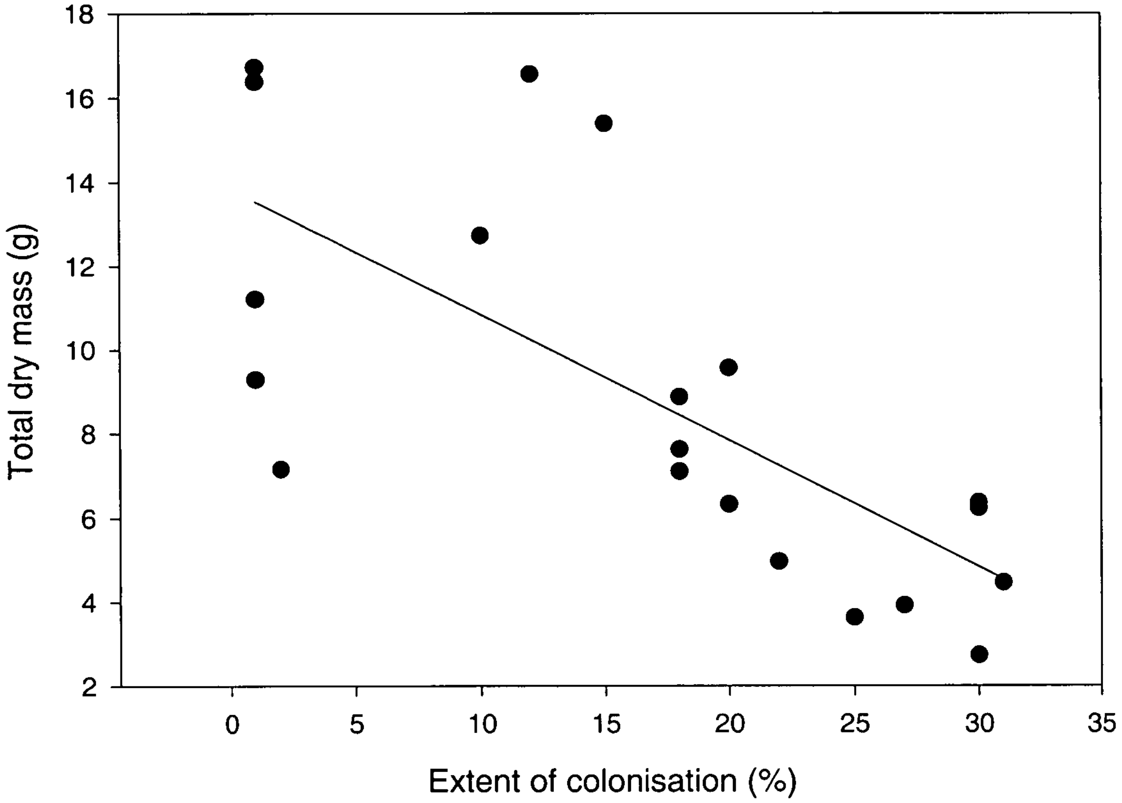

Fig. 4.2.5: Total dry mass (TDM) of *E. camaldulensis* seedlings as a function of extent of colonisation (EXC) by *P. tinctorius* K55 in the EM experiment (experiment 5). Regression equation is: TDM =-0.300+13.839XEXC (r^2 =0.513; $F_{1,18}$ =18.92; P =0.0001).

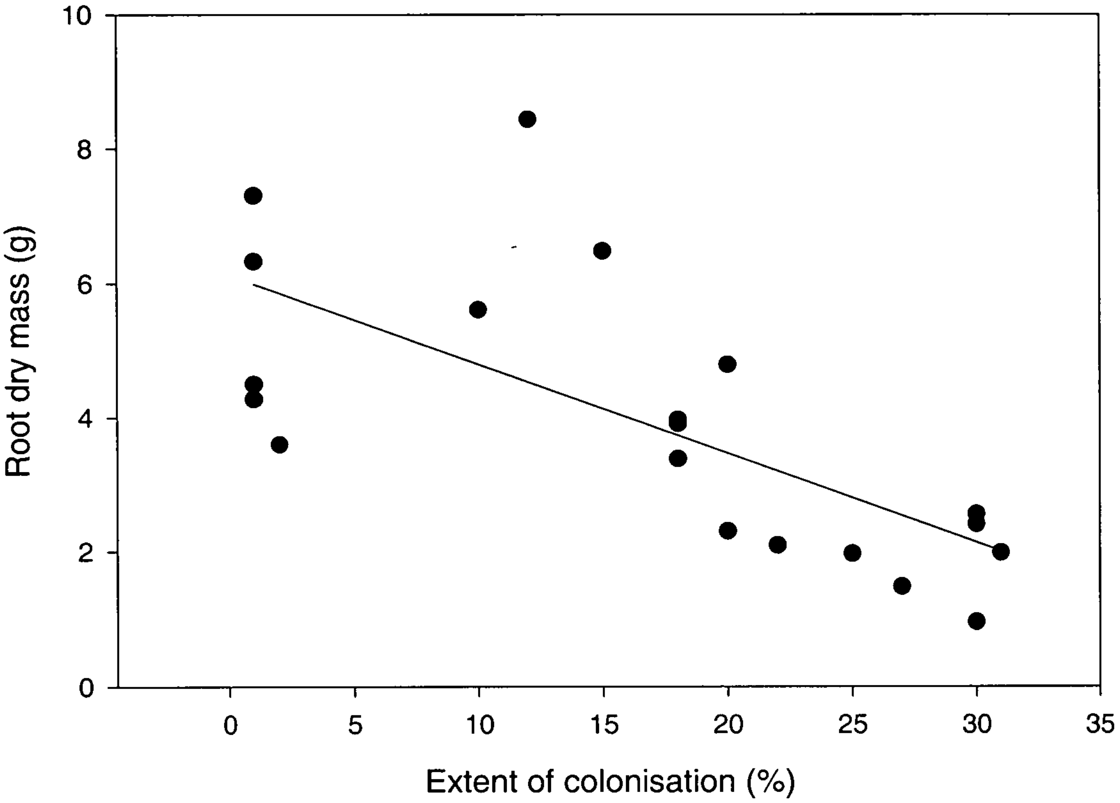

Fig. 4.2.6: Root dry mass (RDM) of *E. camaldulensis* seedlings as a function of extent of colonisation (EXC) by *P. tinctorius* K55 in the EM experiment (experiment 5). Regression equation is: RDM =-0.133+6.125xEXC (r^2 =0.487; $F_{1,18}$ =17.10; P =0.001).

RGR, NAR, SLA, LAR and LMR of the seedlings colonised by all five inoculant fungi were not significantly different from the uninoculated control. At 10 mg l^{-1} P, RMR (Table 4.2.2) and RSR (Table 4.2.3) of the seedlings colonised by *P. tinctorius* isolate K55 were significantly higher than those by either isolate PTE or isolate PT8 but they were not significantly higher than those of other isolates or the uninoculated control.

Table 4.2.2: RMR of *E. camaldulensis* seedlings under six inoculation treatments (five different isolates *of P. tinctorius*, for example, K55, PTE, PT3, PT7, and PT8, and CON, the uninoculated control) and four nutrient treatments (P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4; 20 mg l^{-1} P) in experiment 5 (the EM experiment). Means with different letters are significantly different at *P* ≤0.05 (ANOVA).

Inoculation treatment	RMR			
	P1	P2	P3	P4
K55	0.428*abcdefg*	0.435*abcdefg*	0.457*efg*	0.439*bcdefg*
PTE	0.412*abcdef*	0.455*efg*	0.369*a*	0.378*ab*
PT3	0.459*efg*	0.382*abc*	0.488*g*	0.414*abcdef*
PT7	0.455*efg*	0.372*a*	0.461*fg*	0.436*abcdefg*
PT8	0.387*abcd*	0.394*abcde*	0.376*ab*	0.397*abcdef*
CON	0.409*abcdef*	0.452*defg*	0.418*abcdef*	0.446*cdefg*

Table 4.2.3: RSR of *E. camaldulensis* seedlings under six inoculation treatments (five different isolates *of P. tinctorius*, for example, K55, PTE, PT3, PT7, and PT8, and CON, the uninoculated control) and four nutrient treatments (P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4; 20 mg l^{-1} P in the EM experiment. Means with different letters are significantly different at *P* ≤0.05 (ANOVA).

Inoculation treatment	RSR			
	P1	P2	P3	P4
K55	0.774*abcdefg*	0.794*abcdefg*	0.851*efg*	0.792*bcdefg*
PTE	0.715*abcdef*	0.848*efg*	0.592*a*	0.617*abc*
PT3	0.869*fg*	0.625*abc*	0.968*g*	0.723*abcdef*
PT7	0.856*efg*	0.597*ab*	0.873*fg*	0.781*abcdefg*
PT8	0.641*abcd*	0.655*abcde*	0.607*ab*	0.675*abcdef*
CON	0.705*abcdef*	0.840*defg*	0.729*abcdef*	0.809*cdefg*

4.2.2.4 *Influence of nutrient regime on growth*

The influence of nutrient treatments was positive on growth variables. The four nutrient regimes again had significantly different effects on stem diameter, height, leaf number, leaf area, shoot dry mass, root dry mass and total dry mass (Table 4.2.4).

Table 4.2.4: Means of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g) and total dry mass (TDM, g) of *E. camaldulensis* seedlings in experiment 5 (the EM experiment) under four nutrient treatments: P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P; and P4, 20 mg l^{-1} P. Means with different letters are significantly different at *P* ≤0.05 (ANOVA).

Variable	Nutrient treatment			
	P1	P2	P3	P4
DIAM	3.591*a*	4.346*b*	4.83*c*	5.899*d*
HT	34.93*a*	42.50*b*	49.49*c*	65.86*d*
LFNO	17.43*a*	21.27*b*	25.37*c*	31.80*d*
LFAR	195.5*a*	279.5*b*	402.5*c*	624.9*d*
SDM	2.731*a*	4.117*b*	5.333*c*	9.183*d*
RDM	2.004*a*	2.912*b*	3.988*c*	6.581*d*
TDM	4.730*a*	7.030*b*	9.320*c*	15.76*d*

RGR for different nutrient treatments varied significantly, that is, increased with increasing nutrient availability. Although NAR for 5.0 mg l^{-1} P and 10 mg l^{-1} P were not significantly different from each other, both were significantly lower than that for the 20 mg l^{-1} P treatment and higher than that for the 2.5 mg l^{-1} P treatment. SLA for the 10 mg l^{-1} P was the highest which significantly varied from those for 2.5 mg l^{-1} P and 5.0 mg l^{-1} P. LAR for the 10 mg l^{-1} P was the highest which significantly varied from those for 20 mg l^{-1} P or 5.0 mg l^{-1} P. LMR, RMR and RSR were not affected by different nutrient treatments (Table 4.2.5).

Table 4.2.5: Means of Relative Growth Rate (RGR, g g^{-1} day $^{-1}$), Net Assimilation Rate (NAR, mg cm^{-2} day^{-1}), Specific Leaf Area (SLA, cm^2 g^{-1}), Leaf Area Ratio (LAR, cm^2 g^{-1}), Leaf Mass Ratio (LMR), Root Mass Ratio (RMR) and Root Shoot Ratio (RSR) of *E. camaldulensis* seedlings in experiment 5 (the EM experiment) under four nutrient treatments: P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P; and P4, 20 mg l^{-1} P. Means with different letters are significantly different at *P* ≤0.05 (ANOVA).

Variable	Nutrient treatment			
	P1	P2	P3	P4
RGR	0.063*a*	0.066*b*	0.069*c*	0.073*d*
NAR	1.420*a*	1.553*b*	1.507*b*	1.726*c*
SLA	103.0*a*	97.70*a*	110.5*b*	103.4*ab*
LAR	41.77*b*	39.90*a*	43.28*b*	39.55*a*
LMR	0.440*a*	0.444*a*	0.422*a*	0.413*a*
RMR	0.425*a*	0.415*a*	0.428*a*	0.418*a*
RSR	0.760*a*	0.727*a*	0.770*a*	0.733*a*

4.2.2.5 *Influence of inoculation and nutrient regime on foliar N, P and K uptake*

There was no significant difference between different isolates of *P. tinctorius* and the uninoculated control in the foliar concentration of three nutrient elements by the seedlings (Table 4.2.6). When calculated on the basis of total leaf dry mass per individual seedling, there was no significant difference between the five inocula and the uninoculated control in terms of foliar N, P or K contents (Table 4.2.6). There was no significant relationship between leaf nutrient concentration and mycorrhizal colonisation.

Table 4.2.6: Results of ANOVA of foliar nitrogen (N), phosphorus (P) and potassium (K) uptake of *E. camaldulensis* seedlings in experiment 5 (the EM experiment). Factors are- nutrient and fungus. Figures in bold indicate where effects were significant at *P* ≤0.05 (ANOVA).

Treatment	N		P		K	
	mg gldm^{-1}	mg tldm^{-1}	mg gldm^{-1}	mg tldm^{-1}	mg gldm^{-1}	mg tldm^{-1}
Nutrient	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.172	**<0.001**
Fungus	0.959	0.945	0.569	0.427	0.328	0.259
Nutrient*Fungus	0.477	1.000	0.943	0.728	0.835	0.313

Note: mg gldm^{-1} =mg nutrient per gm leaf dry mass and mg tldm^{-1} =mg nutrient per total leaf dry mass.

Seedlings grown in the 20 mg l^{-1} P nutrient regime had significantly higher foliar concentration of N as compared to those in other nutrient regimes. There was no significant difference between the foliar concentration of N in seedlings grown in 2.5 mg l^{-1} P and 5.0 mg l^{-1} P while seedlings grown in 10 mg l^{-1} P had significantly higher foliar concentration of N than that in 2.5 mg l^{-1} P or that in 5.0 mg l^{-1} P (Fig. 4.2.7). Foliar concentration of P by the seedlings grown in the 20 mg l^{-1} P was significantly higher than that in 2.5 mg l^{-1} only. Different nutrient treatments did not vary significantly in terms of foliar concentration of K by the seedlings.

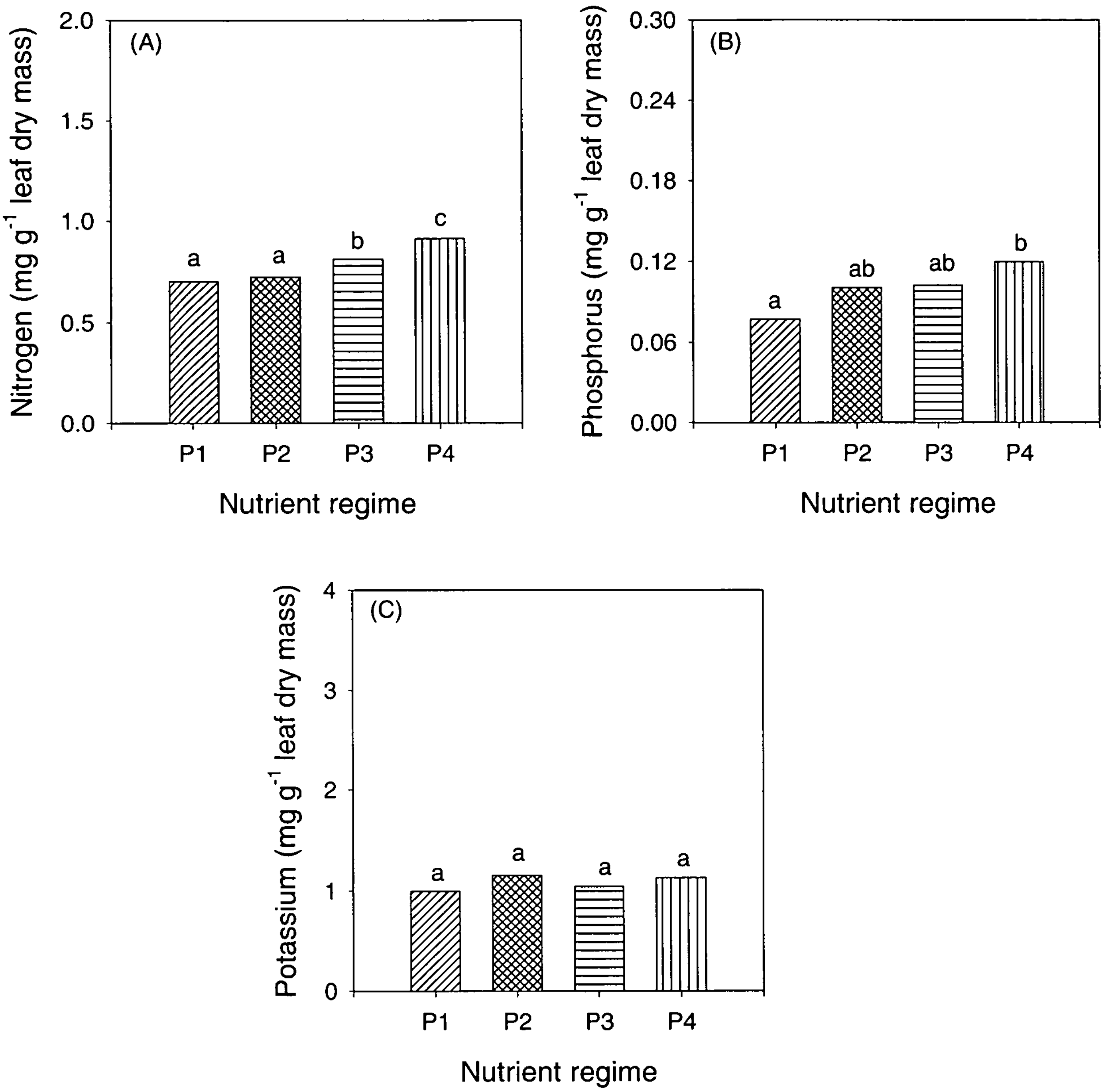

Fig. 4.2.7: Means of foliar (A) nitrogen, (B) phosphorus, and (C) potassium concentrations of *E. camaldulensis* seedlings in the EM experiment (experiment 5) under four nutrient regimes P1, 2.5 mg l^{-1} P; P2, 5.0 mg l^{-1} P; P3, 10 mg l^{-1} P and P4, 20 mg l^{-1} P. Means with different letters are significantly different at P <0.05 (ANOVA).

4.2.3 Discussion

Out of the five fungal isolates used, only *P. tinctorius* K55 resulted in colonisation (up to 27% of the root tips). The other isolates of *P. tinctorius* used in the experiment were not effective colonisers of *E. camaldulensis* seedlings, as they resulted in little colonisation (<1% of the fine root tips). Colonisation by the EM fungal 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. 4.2.3), and in terms of stem diameter (Fig. 4.2.4). 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) are reported to be more favourable for EM formation in *Eucalyptus* by *P. tinctorius* (see Burgess *et al*, 1993; Mason *et al.*, 1999b). EM fungi, in general, require a larger amount of plant photosynthate (as compared to AM 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 is often encountered (Janos, 1996). Often this growth or 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. In the initial two months of the experiment, the availability of light was between 700-800 μmol m^{-2} s^{-1} during daylight hours, but later due to cloudy weather throughout the rest of the experimental period the amount of light available fell down to 400-500 μmol m^{-2} s^{-1} even with the supplementary light provided from mercury vapour lamps. However, although many earlier glasshouse studies of *Eucalyptus* EM did not report the light regimes used, it was found in a very recent study done by Jones *et al.* (1998) that *E. coccifera* successfully formed both EM and AM at 400-500 μmol m^{-2} s^{-1} in a growth cabinet. A mini experiment involving *E. globulus* and same fungal isolates and similar nutrient treatments (which is not described in detail in this thesis) was set up alongside this experiment to assess the formation of mycorrhiza. In that experiment too, most of the colonisation resulted from the inoculation with *P. tinctorius* isolate K55, but there was no positive growth response in seedlings. A similar experiment with *E. globulus* with the isolate *P. tinctorius* PT3 has been reported to have resulted in >70% colonisation where availability of light was maintained at 1800 μmol m^{-2} s^{-1} (Mason *et al.* 1999b). However, the workers in that study did not measure growth responses in mycorrhizal seedlings compared to uninoculated controls. Therefore, it is unclear if such a high colonisation resulted in a growth response in their study. 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 little colonisation (<1% of the fine root tips) and had no significant effect on seedling growth.

As noted earlier, highest colonisation was observed at 2.5 mg l^{-1} P in this study. In the study of Mason *et al.* (1999b), *E. globulus* seedlings were grown at similar P concentration but at a higher N regime (187.5 mg l^{-1}) than that used in this experiment (21 mg l^{-1}). This high N regime has resulted in a higher colonisation in *E. globulus* seedlings. It was unclear from their study whether such a high colonisation resulted in any growth stimulation in mycorrhizal seedlings as they did not measure growth responses in mycorrhizal seedlings compared to uninoculated controls. However, 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 $Ca(H_2PO_4)_2.H_2O$) and N (150 mg per pot in the form of NH_4NO_3) 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.

RGR of seedlings were significantly different among different nutrient treatments. However, variation in NAR and LAR between nutrient treatments did not show any trend. This indicated that even though no trend was seen in the variation of NAR and LAR among different nutrient treatments, their functional product, RGR in different nutrient treatments can still show a particular trend, which, in this case is increased RGR with an increasing nutrient concentration. Similar behaviour of these variables was also observed in the AM experiment.

There was a significant relationship between the extent of colonisation by *P. tinctorius* isolate K55 and nutrient concentration (Fig. 4.2.2). Extent of colonisation by any mycorrhizal fungus decreases with an increasing availability of nutrient (P) in the growth medium and it has been observed in many mycorrhizal experiments (see Bougher *et al.*, 1990; Mason *et al.*, 1999b). There were significantly negative regressions between the extent of colonisation and total dry mass (Fig. 4.2.5), and between the extent of colonisation and root dry mass (Fig. 4.2.6) in the *P. tinctorius* K55 treatment. This further indicated that this treatment contributed to a negative growth response in the seedlings.

Although *P. tinctorius* K55 caused most of the depressive effect on growth of seedlings, foliar N, P and K concentrations of seedlings inoculated with that isolate were not significantly different from the uninoculated control. This is also supported by the fact that RGR, NAR, SLA, LAR and LMR of seedlings in the *P. tinctorius* K55 treatment were not significantly different from the uninoculated control (Table 4.2.1). However, P accumulation in mycorrhizal *Eucalyptus* seedlings has previously been reported together with growth stimulation following EM inoculation (Heinrich and Patrick, 1986; Bougher *et al.*, 1990).

4.3 Implications of the Findings From the Two Experiments

While most AM was formed at 10 mg l^{-1} P, most EM was formed at 2.5 mg l^{-1} P. Inoculation with the AM fungal isolate *G. clarum* BR148-1 and the EM fungal isolate *P. tinctorius* K55 resulted in the highest colonisation. These two fungi were therefore used for the subsequent experiment where seedlings co-inoculated with both fungal isolates were compared against seedlings inoculated with each individual isolate. Nutrient regimes 10 and 2.5 mg l^{-1} P were used in the subsequent experiment as the former was found to be suitable for AM and the latter for EM formation (also either type was present at each of these nutrient regimes).

CHAPTER 5

Effect of Dual Arbuscular-Ectomycorrhizal Inoculation on Mycorrhiza Formation, Growth and Foliar Nutrient Uptake in *E. camaldulensis* Seedlings Under Different Nutrient Regimes (Experiment 6)

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5.0 Introduction

Eucalyptus species have been reported to form both EM and AM associations (Warcup, 1980; Malajczuk *et al.*, 1981; McGee, 1986; Chilvers *et al.*, 1987; Reddell and Warren, 1987; Brundrett and Abbott, 1991). Other tree species such as *Populus* spp. (Brundrett, 1991), *Acacia* spp. (Warcup, 1980), *Alnus* and *Salix*, and some woody legumes (Harley and Harley, 1987) can also form both AM and EM. In a field experiment with *E. dunnii* Maiden, Oliveira *et al.* (1997) found that mycorrhizal colonisation of *Eucalyptus* seedlings depended on the AM or EM inoculum potential of the particular site. They found three patterns: (1) pattern A was on an agricultural site, preceded by the AM-forming soy bean (*Glycine max* (L.) Merr.)- the relatively large incidence of AM five months after planting progressively decreased while that of EM increased; (2) pattern B followed the strategy of AM/EM forming *E. viminalis* Labill.- the incidence of AM remained minimal while that of EM reached a relatively high plateau; and (3) pattern C followed the strategy of EM-forming *Pinus taeda* L.- both AM and EM progressively increased but were never abundant. In the first two patterns, AM was progressively replaced by the EM where there is no occurrence of non-host EM fungi or where host-specific EM fungi are available. But in the third pattern, non-host (that is, *Pinus* specific) EM fungi were not efficient in colonising *E. dunnii* seedlings, and perhaps, for that reason both AM and EM progressively increased, each type without showing any abundance.

Influence of inoculum availability on the occurrence of AM/EM was also recently observed on a tropical tree species, *Uapaca guineensis* M. by Taylor (1998). According to Reddell and Malajczuk (1984), not only inoculum availability, but also the nutrient status of litter, soil and the presence of compounds inhibitory to fungal growth are all critical in determining the abundance of any one or both of the two types of mycorrhizas. Moyersoen and Fitter (1999) found that the pattern of dual AM and EM colonisation depends on the identity of the host species particularly with reference to their habitats, where soil types and moisture regimes play a major role besides inoculum availability.

However, there is some controversy about the relative importance of EM and AM associations in plants which have both (Brundrett *et al.*, 1996c). There have been reports that eucalypt seedlings may initially have AM associations, which are replaced by EM associations as they mature (Gardner and Malajczuk, 1988). Various field reports suggest that both AM and EM may exist in the same root system. Lapeyrie and Chilvers (1985) suspect that predominantly EM tree species such as *Eucalyptus* may be capable of brief AM episodes in the seedling stage and the AM may be important to the early establishment of plants in low nutrient or calcareous soils. They have attributed the succession from AM to EM during the growth of dual EM/AM plants to a competitive displacement of AM by EM. Chilvers *et al.* (1987) considered AM fungi well adapted to rapid primary colonisation and perpetuation within individual roots but inferior to EM fungi for secondary colonisation because of slow hyphal spread via root branches. Cázares and Smith (1996) hypothesised that AM fungi readily colonise typically EM hosts that establish early in plant community succession, in areas where EM propagules are sparse or absent. However, hosts that establish later in plant community succession are less readily colonised by EM. Moyersoen and Fitter (1999) studied a number of tree species having AM/EM colonisation in Korup National Park rainforests in Cameroon and indicated that EM and AM colonisation can interact within the same root system but the mechanisms involved are unclear.

There have been very few studies on dual inoculation of *Eucalyptus* in general and *E. camaldulensis* in particular. Even those studies are contradictory. Amorim and Machovej (1990) (as cited in Lapeyrie *et al.*, 1992) found a depressive growth effect when *E. grandis* W. Hill. ex Maiden seedlings were co-inoculated with AM and EM fungi, whereas a dual inoculation of *P. tinctorius* and AM fungi of *E. camaldulensis* and *E. tereticornis* resulted in increased biomass (by 137.8 and 164.85% respectively), compared with the uninoculated controls (Vishwakarma and Singh, 1996b). However, they did not quantify the nutrient regime(s) they used in their experiment.

This study investigated the dual AM/EM inoculation aspect of *Eucalyptus*. The objective of the experiments described here was to assess the development of both types of mycorrhizas in the same root systems of *E. camaldulensis* at various nutrient concentrations.

The hypotheses tested were:

1) the presence of dual AM/EM inoculants affect colonisation by each;
2) there is an interaction between nutrient availability and dual mycorrhizal colonisation, and it has an effect on growth and nutrient uptake.

5.1 Material and Methods

5.1.1 *Experimental Design*

The experiment contained a factorial combination of four inoculation treatments (AM fungal, EM fungal, AM+EM fungal and an uninoculated control) and three nutrient treatments; 12 treatments in all. Ten randomised blocks each containing one pot per treatment were set up, with a single seedling per pot. Growth conditions were as described in section 2.0 (Chapter 2).

5.1.2 *Fungal Materials*

One AM fungus, *Glomus clarum* BR148-1 and one EM fungus *Pisolithus tinctorius* K55 were used as inoculant fungi (these fungi were found to be suitable for mycorrhiza formation in previous experiments).

5.1.3 *Substrate Preparation*

Preparation of substrate was as described in section 2.5 (Chapter 2).

5.1.4 *Seed Germination, Planting and Fungal Inoculation*

Germination of seeds was carried out as described in section 2.6 (Chapter 2).

Three weeks after sowing one seedling was transplanted from the tray into each pot. The date of transplanting was May 7, 1998. Fungal cultures were prepared and inoculation was carried out as described in sections 2.3, 2.4 and 2.7 (Chapter 2).

5.1.5 *Nutrient Treatment*

Details of preparation of nutrient solution and proportions of N, P and K was same as described in section 2.8 (Chapter 2). Three different nutrient treatments containing 0.5, 2.5 and 10 mg l^{-1} P were applied where all other nutrient elements were proportionally adjusted. The amounts of phosphorus added to pots of each individual treatment were 0.75 mg, 3.75 mg and 15 mg respectively at the end of the final harvest.

5.1.6 *Harvesting and Mycorrhizal Analysis*

An initial harvest was carried out of the seedlings in the germination tray before the transplanting activity as described in section 4.1.16 (Chapter 4). Plant leaf area and dry mass at the beginning of the experiment were 0.466±0.1267 cm^2 and 0.0026±0.0005 g respectively (n= 10 plants). RGR, NAR and other relative growth variables, for example, LAR, SLA, LMR, RMR and RSR were calculated using the formulas mentioned in section 2.12 (Chapter 2). Seedlings were harvested at 14 and 20 weeks respectively (five blocks each time). Harvesting procedure, measurements and method of root washing for mycorrhizal analysis was as described in sections 2.9, 2.10 and 2.11 (Chapter 2).

5.1.7 *Foliar N, P and K analysis*

N, P and K contents in dried leaf samples were carried out as detailed in section 2.13 of Chapter 2.

5.1.8 *Statistical Analysis*

A two-way ANOVA was carried out where the factors were- nutrient regime and inoculation. Statistical comparisons of means was done according to the method described in section 2.14 (Chapter 2).

5.2 Results

5.2.1 *Influence of Inoculation and Nutrient Regime on Mycorrhizal Colonisation*

There was no significant interactive effect of nutrient regime and inoculation on either AM or EM colonisation at any harvest (Table 5.1).

Table 5.1: Results of ANOVA of colonisation by *G. clarum* BR148-1 (between AM alone and AM in the dual inoculation treatments) and by *P. tinctorius* K55 (between EM alone and EM in the dual inoculation treatments) of *E. camaldulensis* seedlings in experiment 6 (the dual inoculation experiment). Figures in bold indicate where effects were significant at $P \leq 0.05$ (ANOVA).

Variable	Nutrient		Fungus		Nutrient*Fungus	
	14-wk harv	24-wk harv	14-wk harv	24-wk harv	14-wk harv	24-wk harv
AM-GC	0.154	0.631	0.463	0.321	0.660	0.913
EM-K55	**<0.001**	**<0.001**	1.000	0.385	0.063	0.094

Note: 14-wk harv =14-week harvest; 24-wk harv =24-week harvest; AM-GC =arbuscular mycorrhizal colonisation by *G. clarum* BR148-1; and EM-K55 =ectomycorrhizal colonisation by *P. tinctorius* K55.

At the 14-week harvest, there was no significant difference in colonisation by *G. clarum* BR148-1 between the AM and the dual inoculation treatments, or by *P. tinctorius* K55 between the EM and the dual inoculation treatment (Table 5.1). Colonisation by *G. clarum* BR148-1 varied significantly among the nutrient treatments with a higher colonisation in the higher nutrient concentration (for example, >50% root length colonised in 10 mg l^{-1} P) (Fig. 5.1). Colonisation by *P. tinctorius* K55 in the EM and dual inoculation treatments varied significantly between 2.5 mg l^{-1} P and 10 mg l^{-1} P with 2.5 mg l^{-1} P having the highest (5%) while 0.5 mg l^{-1} P had <1% of the root tips being colonised (Fig. 5.2).

As with the 14-week harvest, there was no significant difference in colonisation by *G. clarum* BR148-1 between the AM and the dual inoculation treatments, or by *P. tinctorius* K55 between the EM and the dual inoculation treatment at the 24-week harvest (Table 5.1). At 24 weeks, there was no significant difference in AM colonisation between the three nutrient treatments (mean colonisation was up to 40% of total root length) (Table 5.1 and Fig. 5.1). At 24 weeks, EM colonisation at 0.5 mg l^{-1} P still remained <1% while at 2.5 mg l^{-1} P and at 10 mg l^{-1} P values of 10% and 5% of the root tips being mycorrhizal were recorded respectively (Fig. 5.2). Although the 14-week harvest showed that the regression between the extent of AM colonisation and nutrient concentration was significant; increase in colonisation with an increased nutrient concentration, the 24-week harvest showed no significant regression (Fig. 5.3). The regression between the EM colonisation and nutrient concentration was not statistically significant at any of the harvests (r^2 =0.049; $F_{1,28}$ =1.44; P =0.240- for the 14-week harvest, and r^2 =0.122; $F_{1,28}$ =0.35; P =0.561- for the 24-week harvest).

Overall, the two inoculant fungi used in the experiment had significantly different colonisation potential with *G. clarum* BR148-1 having the highest at a maximum of 50% of the total root length and *P. tinctorius* K55 at a maximum of 10 % of total number of root tips.

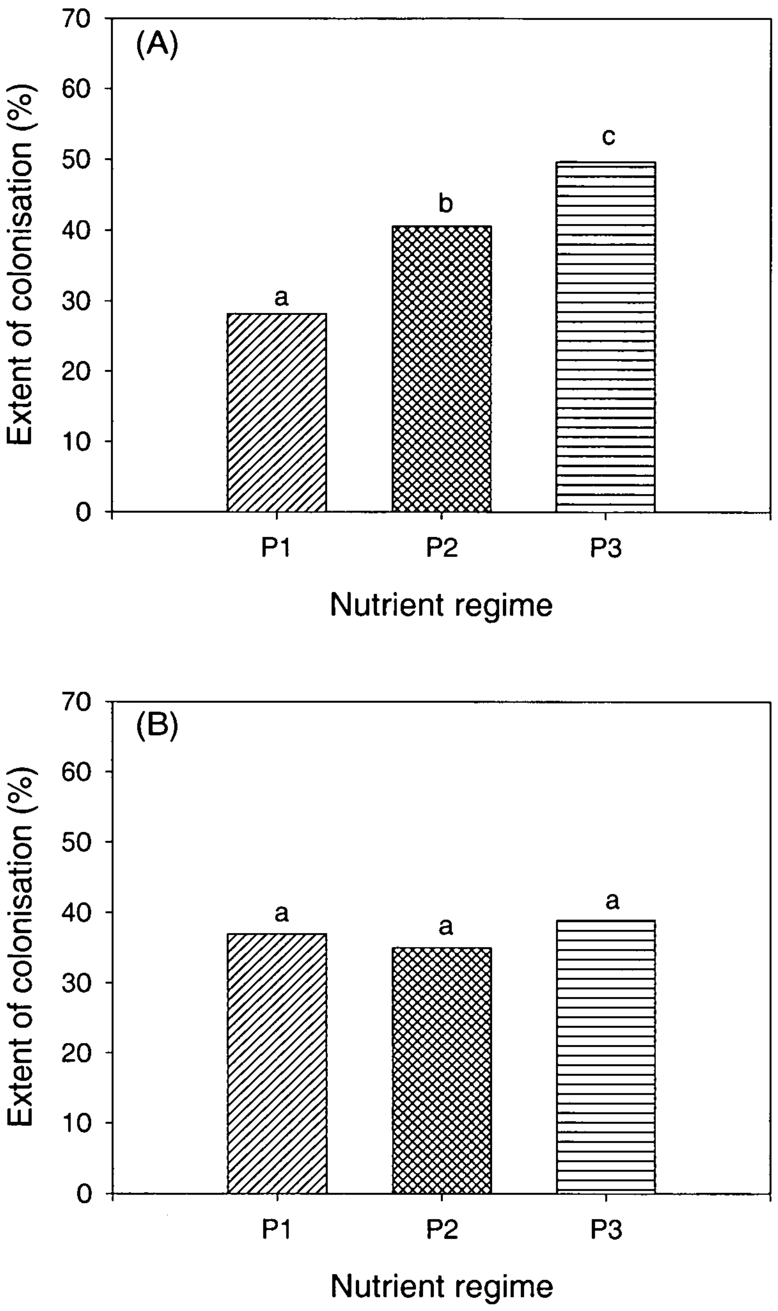


Fig. 5.1: Mean extent of colonisation of roots (% root length colonised) of *E. camaldulensis* seedlings by the AM fungus *G. clarum* BR148-1 under three nutrient regimes- P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; and P3, 10 mg l^{-1} P at (A) the 14 week harvest, and (B) the 24-week harvest in the dual inoculation experiment (experiment 6). Values are treatment means and means with different letters are significantly different at P <0.05 (ANOVA).

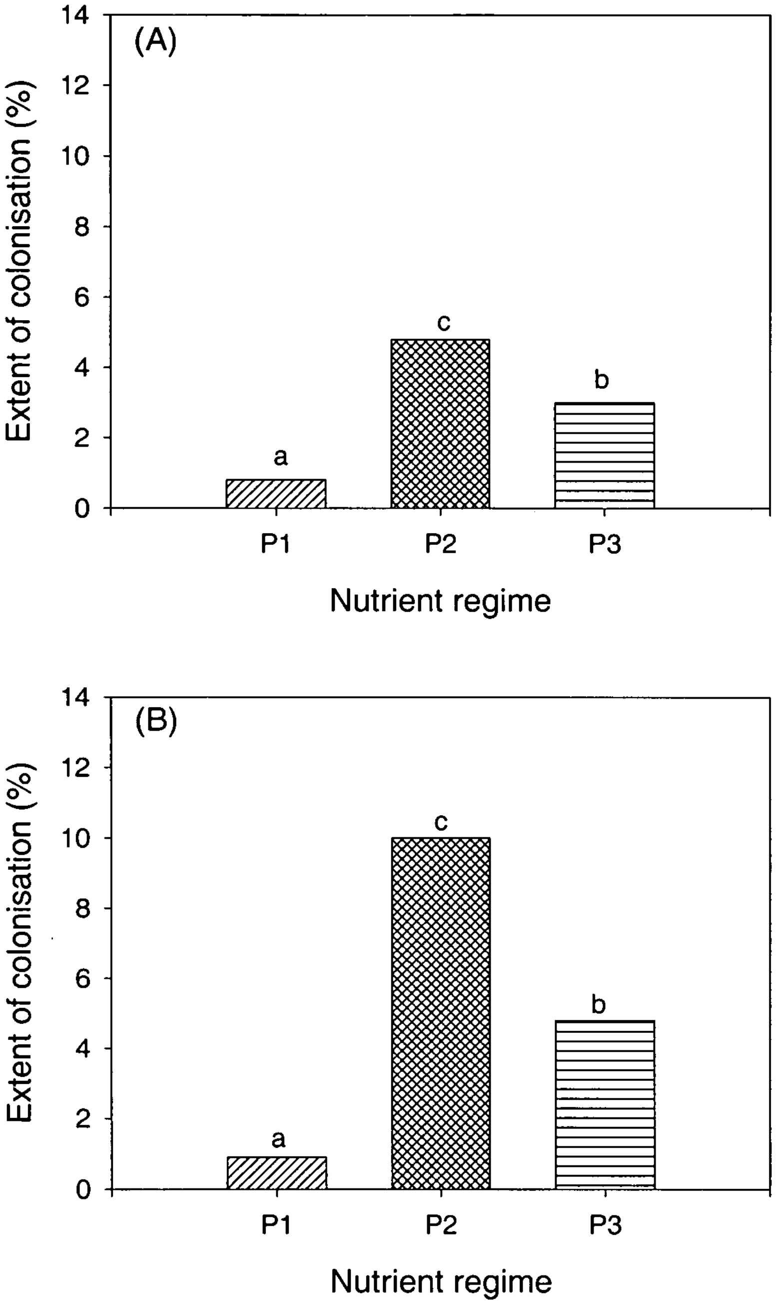
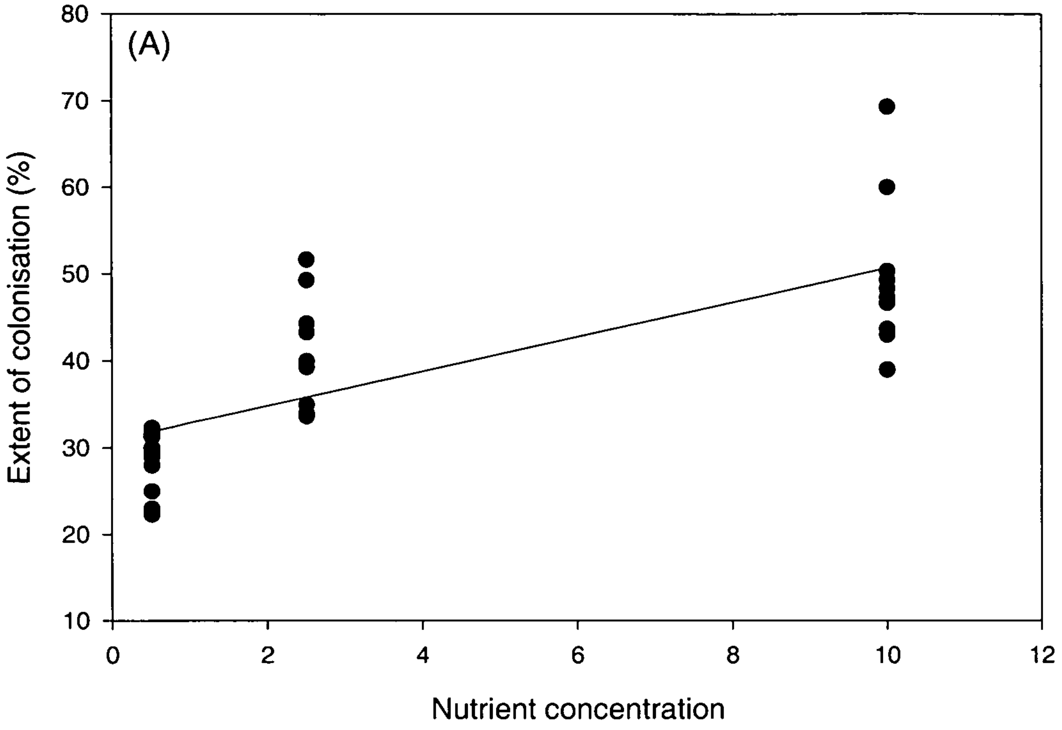


Fig. 5.2: Mean extent of colonisation of the roots (% root length colonised) of *E. camaldulensis* seedlings by the EM fungus *P. tinctorius* K55 under three nutrient regimes- P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; and P3, 10 mg l^{-1} P at (A) the 14-week harvest, and (B) the 24-week harvest of the dual inoculation experiment (experiment 6). Values are treatment means and means with different letters are significantly different at $P < 0.05$ (ANOVA).



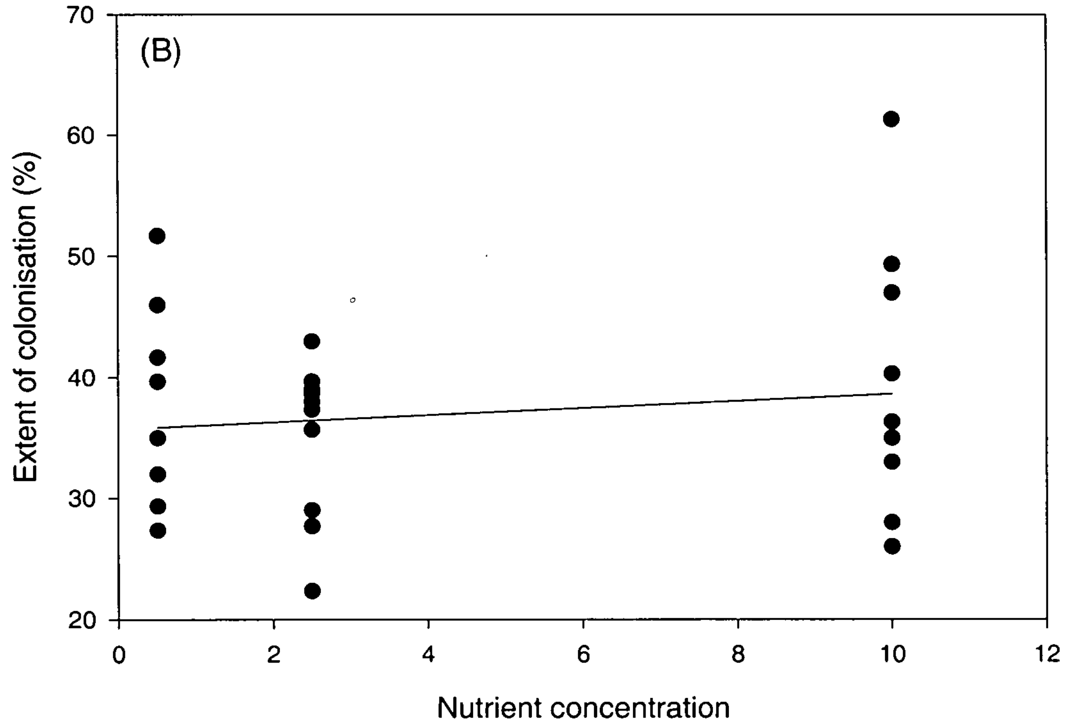


Fig. 5.3: Extent of colonisation (EXC) in *E. camaldulensis* seedlings as a function of nutrient concentration (PCN) under *G. clarum* BR148-1 treatment in the dual inoculation experiment: at (A) the 14-week harvest, and (B) the 24-week harvest in the dual inoculation experiment (experiment 6). Regression equations are: (A) EXC =1.977+30.923xPCN (r^2 =0.556; $F_{1,28}$ =35.06; P =0.0001), and (B) EXC = 0.293+35.709xPCN (r^2 =0.020; $F_{1,28}$ =0.58; P =0.453). Nutrient concentrations are illustrated in terms of P.

5.2.2 *Influence of Mycorrhizal Inoculation on Growth*

At the 14 week harvest, there was a significant interactive effect of nutrient and fungus on shoot dry mass (Table 5.2). In the 0.5 mg l^{-1} P nutrient treatment, shoot dry mass of the seedlings in the dual inoculation treatment was significantly ($P<0.05$) higher than that for the AM inoculation treatment. Although shoot dry mass of the seedlings at 2.5 mg l^{-1} P varied significantly between AM and EM or dual and EM (with EM, higher shoot dry mass), at 10 mg l^{-1} P shoot dry mass did not differ significantly between the three fungal inoculation treatments. Either the AM or the dual inoculation treatment had significantly lower shoot dry mass as compared to the EM inoculation treatment or the uninoculated control at each of the three nutrient treatments (Fig. 5.4). There was no significant difference between the EM inoculation treatment and the uninoculated control with respect to shoot dry mass of the seedlings at any of the three nutrient treatments (Fig. 5.4). However, there was no significant interactive effect of nutrient regime and inoculation on any variable of seedling growth at the 24 week (final) harvest.

Table 5.2: Results of ANOVA of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g), relative growth rate (RGR, g $g^{-1}day^{-1}$), net assimilation rate (NAR, mg cm^{-2} day^{-1}), leaf area ratio (LAR, cm^2 g^{-1}), specific leaf area (SLA, cm^2 g^{-1}), leaf mass ratio (LMR), root mass ratio (RMR) and root shoot ratio (RSR) of *E. camaldulensis* seedlings in experiment 6 (the dual inoculation experiment). Figures in bold indicate where effects were significant at $P \leq 0.05$ (ANOVA).

Variable	Nutrient		Fungus		Nutrient*Fungus	
	14-wk harv	24-wk harv	14-wk harv	24-wk harv	14-wk harv	24-wk harv
DIAM	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.461	0.955
HT	**<0.001**	**<0.001**	**0.001**	0.075	0.477	0.441
LFNO	**<0.001**	**<0.001**	0.246	0.741	0.276	0.886
LFAR	**<0.001**	**<0.001**	**<0.001**	0.170	0.452	0.887
SDM	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.038**	0.774
RDM	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.333	0.954
TDM	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.103	0.879
RGR	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.103	0.738
NAR	**0.016**	**<0.001**	**<0.001**	**0.043**	0.658	0.721
LAR	0.368	**<0.001**	**<0.001**	**<0.001**	0.722	0.502
SLA	0.224	0.405	**<0.001**	**0.005**	0.810	0.903
LMR	0.057	**0.015**	0.059	0.058	0.309	0.565
RMR	0.268	**0.003**	0.057	0.156	0.243	0.932
RSR	0.956	**0.002**	0.119	0.259	0.070	0.990

Note: 14-wk harv =14-week harvest and 24-wk harv =24-week harvest.

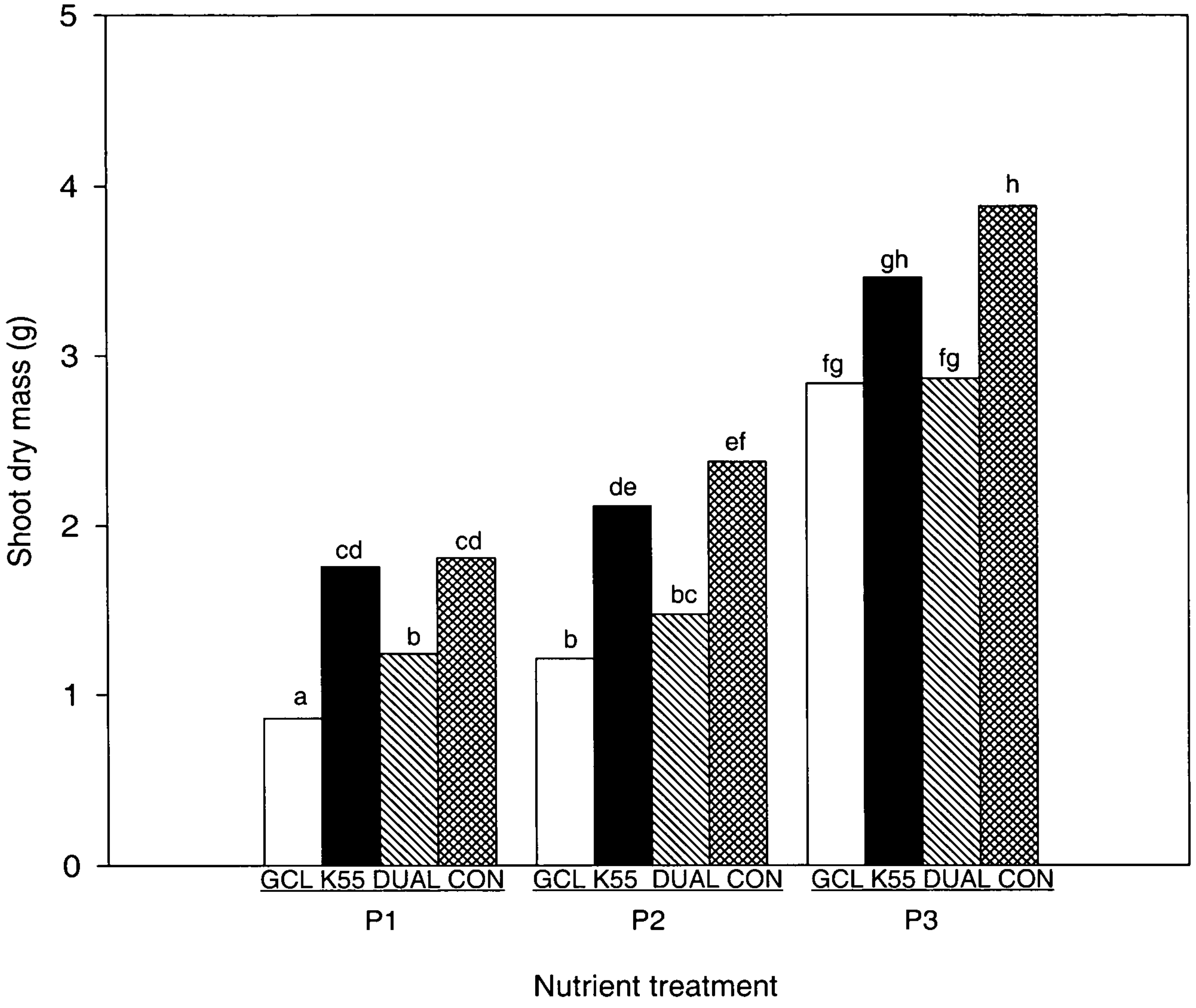


Fig. 5.4: Mean shoot dry mass of *E. camaldulensis* seedlings at the 14-week harvest in the dual inoculation experiment (experiment 6) under three nutrient regimes; P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; P3, 5.0 mg l^{-1} P and P4, 10 mg l^{-1} P and four inoculation treatments: GCL, *G. clarum* BR148-1; K55, *P. tinctorius* K55; DUAL, GCL with K55; and CON, the uninoculated control. Means with different letters are significantly different at P <0.05 (ANOVA).

At the 14 week harvest, mycorrhizal colonisation resulted in negative growth responses in terms of stem diameter, height and leaf area (Fig. 5.5) and also in terms of shoot dry mass, root dry mass and total dry mass (Fig. 5.6). Although both AM and dual inoculation treatments contributed to the negative effects on growth, the dual inoculation treatment produced significantly larger leaf area (Fig. 5.5) and shoot dry mass, root dry mass and total dry mass (Fig. 5.6) as compared to the AM inoculation treatment. There was no significant difference between the effects of EM inoculation treatment and the uninoculated control on any growth variable of the seedlings. The regressions between total dry mass and the extent of AM colonisation (Fig. 5.7), and between root dry mass and the extent of AM colonisation (Fig. 5.8) were statistically significant at the 14-week harvest; increased dry mass at an increasing extent of colonisation.

The 24 week harvest indicated that mycorrhizal colonisation has resulted in negative growth responses in terms of stem diameter, shoot dry mass, root dry mass and total dry mass (Fig. 5.9). The AM and dual inoculation treatments were again associated with all of the depressive effects on different variables of seedling growth. While these two inoculation treatments did not have a significantly different effect on stem diameter and shoot dry mass, the dual inoculation treatment was associated with significantly higher root dry mass and total dry mass as compared to the AM inoculation treatment. As at the 14-week harvest, there was no significant difference between the effects of EM inoculation treatment and the uninoculated control on any of the variables.

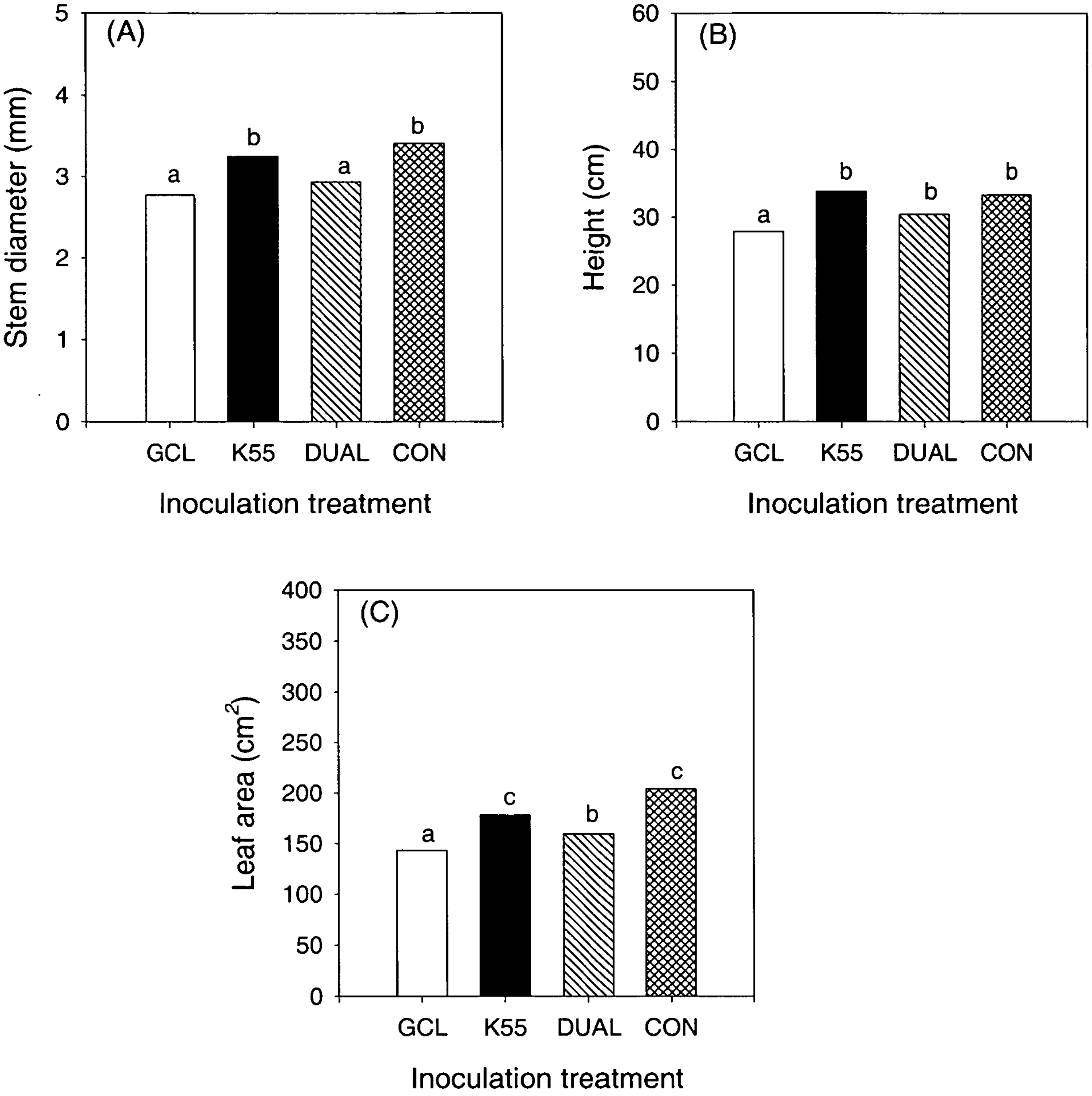


Fig. 5.5: Means of (A) stem diameter, (B) height, and (C) leaf area of *E. camaldulensis* seedlings at the 14-week harvest in the dual inoculation experiment (experiment 6) under four inoculation treatments. GCL, with *G. clarum* BR148-1; K55, with *P. tinctorius* K55; DUAL, with GCL and K55; and CON, the uninoculated control. Means with different letters are significantly different at *P*, <0.05 (ANOVA).

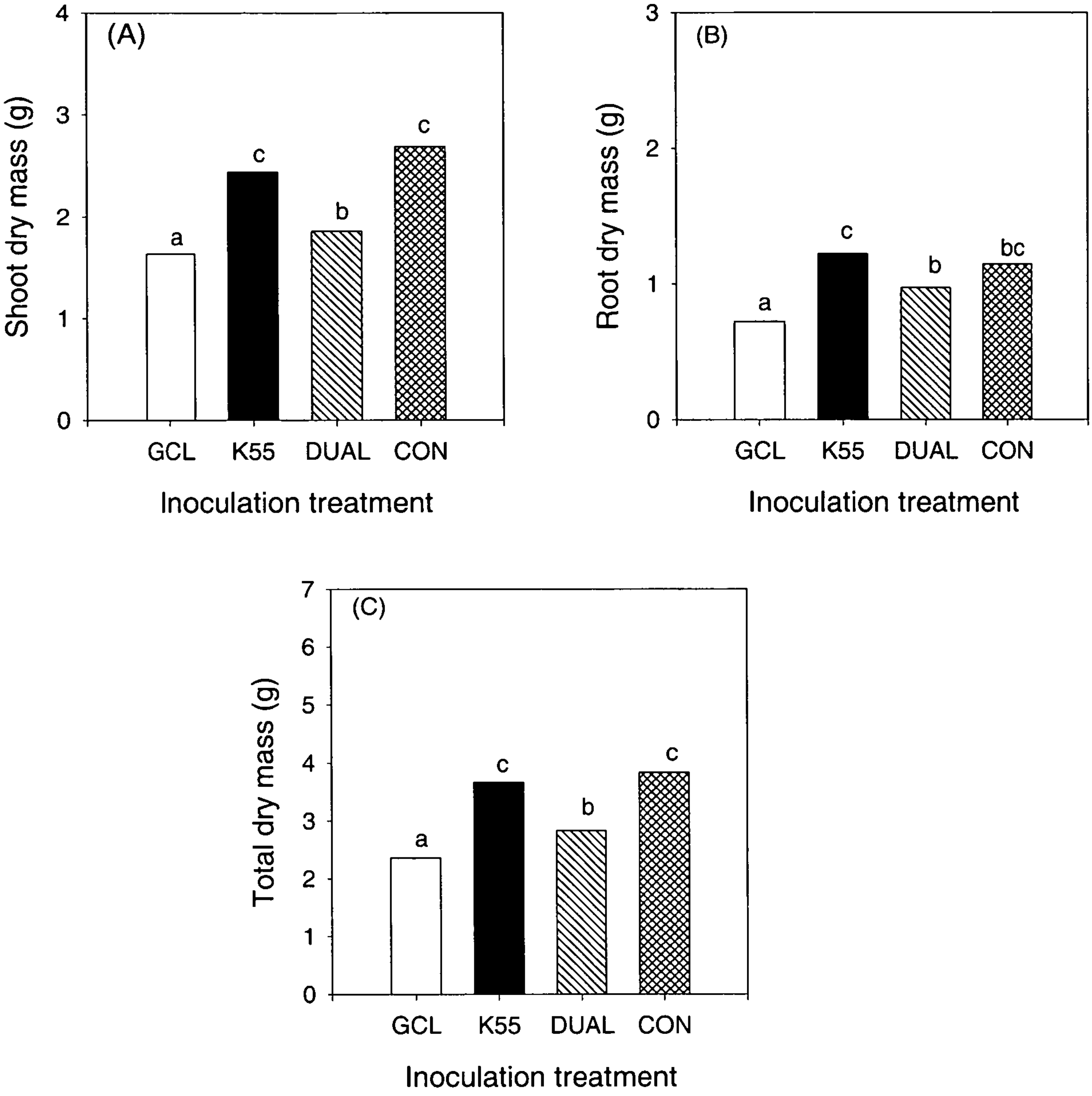


Fig. 5.6: Means of (A) shoot dry mass, (B) root dry mass, and (C) total dry mass of *E. camaldulensis* seedlings at the 14-week harvest in the dual inoculation experiment (experiment 6) under four inoculation treatments. GCL, with *G. clarum* BR148-1; K55, with *P. tinctorius* K55; DUAL, with GCL and K55; and CON, the uninoculated control. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

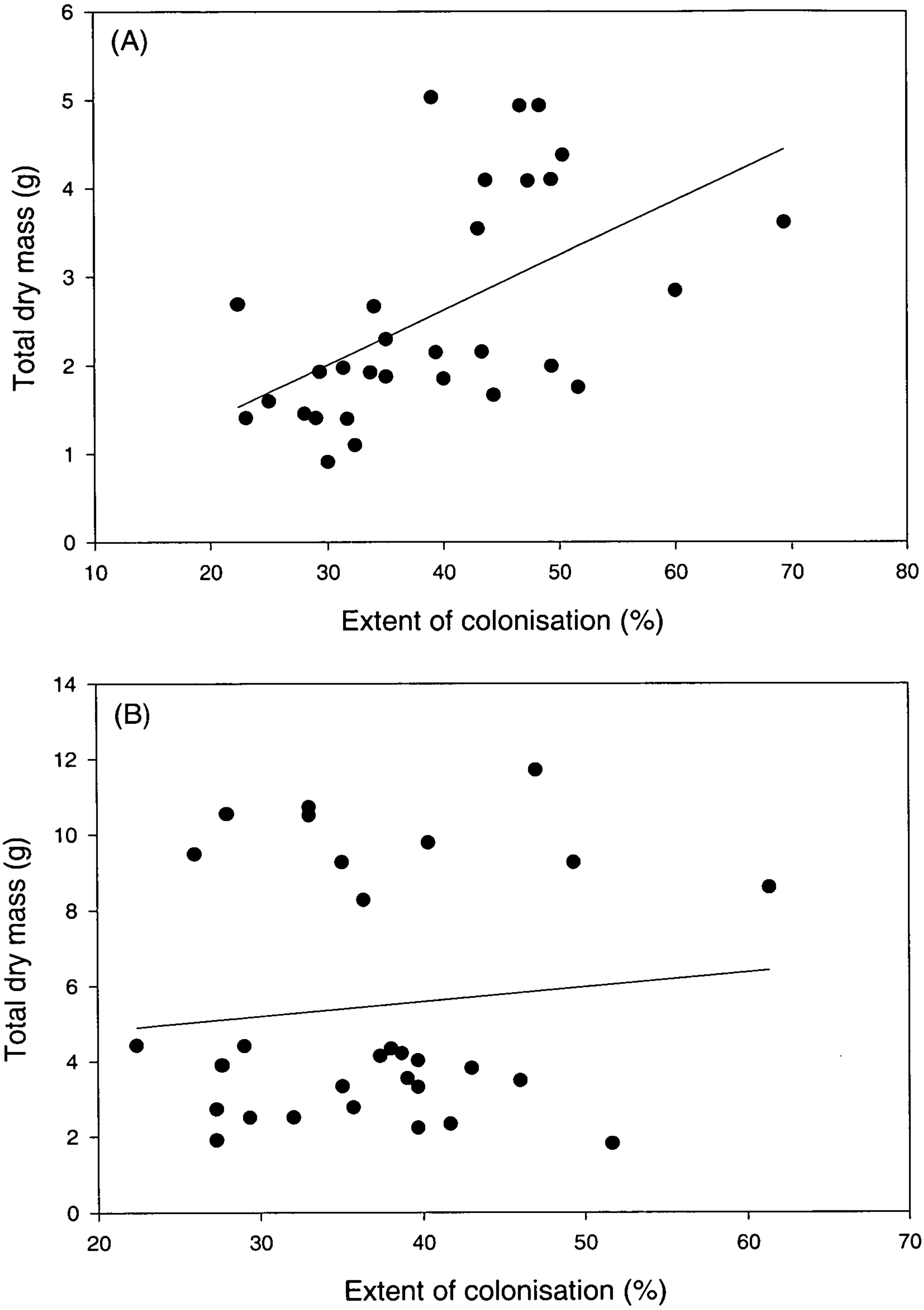


Fig. 5.7: Total dry mass (TDM) of *E. camaldulensis* seedlings as a function of extent of colonisation (EXC) by *G. clarum* BR148-1 in the dual inoculation experiment: at (A) the 14-week harvest, and (B) the 24-week harvest in the dual inoculation experiment (experiment 6). Regression equations are: (A) TDM = 0.062+0.152xEXC (r^2 =0.299; $F_{1,28}$ =11.94; P =0.002), and (B) TDM =0.039+4.027xEXC (r^2 =0.010; $F_{1,28}$ =0.29; P =0.592).

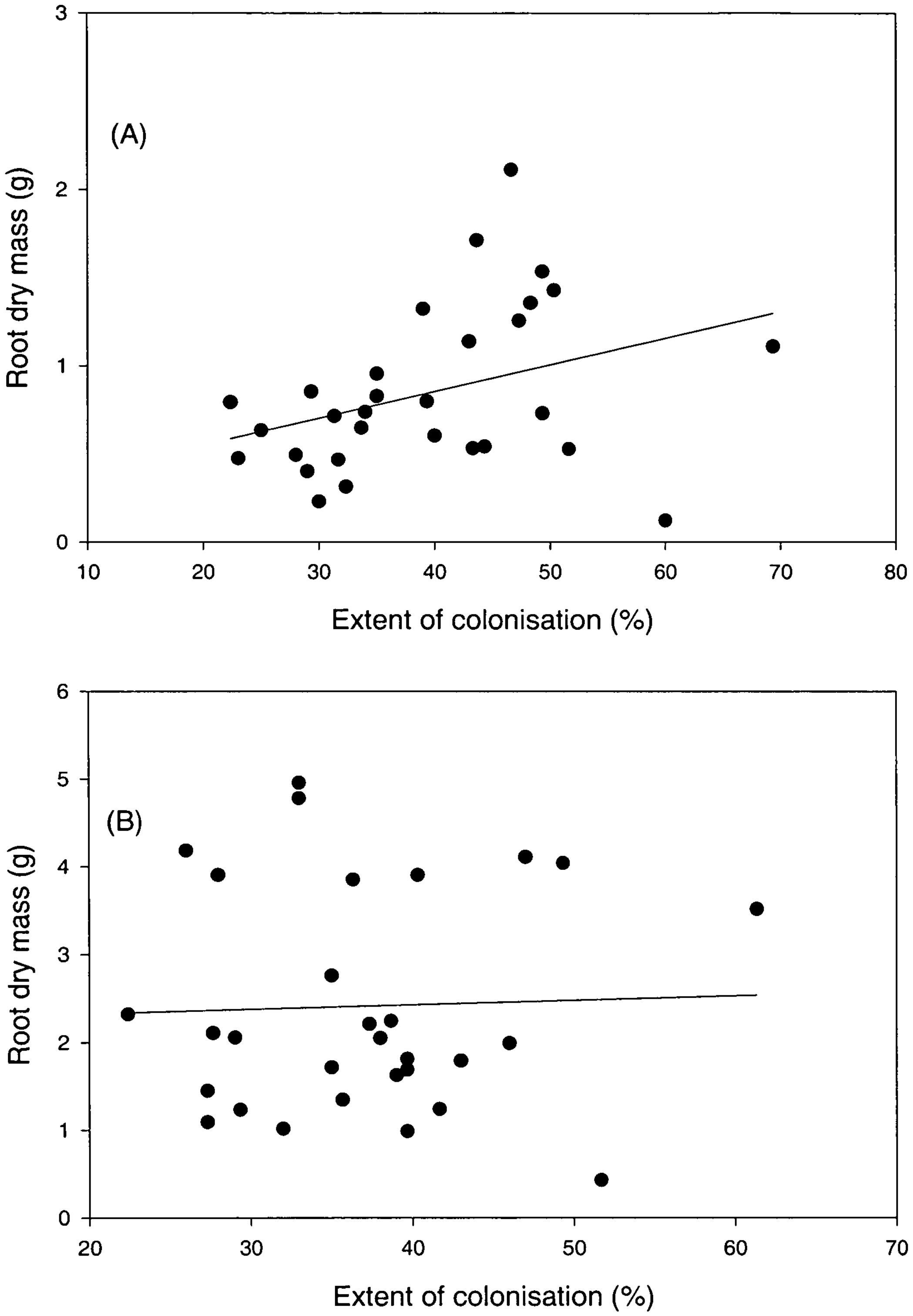


Fig. 5.8: Root dry mass (RDM) of *E. camaldulensis* seedlings as a function of extent of colonisation (EXC) by *G. clarum* BR148-1 in the dual inoculation experiment: at (A) the 14-week harvest, and (B) the 24-week harvest in the dual inoculation experiment (experiment 6). Regression equations are: (A) RDM = 0.254+0.152xEXC (r^2 =0.128; $F_{1,28}$ =4.11; P =0.050), and (B) RDM =5.136+2.225xEXC (r^2 =0.001; $F_{1,28}$ =0.03; P =0.855).

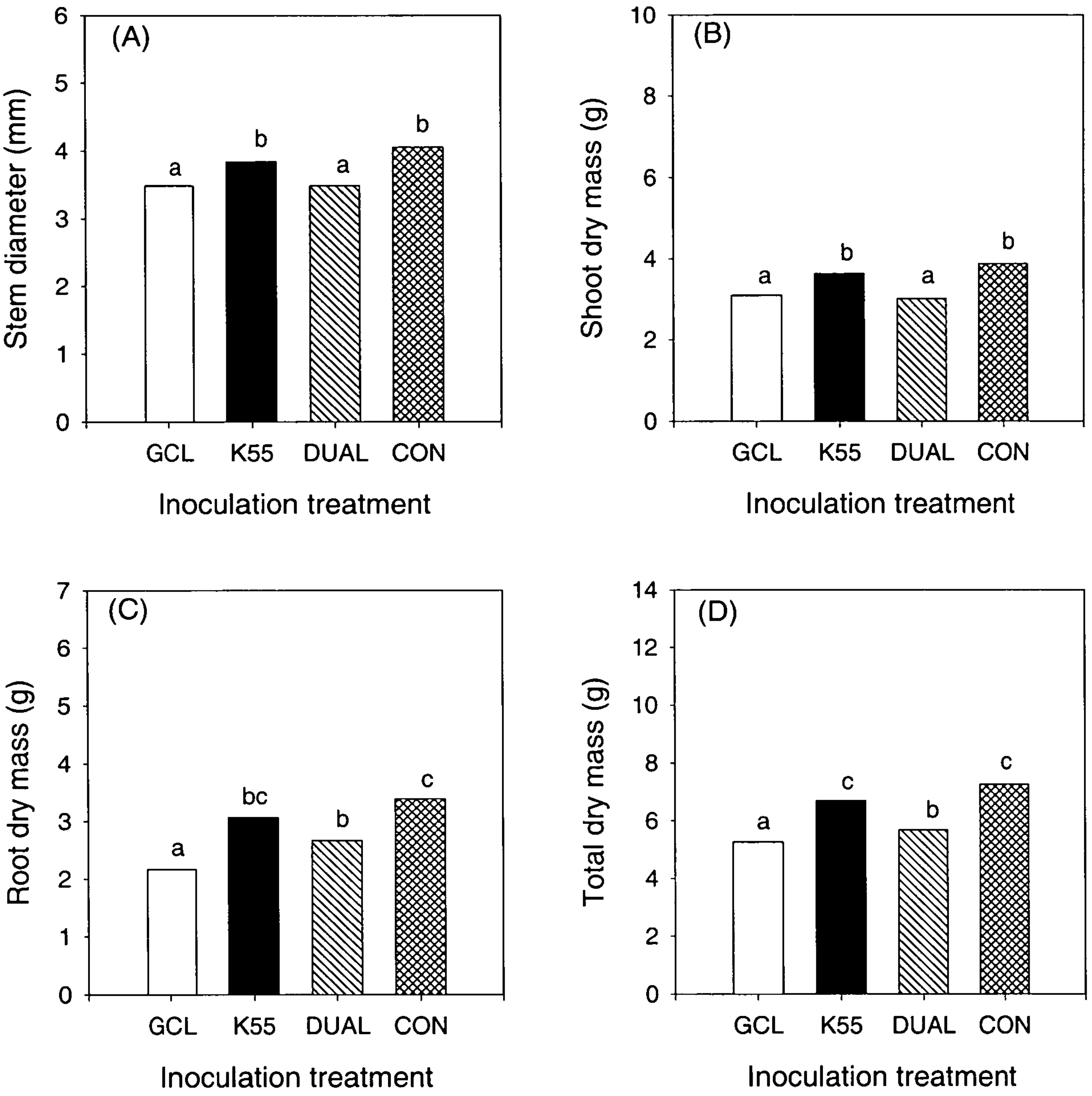


Fig. 5.9: Means of (A) stem diameter, (B) shoot dry mass, (C) root dry mass, and (D) total dry mass of *E. camaldulensis* seedlings at the 24 -week harvest in the dual inoculation experiment (experiment 6) under four inoculation treatments. GCL, with *G. clarum* BR148-1; K55, *P. tinctorius* K55; DUAL, with GCL and K55; and CON, the uninoculated control. Means with different letters are significantly different at P <0.05 (ANOVA).

At the 14-week harvest, RGR of the seedlings in the AM and dual inoculation treatments were significantly reduced as compared to either the EM inoculation treatment or the uninoculated control. The dual inoculation treatment contributed to significantly higher RGR as compared to the AM treatment (Table 5.3). NAR of seedlings from different inoculation treatments varied significantly among themselves with the EM treatment contributing to the highest value followed by the uninoculated control, the dual inoculation treatment and the AM treatment (Table 5.3). SLA for AM and dual inoculation treatments were significantly higher compared to either the EM or the uninoculated control. LAR of seedlings among the three inoculation treatments except the EM treatment did not vary significantly from each other, but they were significantly higher as compared to the EM inoculation treatment. LMR, RMR and RSR of the seedlings were not affected by the various inoculation treatments (Table 5.3).

Table 5.3: Means of Relative Growth Rate (RGR, g g^{-1} day $^{-1}$), Net Assimilation Rate (NAR, mg cm^{-2} day^{-1}), Specific Leaf Area (SLA, cm^2 g^{-1}), Leaf Area Ratio (LAR, cm^2 g^{-1}), Leaf Mass Ratio (LMR), Root Mass Ratio (RMR) and Root Shoot Ratio (RSR) of *E. camaldulensis* seedlings at the 14-week harvest of experiment 6 (the dual inoculation experiment) under four inoculation treatments: GCL, *G. clarum* BR148-1; K55, *P. tinctorius* K55; DUAL, including GCL and K55; and CON, the uninoculated control. Means with different letters are significantly different at $P \leq 0.05$ (ANOVA).

Variable	Inoculation treatment			
	GCL	K55	DUAL	CON
RGR	0.068*a*	0.074*c*	0.071*b*	0.074*c*
NAR	0.962*a*	1.250*d*	1.059*b*	1.166*c*
SLA	115.7*b*	95.60*a*	112.7*b*	95.30*a*
LAR	60.95*b*	48.37*a*	55.99*b*	52.83*b*
LMR	0.529*a*	0.514*a*	0.501*a*	0.557*a*
RMR	0.316*a*	0.328*a*	0.340*a*	0.294*a*
RSR	0.481*a*	0.495*a*	0.528*a*	0.422*a*

At the 24 week harvest, the AM and dual inoculation treatments resulted in significantly reduced values for RGR, NAR, SLA and LAR as compared to those for either the EM inoculation treatment or the uninoculated control. The AM inoculation treatment produced significantly lower RGR as compared to the dual inoculation treatment. Again, there was no significant difference between the effects of EM inoculation treatment and the uninoculated control on any of these variables. In addition, LMR, RMR and RSR of the seedlings were not affected by the various inoculation treatments (Table 5.4).

Table 5.4: Means of Relative Growth Rate (RGR, g g^{-1} day $^{-1}$), Net Assimilation Rate (NAR, mg cm^{-2} day^{-1}), Specific Leaf Area (SLA, cm^2 g^{-1}), Leaf Area Ratio (LAR, cm^2 g^{-1}), Leaf Mass Ratio (LMR), Root Mass Ratio (RMR) and Root Shoot Ratio (RSR) of *E. camaldulensis* seedlings at the 24-week harvest of experiment 6 (the dual inoculation experiment) under four inoculation treatments: GCL, *G. clarum* BR148-1; K55, *P. tinctorius* K55; DUAL, including GCL and K55; and CON, the uninoculated control. Means with different letters are significantly different at *P* ≤0.05 (ANOVA).

Variable	Inoculation treatment			
	GCL	K55	DUAL	CON
RGR	0.045*a*	0.046*c*	0.045*b*	0.047*c*
NAR	0.852*a*	1.040*b*	0.903*a*	1.103*b*
SLA	110.3*b*	100.1*a*	113.6*b*	101.3*a*
LAR	42.82*b*	35.07*a*	40.49*b*	33.57*a*
LMR	0.390*a*	0.356*a*	0.357*a*	0.336*a*
RMR	0.436*a*	0.470*a*	0.486*a*	0.480*a*
RSR	0.815*a*	0.915*a*	0.964*a*	0.954*a*

5.2.3 *Influence of Nutrient Regime on Growth*

The influence of nutrient treatments was positive on growth at both of the harvests. The three nutrient regimes had significantly different effects on stem diameter, height, leaf number, leaf area, shoot dry mass, root dry mass and total dry mass (Table. 5.5). Only final harvest data are illustrated.

Table 5.5: Means of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g) and total dry mass (TDM, g) of *E. camaldulensis* seedlings at the 24-week harvest in experiment 6 (the dual inoculation experiment) under three nutrient treatments: P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P and P3, 10 mg l^{-1} P. Means with different letters are significantly different at *P* ≤0.05 (ANOVA).

Variable	Nutrient treatment		
	P1	P2	P3
DIAM	2.914*a*	3.544*b*	4.698*c*
HT	25.00*a*	32.20*b*	59.80*c*
LFNO	15.25*a*	19.30*b*	33.40*c*
LFAR	106.7*a*	160.3*b*	461.4*c*
SDM	1.525*a*	2.361*b*	6.313*c*
RDM	1.519*a*	2.226*b*	4.713*c*
TDM	3.044*a*	4.587*b*	11.03*c*

For both harvests, RGR differed significantly between nutrient treatments with lower RGR recorded for the lower P treatment and higher RGR for the higher P treatment. At the 14 week harvest, NAR between 0.5 mg l^{-1} P and 2.5 mg l^{-1} P and also between 2.5 mg l^{-1} P and 10 mg l^{-1} P did not differ significantly (Table 5.6). At the 14 week harvest, LAR, SLA, LMR, RMR and RSR were not affected by different nutrient treatments (Table 5.6).

As at the 14-week harvest, NAR between 0.5 mg l^{-1} P and 2.5 mg l^{-1} P and also between 2.5 mg l^{-1} P and 10 mg l^{-1} P did not differ significantly at the 24-week harvest (Table 5.7). LAR and LMR for 10 mg l^{-1} P was significantly higher compared to those for either 0.5 mg l^{-1} P or 2.5 mg l^{-1} P nutrient treatment. RMR and RSR for 10 mg l^{-1} P were significantly lower compared to those for either 0.5 mg l^{-1}

P or 2.5 mg l^{-1} P. SLA was not affected by different nutrient treatments even at the end of 24 weeks of the growth of seedlings (Table 5.7).

Table 5.6: Means of Relative Growth Rate (RGR, g g^{-1} day $^{-1}$), Net Assimilation Rate (NAR, mg cm^{-2} day^{-1}), Specific Leaf Area (SLA, cm^2 g^{-1}), Leaf Area Ratio (LAR, cm^2 g^{-1}), Leaf Mass Ratio (LMR), Root Mass Ratio (RMR) and Root Shoot Ratio (RSR) of *E. camaldulensis* seedlings at the 14-week harvest of experiment 6 (the dual inoculation experiment) under three nutrient treatments: P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; and P3, 10 mg l^{-1} P. Means with different letters are significantly different at $P \leq 0.05$ (ANOVA).

Variable	Inoculation treatment		
	P1	P2	P3
RGR	0.068*a*	0.070*b*	0.077*c*
NAR	1.063*a*	1.100*ab*	1.165*b*
SLA	100.5*a*	104.5*a*	109.4*a*
LAR	53.80*a*	53.32*a*	56.49*a*
LMR	0.542*a*	0.514*a*	0.520*a*
RMR	0.318*a*	0.322*a*	0.317*a*
RSR	0.475*a*	0.486*a*	0.483*a*

Table 5.7: Means of Relative Growth Rate (RGR, g g^{-1} day $^{-1}$), Net Assimilation Rate (NAR, mg cm^{-2} day^{-1}), Specific Leaf Area (SLA, cm^2 g^{-1}), Leaf Area Ratio (LAR, cm^2 g^{-1}), Leaf Mass Ratio (LMR), Root Mass Ratio (RMR) and Root:Shoot Ratio (RSR) of *E. camaldulensis* seedlings at the 24-week harvest of experiment 6 (the dual inoculation experiment) under three nutrient treatments: P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; and P3, 10 mg l^{-1} P. Means with different letters are significantly different at $P \leq 0.05$ (ANOVA).

Variable	Nutrient treatment		
	P1	P2	P3
RGR	0.042*a*	0.045*b*	0.050*c*
NAR	0.932*a*	1.002*b*	0.990*ab*
SLA	105.5*a*	104.3*a*	109.1*a*
LAR	36.18*a*	35.39*a*	42.39*b*
LMR	0.346*a*	0.344*a*	0.389*b*
RMR	0.491*b*	0.488*b*	0.425*a*
RSR	1.003*b*	0.976*b*	0.757*a*

5.2.4 *Influence of Inoculation and Nutrient Regime on Foliar N, P and K uptake*

At 14 weeks, there were significant interactive effects of inoculation and nutrient treatments on both leaf K concentration (mg g^{-1} leaf dry mass) and total leaf K contents (mg per total leaf dry mass) (Table 5.8). Foliar K concentration for *G. clarum* BR148-1 inoculated seedlings were significantly higher compared to the uninoculated control at 0.5 mg l^{-1} P (Fig. 5.10). Although *G. clarum* BR148-1 and dual inoculation treatments did not differ significantly from each other at any nutrient treatment, *G. clarum* BR148-1 inoculated seedlings had significantly higher foliar K concentration compared to *P. tinctorius* K55 inoculated seedlings at 0.5 mg l^{-1} P and 10 mg l^{-1} P nutrient treatments (Fig. 5.10). However, foliar K content for *G. clarum* BR148-1 inoculated seedlings were significantly lower compared to the uninoculated control at all nutrient treatments (Fig. 5.10). *G. clarum* BR148-1 and dual inoculation treatments did not differ significantly from each other at any nutrient treatment. *P. tinctorius* K55 inoculated seedlings had significantly lower foliar K content compared to the uninoculated control at 10 mg l^{-1} P nutrient treatment (Fig. 5.10). However, there was no interactive effect of inoculation and nutrient treatments at the 24-week harvest (Table 5.8).

Table 5.8: Results of ANOVA of foliar nitrogen (N), phosphorus (P) and potassium (K) uptake of *E. camaldulensis* seedlings in experiment 6 (the dual inoculation experiment). Factors are- nutrient and fungus. Figures in bold indicate where effects were significant at *P* ≤0.05 (ANOVA).

Treatment	The 14-week harvest					
	N		P		K	
	mg $gldm^{-1}$	mg $tldm^{-1}$	mg $gldm^{-1}$	mg $tldm^{-1}$	mg $gldm^{-1}$	mg $tldm^{-1}$
Nutrient	**<0.001**	**<0.001**	0.206	**<0.001**	0.297	**<0.001**
Fungus	**0.019**	**0.001**	**<0.001**	0.334	**0.037**	**<0.001**
Nutrient*Fungus	0.467	0.585	0.862	0.621	**0.005**	**0.049**
	The 24-week harvest					
Nutrient	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Fungus	**0.018**	0.946	**<0.001**	0.319	0.102	0.230
Nutrient*Fungus	0.928	0.762	0.715	0.850	0.227	0.224

Note: mg $gldm^{-1}$ =mg nutrient per g leaf dry mass and mg $tldm^{-1}$ =mg nutrient per total leaf dry mass (g).

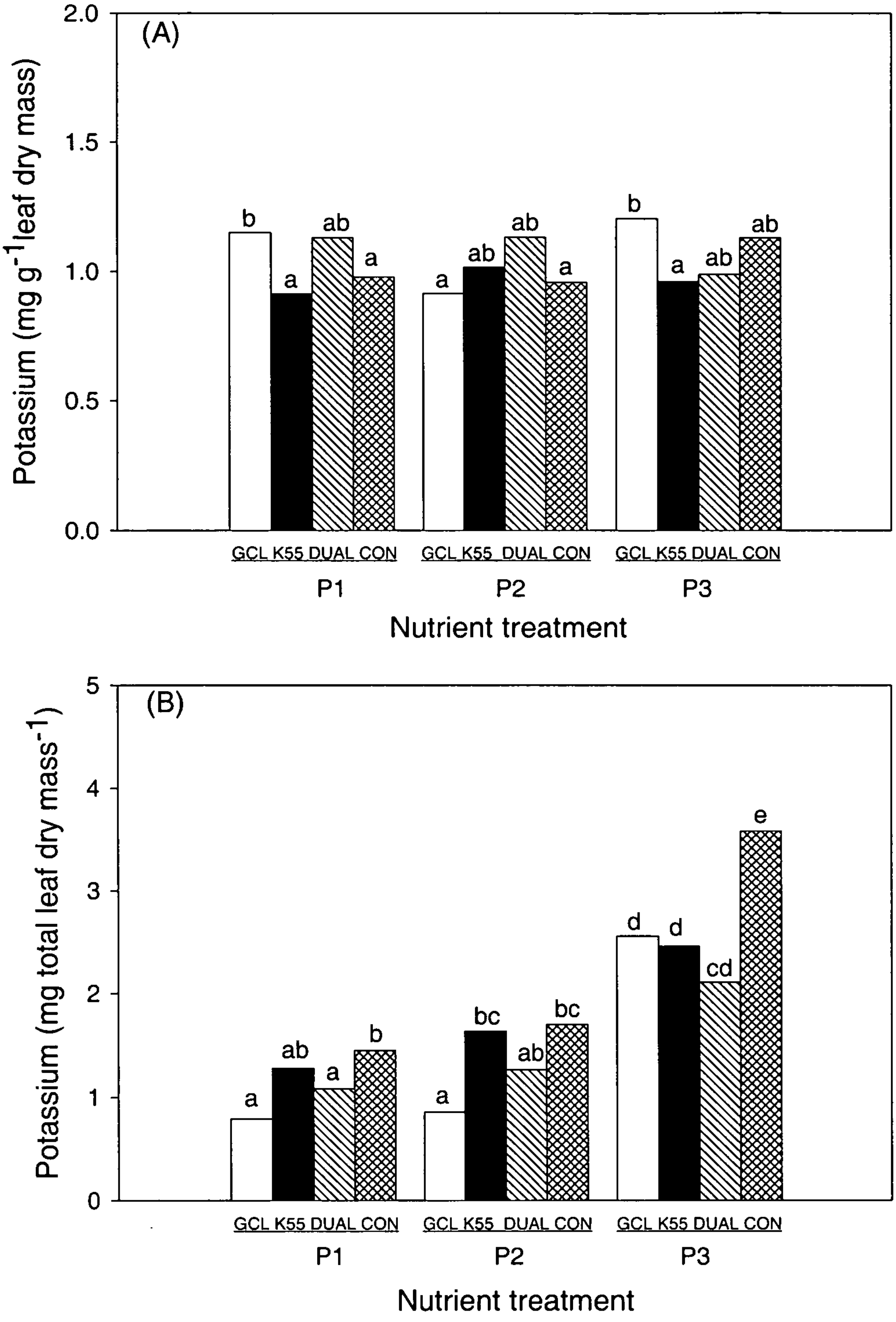


Fig. 5.10: Means of foliar (A) potassium concentration and (B) potassium content of *E. camaldulensis* seedlings at the 14-week harvest in the dual inoculation experiment (experiment 6) under three nutrient regimes; P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; P3, 5.0 mg l^{-1} P and P4, 10 mg l^{-1} P and four inoculation treatments: GCL, *G. clarum* BR148-1; K55,*P. tinctorius* K55; DUAL, GCL with K55; and CON, the uninoculated control. Means with different letters are significantly different at *P* <0.05 (ANOVA).

At 14 weeks, the dual inoculation treatment had the highest foliar concentration of N which varied significantly from the EM and the uninoculated control but not from the AM inoculation treatment. Foliar concentration of P between the AM and the dual inoculation treatments did not differ significantly but each was significantly higher than either the EM treatment or the uninoculated control. Foliar K concentration for the AM and the dual inoculation treatments was significantly higher than that for the EM treatment but not for the uninoculated control (Fig. 5.11).

When calculated on the basis of total leaf dry mass per individual seedling, N and K contents in AM and dual inoculated seedlings were significantly lower compared to the EM or the uninoculated control at the 14-week harvest (Fig. 5.11). However, this difference disappeared at the 24-week harvest, and the three inoculation treatments did not differ significantly from the uninoculated control (Table 5.8).

At 24 weeks, seedlings in the AM and the dual inoculation treatments had significantly higher foliar concentration of N and P as compared to the EM treatment or the uninoculated control. There was no significant difference between the uninoculated control and the EM treatment, and the four inoculation treatments did not differ significantly in terms of K concentration (Fig. 5.12).

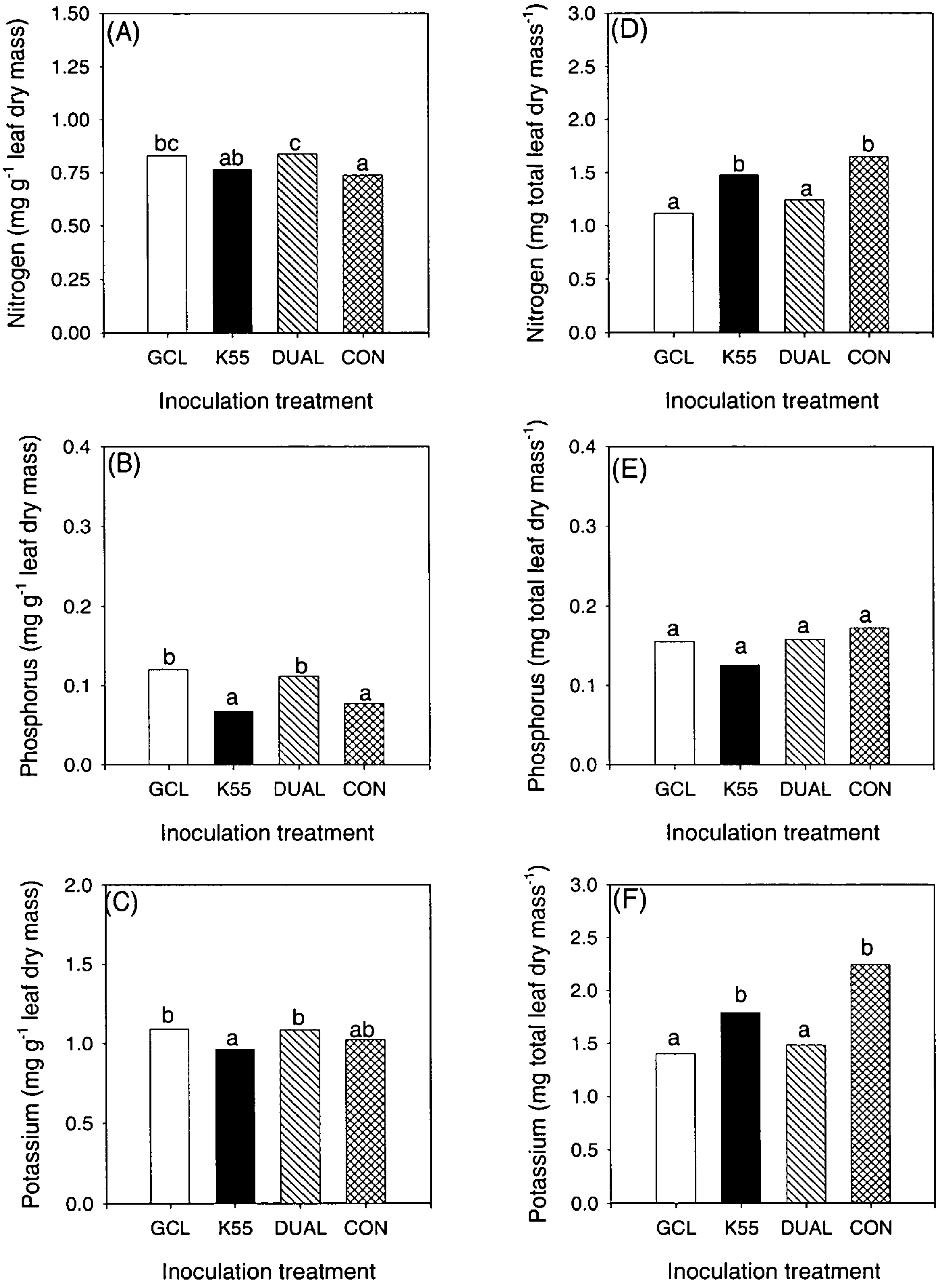


Fig. 5.11: Means of foliar (A) nitrogen, (B) phosphorus, and (C) potassium concentrations, and (D) nitrogen, (E) phosphorus and (F) potassium contents of *E. camaldulensis* seedlings at the 14-week harvest in the dual inoculation experiment (experiment 6) under four inoculation treatments. GCL, *G. clarum* BR148-1; K55, *P. tinctorius* K55; DUAL, a dual inoculation treatment including GCL and K55; and CON, the uninoculated control. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

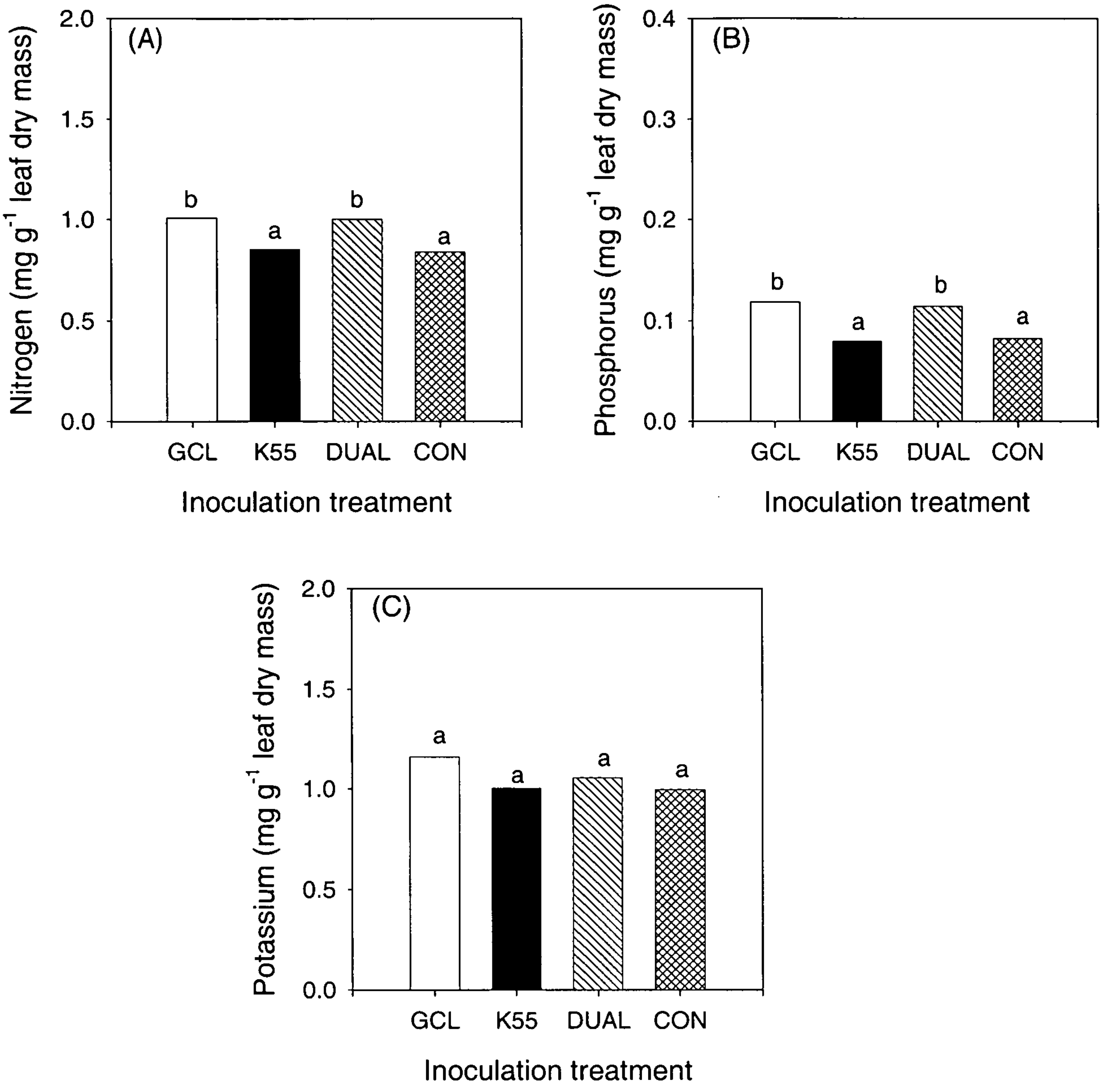


Fig. 5.12: Means of foliar (A) nitrogen, (B) phosphorus, and (C) potassium concentrations of *E. camaldulensis* seedlings at the 24-week harvest in the dual inoculation experiment (experiemnt 6) under four inoculation treatments. GCL, *G. clarum* BR148-1; K55, *P. tinctorius* K55; DUAL, a dual inoculation treatment combining GCL and K55; and CON, the uninoculated control. Means with different letters are significantly different at P <0.05 (ANOVA).

At 14 weeks, there was no significant difference between the three nutrient treatments in the foliar concentrations of P and K, but N concentration in the 10 mg l^{-1} P treatment was significantly higher compared to the other two nutrient treatments (0.5 mg l^{-1} P and 2.5 mg l^{-1} P) (Fig. 5.13).

At 24 weeks, there was a significant difference between the three nutrient treatments in the foliar concentrations of N and P, but K concentration did not differ significantly between 0.5 mg l^{-1} P and 2.5 mg l^{-1} P, with the value in the 10 mg l^{-1} P being significantly higher than in the other two nutrient treatments (Fig. 5.14).

There was no significant relationship between AM or EM colonisation and foliar N, P or K concentration at any harvest.

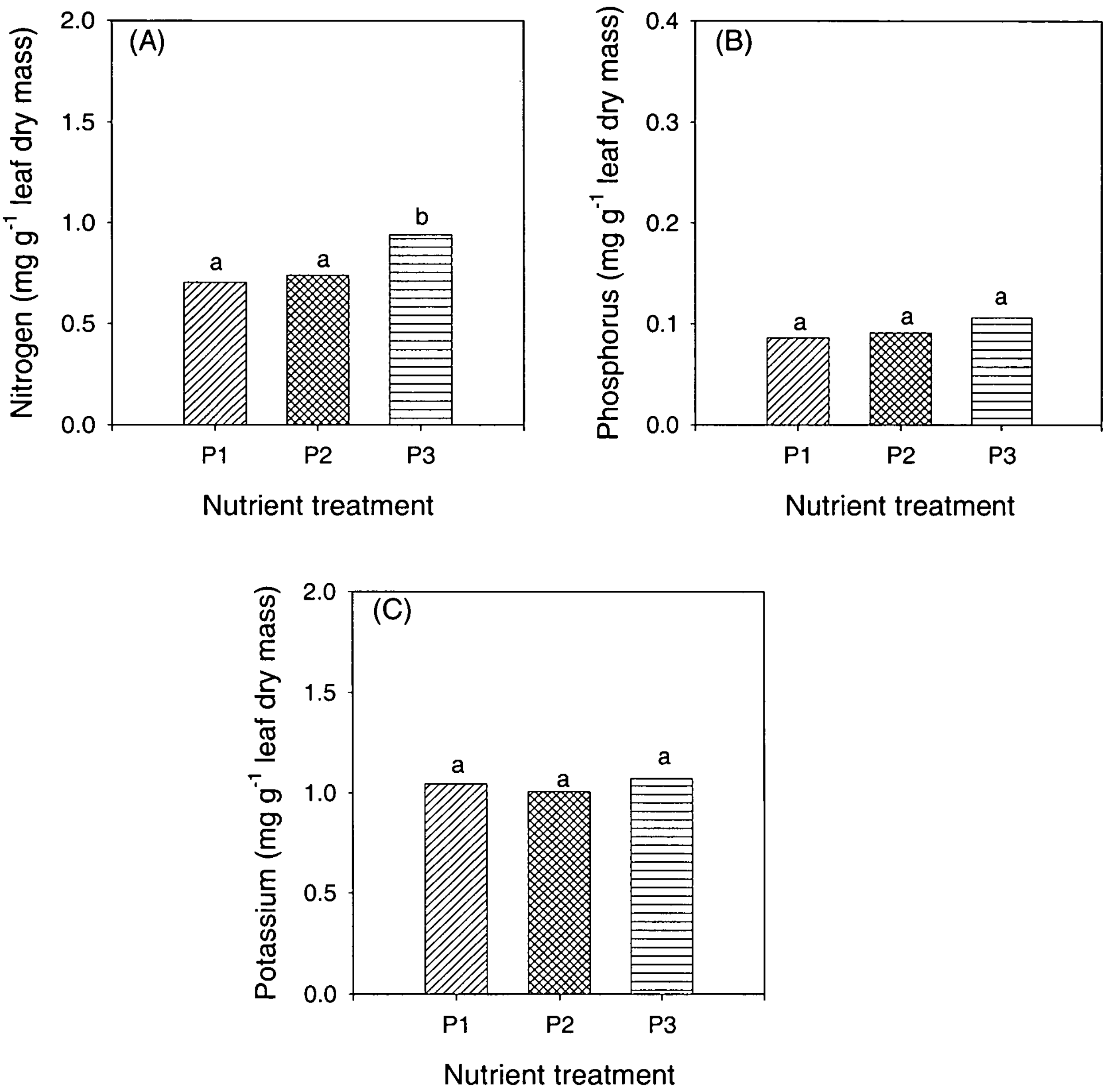


Fig. 5.13: Means of foliar (A) nitrogen, (B) phosphorus, and (C) potassium concentrations of *E. camaldulensis* seedlings at the 14-week harvest in the dual inoculation experiment (experiment 6) under three nutrient regimes. P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; and P3, 10 mg l^{-1} P. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

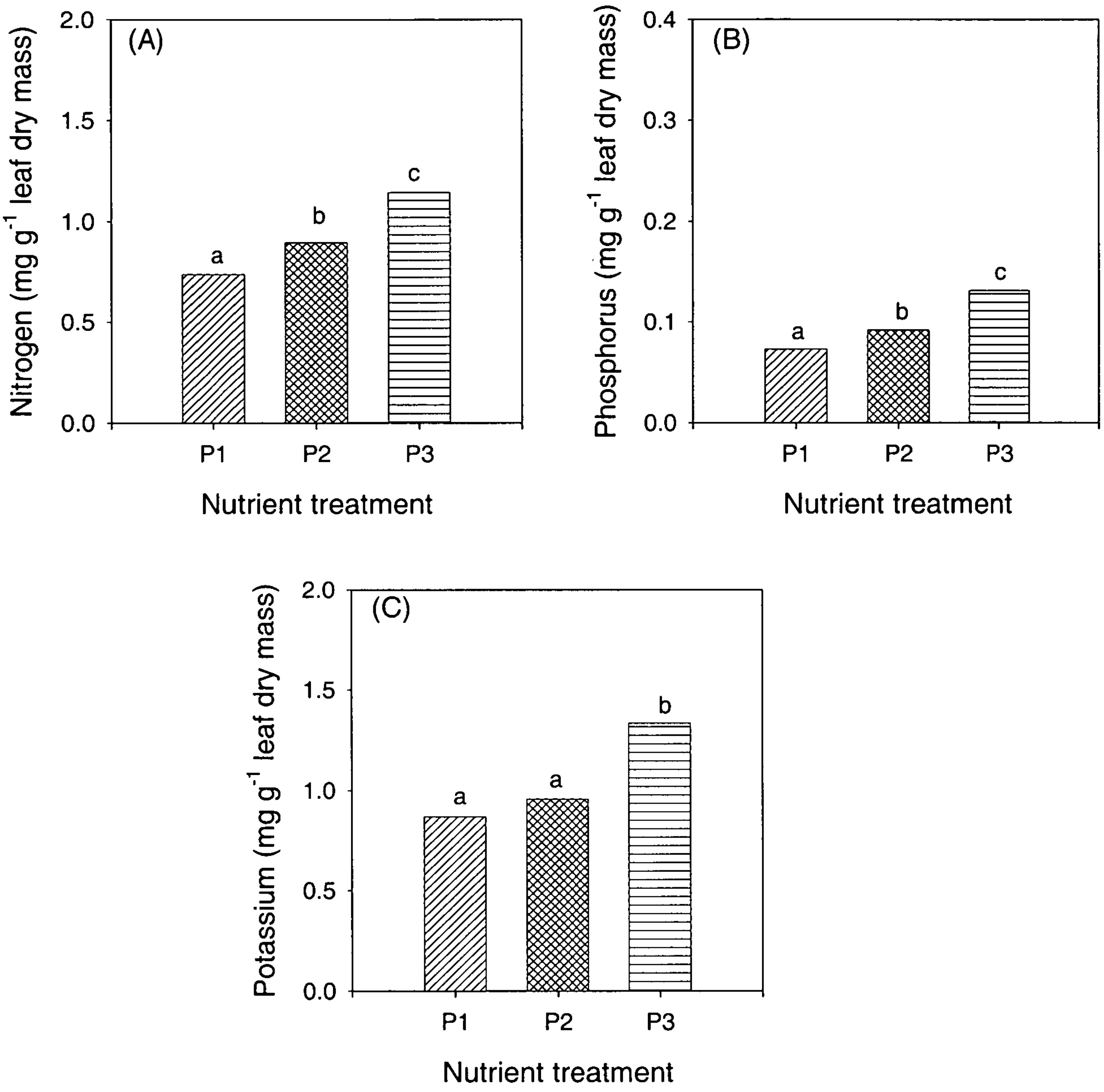


Fig. 5.14: Means of foliar (A) nitrogen, (B) phosphorus, and (C) potassium concentrations of *E. camaldulensis* seedlings at the 24 week-harvest in the dual inoculation experiment (experiment 6) under three nutrient regimes. P1, 0.5 mg l^{-1} P; P2, 2.5 mg l^{-1} P; and P3, 10 mg l^{-1} P. Means with different letters are significantly different at $P < 0.05$ (ANOVA).

5.3 Discussion

Although there was significant variation in AM colonisation of *E. camaldulensis* seedlings at different nutrient regimes at the 14-week harvest (Fig. 5.1), they were not significantly different at the 24-week harvest. There was a significant relationship between the extent of AM colonisation and nutrient concentration (Fig. 5.3) only at the 14-week harvest. This indicated that nutrient regimes where P concentrations were in the range of 0.5 to 10 mg l^{-1} was still within a minimum limit for an adequate rate of AM colonisation. This does not contradict the results found in the earlier experiments where a drop in colonisation rate was observed when nutrient concentration exceeded from 10 to 20 mg l^{-1} P. However, the variation between AM colonisation at different nutrient regimes levelled off at the end of 24 weeks of growth. Seedlings supplied with lower nutrient regimes (0.5 and 2.5 mg l^{-1} P) probably accumulated enough nutrients over time (24 weeks) to support a higher extent of AM colonisation which has resulted from a concurrently higher rate of gain in root biomass (RMR) compared to seedlings supplied with 10 mg l^{-1} P nutrient solution. However, at the highest nutrient concentration (10 mg l^{-1} P), accumulation of nutrients over time perhaps tended to inhibit the extent of AM colonisation. Extent of EM colonisation at the 14-week harvest was very low (average 3% of the root tips) and were found mostly in 2.5 mg l^{-1} P (up to 5%) and 10 mg l^{-1} P (3%) which slightly improved over 24 weeks with 2.5 mg l^{-1} P giving 10 % and 10 mg l^{-1} P, 5%. It was found that the rate of extent of EM colonisation well exceeded that of gain in root biomass over 24 weeks. This has happened probably because of an affinity of the EM inoculant to colonise roots of relatively older seedlings which is in agreement with what Lapeyrie and Chilvers (1985) found in their AM/EM experiment with *E. dumosa*. Observation of a higher EM colonisation in *Eucalyptus* seedlings as they matured in the field has been reported in a number of studies (see Bellei *et al.*, 1992; Gardner and Malacjzuk, 1988).

The AM and dual mycorrhizal colonisation led to a negative growth response at both harvests of the *E. camaldulensis* seedlings (Figs. 5.5, 5.6 and 5.9). At both harvests, the dual inoculation treatment contributed to a significantly higher total dry mass of the seedlings than the AM treatment; however both the AM and the dual inoculation treatments were associated with a significantly lower dry mass compared to the uninoculated control. Both shoot and root dry mass for the dual inoculation treatment were significantly higher than the AM treatment in the 14-week harvest, so that the resulting total dry mass was also higher. Although only root dry mass for the dual inoculation treatment was higher compared to the AM treatment in the 24-week harvest, total dry mass was also higher. This indicated that the dual inoculation may have resulted in a relatively high accumulation of biomass in the below-ground part of the seedlings at the end of 24 weeks of growth compared to that at 14 weeks of growth of seedlings. Similar results were found in the experiments of Amorim and Muchovej (1990) (as cited by Lapeyrie *et al.*, 1992) where seedlings of *E. grandis* were inoculated with a mixed AM-EM inoculum. After two months, EM inoculation stimulated plant growth compared with the uninoculated control and stimulated growth of seedlings co-inoculated with AM fungal isolates compared with the treatment with AM alone. In this experiment, the AM colonisation was around 40% (of the total root length). Although the EM inoculation resulted in a low colonisation (<10% of the root tips), it appeared to have an effect on seedlings in the dual inoculation treatment enabling them to attain more biomass as compared to those in the AM inoculation treatment. It is very difficult to explain here whether the dual inoculation was beneficial or otherwise. EM mantle is known to prevent pathogens and other fungi from entering roots (Zak, 1964; Marx, 1971). It would be tempting to argue that, even with a very limited EM formation, there was a kind of inhibitory effect by the EM fungus on the AM fungus. However, this cannot be explained in terms of colonisation rate by the AM fungus. AM colonisation in the dual inoculation treatment did not differ significantly from that in the AM inoculation treatment. Therefore, an enhanced attainment of biomass in the dual inoculation treatment as compared to the AM treatment may be considered as a positive effect of AM/EM interaction at least relative to the results from the study of Vishwakarma and Singh

(1996b) where dual (AM/EM) inoculation of both *E. camaldulensis* and *E. tereticornis* seedlings resulted in significantly higher biomass compared to either the single inoculation treatments or the uninoculated control.

A preliminary ultrastructural study by Boudarga (1989) (as cited in Lapeyrie *et al.*, 1992) showed that both types of mycorrhiza could be found in an active state, even when they are involved in a dual association. Therefore, both types of mycorrhiza were probably active in the seedlings under the dual inoculation treatment which contributed to an enhanced dry mass. The average rate of colonisation by both AM and EM fungi in this experiment was relatively low as compared to the previous experiments where same fungi were used. Again, this could probably be related to a low availability of light which, on average, was even lower for most of the time (400-500 μmol m^{-2} s^{-1}) in this experiment compared to the previous experiments. However as mentioned elsewhere in this thesis, growth responses in mycorrhizal seedlings do not always correlate to the extent of colonisation by fungi.

RGR of seedlings in the dual inoculation treatment was significantly higher than that in the AM inoculation treatment at both harvests. This conforms to the results obtained in terms of root dry mass and total dry mass above. Again it indicated that dual (fungal) inoculated seedlings were relatively more efficient at assimilating photosynthate. In some studies it has been found that EM fungi replace AM fungi in a succession as seedlings of *Eucalyptus* mature in the field (Lapeyrie and Chilvers, 1985; Lapeyrie *et al.*, 1992). The general explanation is that plants having dual mycorrhizal associations initially form AM because of a relatively low carbon demand by the AM fungi, and that EM take over as plants mature when they are able to support the reputedly higher carbon demand of the EM fungi. Chilvers *et al.* (1987) opined that such a change could be because of the competition for colonisation sites. However, one important difference between the two types of fungi is their secondary inoculum potential. In a dual inoculation experiment with *E. dumosa*, Chilvers *et al.* (1987) observed that as the seedlings grew older, there was relatively little secondary AM colonisation with the absolute increase in length of

AM root being consistent with extension of existing colonisation. By contrast, nearly all of the increase in EM tissue was due to increased number of colonised apices, in which secondary colonisation probably played a major role.

According to a recent study on a tropical tree species, *U. guineensis* by Taylor (1998), whether AM can be replaced by EM or *vice versa* as seedlings grow in the field, depends on the inoculum potential of either type. Therefore, depending on the availability of inoculum of both AM and EM in the growth medium, both types of mycorrhiza can remain active. Occurrence of either AM or EM may also depend on soil nutrient condition, for example, AM can be found more in mineral- or nutrient-rich soils, while EM can be found in soils rich in organic matter (Smith and Read, 1997).

Although NAR for the dual inoculation treatment was significantly higher compared to the AM inoculation treatment at the 14-week harvest (Table 5.3), they did not vary significantly at the 24-week harvest (Table 5.4). However, RGR between the two treatments varied significantly irrespective of the harvests with the dual inoculation treatment contributing to significantly higher RGR compared to the AM inoculation treatment. As seedlings grew from 14 weeks to 24 weeks, there was an increase in the extent of EM formation. Part of the photosynthetic assimilates from leaves could thus have been drawn to roots to support EM fungal expansion which reduced NAR of the dual inoculated seedlings compared to the AM inoculated seedlings at the 24-week harvest. Despite this, RGR in the dual inoculation treatment was significantly higher than that in the AM treatment. Interestingly, SLA of seedlings between these two inoculation treatments did not differ significantly at any harvest.

Increased RGR with an increasing nutrient concentration indicated that different nutrient treatments had a discernible effect on seedling growth. However, variation in NAR and LAR of seedlings at both harvests did not follow a similar trend. Again this indicated that even though NAR and LAR in different nutrient treatments may not be significantly different from each other, their functional product, RGR were significantly different in different nutrient treatments.

Although total leaf N and P contents in AM and dual inoculated seedlings did not differ significantly from the EM or the uninoculated control at 24 weeks, these treatments resulted in a higher foliar concentration of N and P at both harvests. This is also supported by the occurrence of a relatively high SLA and LAR of seedlings in these inoculation treatments (Table 5.3 and Table 5.4). Despite a higher SLA and LAR, NAR in those treatments were significantly reduced. This indicated that a major part of the photosynthate was drawn below ground by the fungi (which resulted in a reduced NAR), and was used for their growth and maintenance. This evidence probably consolidates the fact that there was a net carbon drain on the seedlings in these inoculation treatments.

CHAPTER 6

Structures of Arbuscular- and Ecto-mycorrhizas in *Eucalyptus camaldulensis*

CHAPTER 6

Structures of Arbuscular- and Ecto-mycorrhizas in *Eucalyptus camaldulensis*

6.0 Introduction

Colonisation of host root tissues by an AM fungus follows a morphologically well defined series of events which begins with the formation on the root surface of an appressorium, from which the hypha penetrates the root tissue, and culminates in the intense proliferation of mycelium (inter- and intra-radical hyphae, coiled hyphae) in the cortical parenchyma and the differentiation of specialised, highly branched arbuscules (Gianinazzi-Pearson *et al.*, 1996). In contrast, typical defence responses are associated with incompatibility in plant genotypes resistant to AM fungi and non-mycorrhizal plants, in which root interactions and fungal development are arrested at the stage of appressorium formation on the root surface (Gianinazzi-Pearson *et al.*, 1996). Aborted colonisation by AM fungi on non-mycorrhizal pea roots (Duc *et al.*, 1989) is associated with abnormally thick wall appositions in epidermal and hypodermal cell walls adjacent to appressoria (Gianinazzi-Pearson *et al.*, 1991). The wall thickenings have a heterogeneous structure, stain strongly with toluidine blue and autofluoresce, indicating phenolic accumulation (Gollotte *et al.*, 1993).

Morphological aspects of EM roots have been studied in a number of plant-fungus combinations and recently reviewed by Smith and Read (1997). Morphogenesis of ectomycorrhiza follows a complex sequence. The precolonisation stage involves hyphal growth and branching close to the host followed by adhesion of hyphal branches to the root surface (Tagu and Martin, 1996). Major morphogenetic events for the fungal symbiont are: (1) the aggregation of hyphae to produce a tissue-like structure (the mantle) and (2) a labyrinthine growth of hyphae between the epidermal or cortical cells to produce the Hartig net (Bonfante *et al.*, 1998). The initial changes in the host plant include: (1) stimulation of lateral root formation by fungal auxins, (2) radial elongation of epidermal cells and (3) arrest of cell divisions once the mantle has enclosed the root (Peterson and Bonfante, 1994). Fully developed EM formation between *Pisolithus tinctorius* and *Eucalyptus* spp. (*E. globulus* and *E. grandis*) is described by Horan *et al.* (1988) as one having a dense mantle, a blunt apex, a reduced root cap and meristem, vascular differentiation close to the apex and radially elongated epidermal cells with a Hartig net of intercellular hyphae. In a few studies, the extent of mantle and Hartig net development in EM has been found to be dependent on environmental conditions [for example, temperature (Marx *et al.*, 1970), pH (Metzler and Oberwinkler, 1987)], plant genotype and maturity (Tonkin *et al.*, 1989), the ecotypes of a given fungal species (Malajczuk *et al.*, 1990) and fungal variants (Wong *et al.*, 1989). High N concentration is reported to affect Hartig net development in EM (Brunner and Scheidegger, 1994).

In the experiments reported in this thesis, AM colonisation has resulted in a negative growth response for *E. camaldulensis* seedlings. A low degree of EM colonisation of seedlings has also been observed with no associated growth response. The aim of the investigation was to assess the AM and EM roots in order to determine whether mycorrhiza formation and structures present were typical of a normal symbiosis.

The objective of this investigation, therefore, was to describe formation and development of the AM and EM structures found in *E. camaldulensis* roots using light microscopy. Seedlings of *Eucalyptus globulus* Labill. were also inoculated with the same EM inoculant (*P. tinctorius* K55) for comparative purposes.

6.1 Methodology

6.1.1 *Materials*

6.1.1.1 *Arbuscular mycorrhiza*

Roots were stained with trypan blue as described in section 2.10 and examined for evidence of structures associated with AM colonisation. Stained root samples were removed in September 1997 from experiment 4 (14-week old samples) and in October 1998 from experiment 6 (24-week old samples). In experiment 4, roots were sampled from three inoculation treatments (*Glomus clarum* BR148-1, *Gigaspora rosea* FL105.5 and a mixed AM culture from Bangladesh) and four nutrient treatments (Ingestad's 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P, and 20 mg l^{-1} P), giving a total of 12 treatment combinations. Roots were sampled at random from pots in three replicate blocks (that is, a total of 36 pots). In experiment 6, roots were sampled from two inoculation treatments (*G. clarum* BR148-1 alone and *G. clarum* BR148-1 with *P. tinctorius* K55), and three nutrient treatments (Ingestad's 0.5 mg l^{-1} P, 2.5 mg l^{-1} P, and 10 mg l^{-1} P), giving six treatment combinations. Roots were sampled at random from pots in three replicate blocks (that is, a total of 18 pots).

6.1.1.2 *Ectomycorrhiza*

Root samples were removed in October 1997 from experiment 5 (18-week old samples) and in October 1998 from experiment 6 (24-week old samples). In experiment 5, roots were sampled from one inoculation treatment (*Pisolithus tinctorius* isolate K55, because other isolates of *P. tinctorius* did not result in significant colonisation; <1% of the root tips being colonised) and four nutrient treatments (Ingestad's 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P, and 20 mg l^{-1} P), thus giving four treatment combinations. Roots were sampled at random from pots in three replicate blocks (that is, a total of 12 pots). In experiment 6, roots were sampled from two inoculation treatments (*P. tinctorius* K55 alone and *P. tinctorius* K55 with *G. clarum* BR 148-1) and three nutrient treatments (Ingestad's 0.5 mg l^{-1} P, 2.5 mg l^{-1} P, and 10 mg l^{-1} P), thus giving six treatment combinations. Roots were sampled at random from pots in three replicate blocks (that is, a total of 18 pots).

Also included for comparative purposes were seedlings of *E. globulus*, inoculated at the same time and grown alongside experiments 5 and 6. Root samples were removed in October 1997 (18-week old samples) and in October 1998 (24-week old samples). In 1997, seedlings were inoculated with the same isolate (*P. tinctorius* isolate K55) and grown under two nutrient regimes (Ingestad's 2.5 mg l^{-1} P, 5.0 mg l^{-1} P), thus giving two treatment combinations. Roots were sampled at random from pots in five replicate blocks (that is, a total of 10 pots). In 1998, seedlings were inoculated with the same isolate (*P. tinctorius* isolate K55) and grown under two nutrient regimes (Ingestad's 0.5 mg l^{-1} P, 2.5 mg l^{-1} P), thus giving two treatment combinations. Roots were sampled at random from five replicate blocks (that is, a total of 10 pots). It is to be noted here that, *E. globulus* seedlings inoculated with this isolate resulted in 35-40 % colonisation of the root tips in 1997 and 20-25% in 1998.

As another isolate of *P. tinctorius* (isolated from under a *E. camaldulensis* plantation in Vietnam) was obtained later in 1997, two replicate blocks of *E. camaldulensis* seedlings inoculated with this isolate and grown in the four nutrient regimes (Ingestad's 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P, and 20 mg l^{-1} P) were also set up one week after the beginning of experiment 5. The seedlings were harvested one week later than the experiment 5 so that they were of the same age as those in experiment 5 (that is, 18 weeks) at the time of harvest. Roots were sampled at random from pots in two replicate blocks (that is, a total of 8 pots). It is to be noted here that, *E. camaldulensis* seedlings inoculated with this isolate resulted in only 5-10 % colonisation of the root tips. Because of the low colonisation root samples from these seedlings were included in this study.

In all root sampling, non-mycorrhizal root tips of both *E. camaldulensis* and *E. globulus* were included in the samples for comparative purposes. All samples were preserved in 2% glutaraldehyde and stored at 4°C.

6.1.2 *Processing for Microscopy*

6.1.2.1 *Arbuscular mycorrhiza*

After staining, roots were mounted in polyvinyl alcohol lacto-glycerol (PVLG) (Koske and Tessier, 1983) on slides and squashed under coverslips to enable observation of AM structures under a compound microscope.

6.1.2.2 *Ectomycorrhiza*

Longitudinal and cross sections of root tips were removed using a base sledge microtome with a Peltier cooled freezing stage (Mectron Instruments Ltd.). This technique was useful because it is a rapid method of preparing sections for diagnostic purposes and it enables the observation of fats, lipids, and other tissue components (Culling, 1974). The sections were mounted on slides in PVLG under coverslips (without squashing them) for observation under a compound microscope.

6.1.3 *Microscopy and Imaging*

All root samples were first observed using a Wild M5 stereo dissecting microscope (x5 to x50 magnification). Root squashes and sections were observed using an Olympus BH2 compound microscope (x125 to x500 magnification). Materials were observed under normal brightfield illumination or Differential Interference Contrast (DIC). In 1997, colour images for AM and black and white images for EM were recorded with a 35 mm automatic photo system camera mounted on the compound microscope. In 1998, colour images for both AM and EM were captured with a Sony DXC151 video camera and transferred to the computer as electronic files using the software MGI Videowave (Version 5). Samples from the 1997 experiments were also included for video imaging in 1998.

6.2 Results

6.2.1 *AM Colonisation Structure*

All three AM inocula tested in experiments 4 and 6 appeared to have produced mycorrhizal structures in the host roots typical of normal mycorrhizas. The nutrient treatments did not appear to have any effect on the kind of structures produced in either experiment although they had significant effects on the extent of colonisation (see chapters 4 and 5).

Plate 6.1 shows the intensive colonisation of the roots of *E. camaldulensis* seedlings by *Gigaspora rosea* isolate FL105-5 in the form of arbuscular and hyphal colonisation. This fungus produced a lot of appressoria-like structures (Plate 6.1b) on the root surface. Intracellular colonisation by *G. rosea* FL105-5 was characterised by intensive colonisation with coiled wide-diameter hyphae (Plate 6.1c) and arbuscules (Plate 6.1c,d). Lipid globules accumulated in intraradical hyphae (Plate 6.1d) indicating the presence of active fungal metabolism in the symbiosis. All these structures (appressoria, coiled hyphae and arbuscules) are recognised as the basic functional organs in the symbiosis.

There was also intensive colonisation of the roots of *E. camaldulensis* seedlings by *Glomus clarum* isolate BR148-1 in the form of intraradical spores, vesicles, arbuscules and hyphae (Plate 6.2). This fungus was characterised by the presence of intraradical spores and vesicles (Plate 6.2a,c). This fungus also produced a large amount of external hyphae (Plate 6.2b) compared to the other inocula. Intracellular hyphae and arbuscules were also present (Plate 6.2d), and again, these structures are recognised as the basic functional organs in the symbiosis.

As might have been expected, mycorrhizal structures formed by the mixed AM culture from Bangladesh were varied (Plate 6.3). Some roots possessed intraradical spores (Plate 6.3a). These intraradical spores were probably formed by *Glomus manihotis* as spore extracts of the soil from the mixed AM culture from Bangladesh contained large numbers of *G. manihotis* spores (see section 3.4 in the chapter 3). Poorly stained, irregularly shaped, thin-walled vesicles (Plate 6.3c) are thought to be characteristic of some *Acaulospora* spp. (Brundrett *et al.*, 1996c). In most roots, extensive formation of intracellular hyphae and arbuscules was seen in the mycorrhiza formed by this mixed culture (Plate 6.3b-d). Generally, the mycorrhizal colonisation observed varied from one root fragment to another and it can be assumed that several different fungi were colonising the roots.

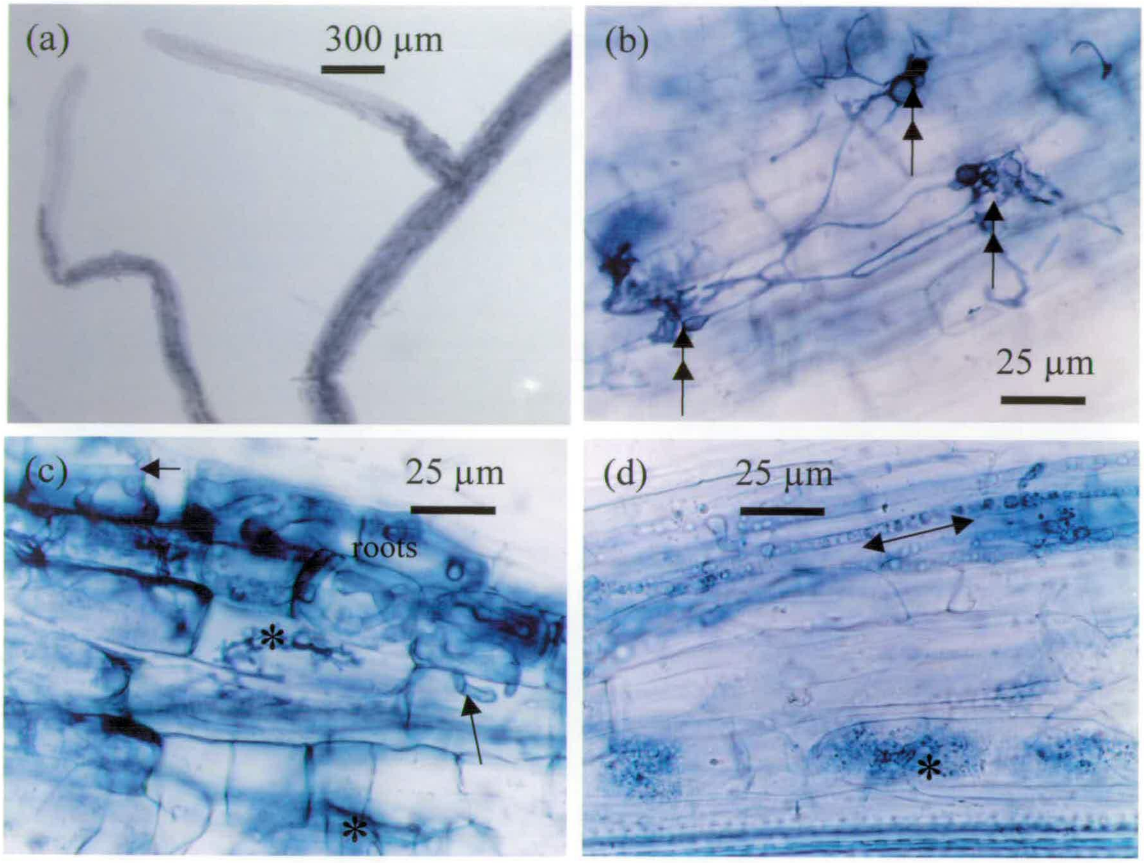


Plate 6.1: Roots of *E. camaldulensis* seedlings colonised by *G. rosea* FL105-5: (**a**) low power view showing intensive colonisation by coiled hyphae and arbuscules - note that colonisation has not yet occurred in the root tips; (**b**) appressoria formation on the root surface(—▶▶); (**c**) coiled hyphae (—▶) in outer cortical cells and arbuscules (*) in inner cortical cells; (**d**) formation of lipid globules in hyphae (↔) and arbuscules (*).

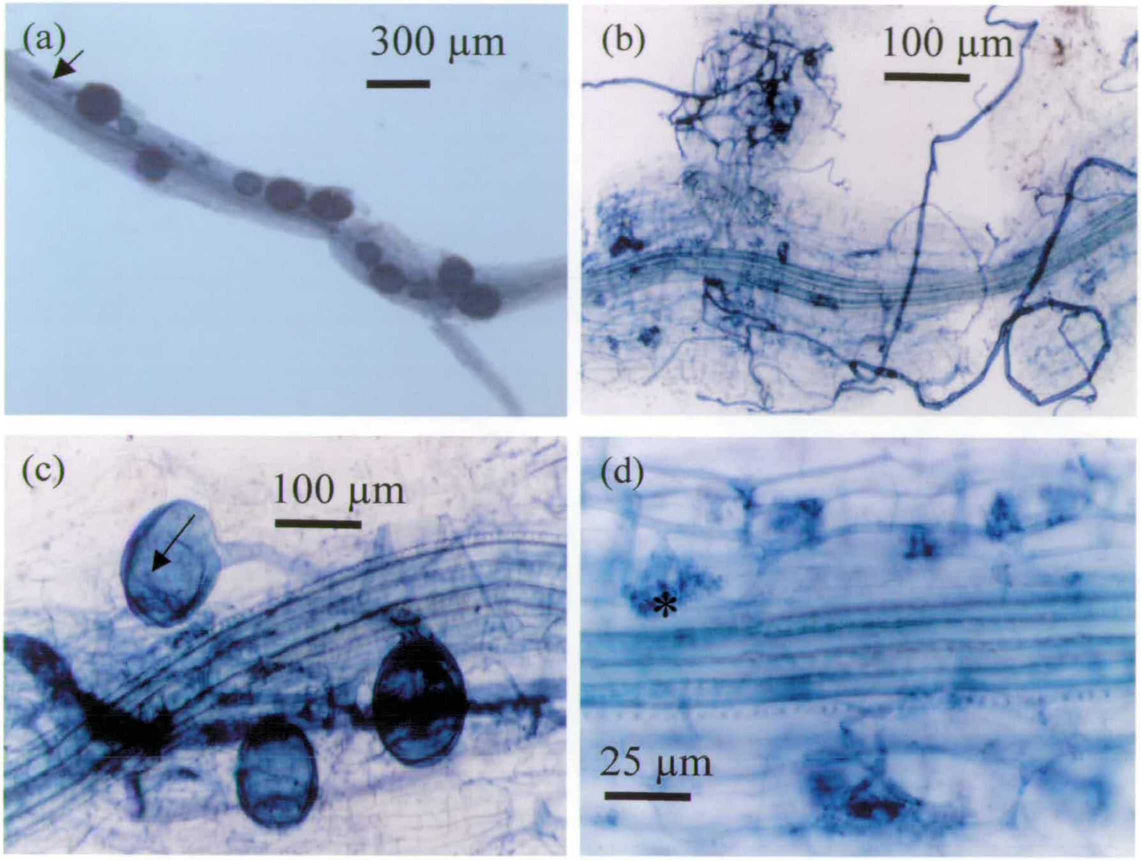


Plate 6.2: Roots of *E. camaldulensis* seedlings colonised by *G. clarum* BR148-1: (**a**) low power view showing dense colonisation by intraradical spores and vesicles (⟶); (**b**) profusion of external hyphae; (**c**) intraradical spores, vesicles and internal hyphae; and (**d**) arbuscules (*) and intracellular hyphae.

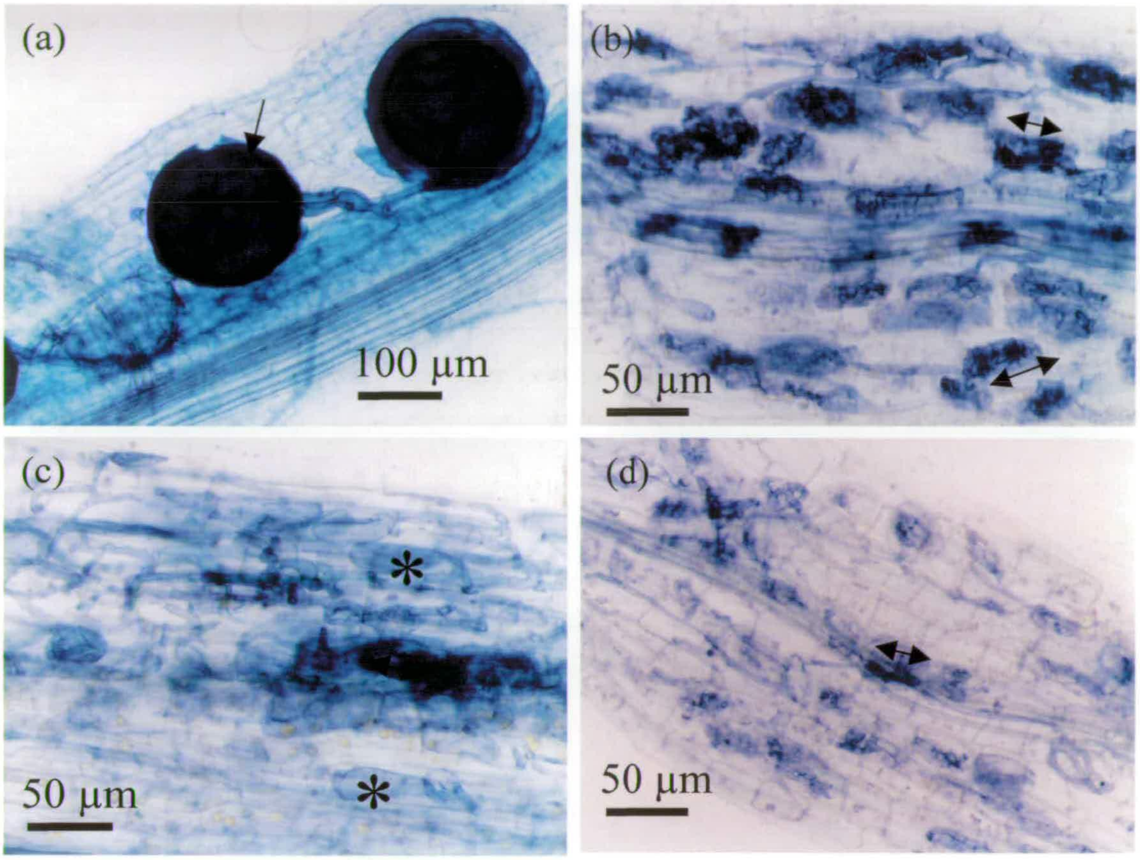


Plate 6.3: Roots of *E. camaldulensis* seedlings colonised by the mixed AM culture from Bangladesh: (**a**) intraradical spores (⟶) possibly formed by *Glomus manihotis*; (**b**) and (**c**) arbuscules (⟷); and poorly stained, irregularly shaped, thin-walled vesicles (*) probably formed by *Acaulospora* spp.; (**d**) inter- and intra-cellular hyphal network forming a distinct pattern of colonisation.

6.2.2 *EM Colonisation Structure*

Although the nutrient treatments had significant effects on the extent of colonisation (see chapters 4 and 5) they did not appear to have any effect on the kind of EM structures produced in either experiment.

As reported in chapter 4, EM colonisation of *E. camaldulensis* only occurred with isolate *P. tinctorius* K55. Initial observation of these mycorrhizas under the stereo microscope indicated that many of the mycorrhizal root tips were not covered by a fungal mantle (Plate 6.4c). In contrast, mycorrhizas formed by the isolate *P. tinctorius* K55 with *E. globulus* seedlings (set up at the same time) were found to be fully enveloped by a fungal mantle (Plate 6.4a). This absence of a mantle on some *E. camaldulensis* mycorrhizas was confirmed by examining longitudinal sections of such roots under a compound microscope (Plate 6.4d) and comparing them with longitudinal sections of *E. globulus* mycorrhizas (Plate 6.4b).

Further examination of longitudinal and cross sections of mycorrhizal root tips from the 1997 experiment showed that EM colonisation in *E. globulus* seedlings led to an occurrence of a typical ectomycorrhiza with the presence of a mantle, a fully developed paraepidermal Hartig net and elongated epidermal cells (Plate 6.4b and Plate 6.5e,f). It should also be noted that the labyrinthine branching of the Hartig net could be observed where a plan view of the Hartig net was seen (Plate 6.5e,f). EM colonisation in *E. camaldulensis* seedlings was characterised by a mantle, but with a poorly developed Hartig net, little elongation of the epidermal cells and the presence of many globules or vacuoles in the epidermal cells (Plate 6.4d and Plate 6.5a,b).

Longitudinal and cross sections were also examined of *E. camaldulensis* seedlings inoculated with *P. tinctorius* Vietnam isolate in 1997. Unlike *E. camaldulensis-P. tinctorius* K55 mycorrhizas, fewer root tips were found to have grown out of the fungal mantle. However, like *E. camaldulensis-P. tinctorius* K55 mycorrhizas, these mycorrhizas also showed a poorly developed Hartig net, little elongation of

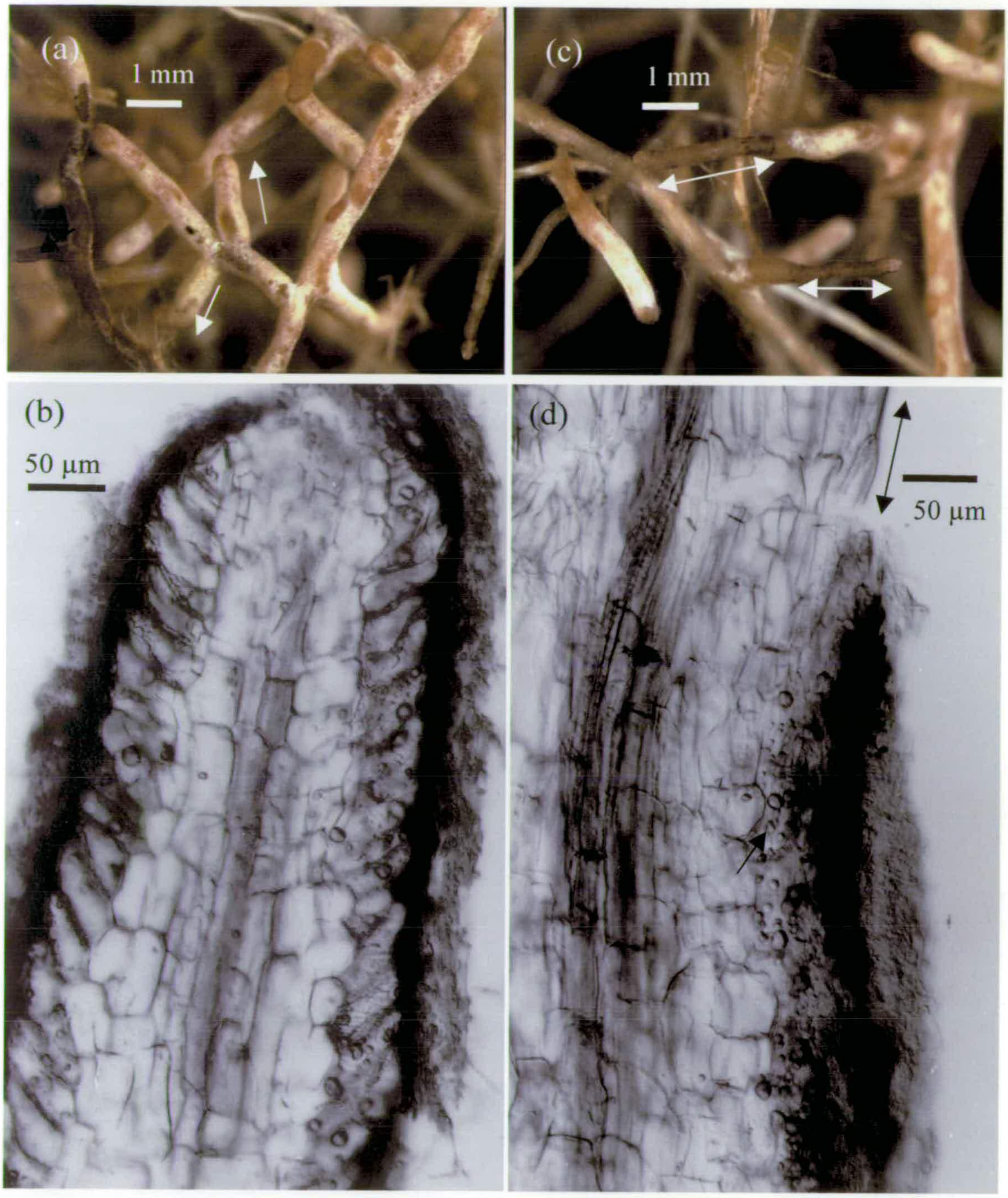


Plate 6.4: Comparison of EM of *E. camaldulensis* and *E. globulus* formed with *P. tinctorius* isolate K55: (**a**) low power view of *E. globulus* mycorrhiza with mantle enveloping root tips, and (**b**) a longitudinal section of one *E. globulus* root tip showing mantle and Hartig net development and some globules in epidermal cells; and (**c**) low power view of *E. camaldulensis* mycorrhizas showing root tips which have grown out from the mantle (↔), and (**d**) a longitudinal section of one *E. camaldulensis* root tip showing absence of mantle towards root tip (↔), poor development of Hartig net and profusion of globules in epidermal cells (⟶).

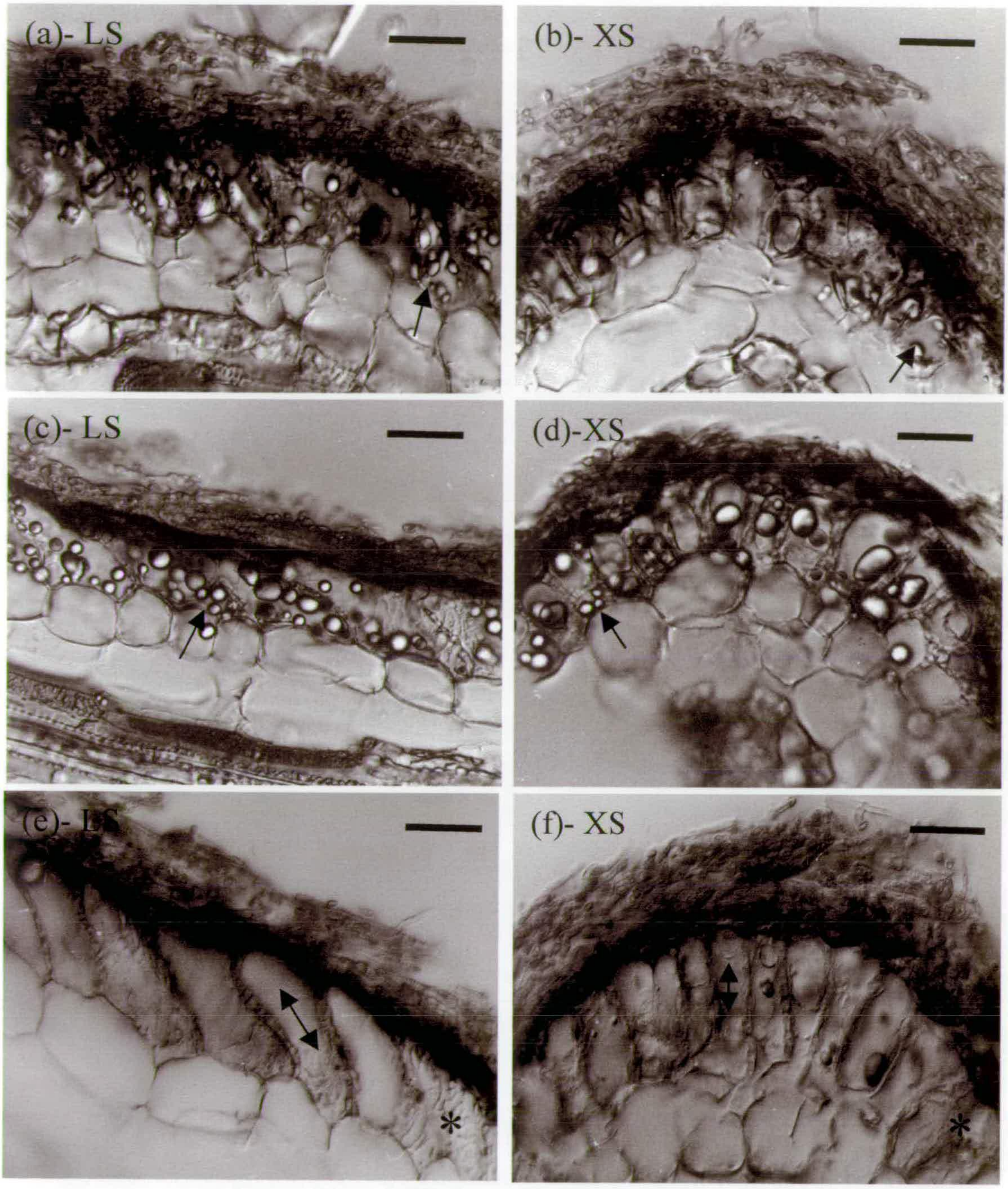


Plate 6.5: (**a**) Longitudinal section and (**b**) cross section of *E. camaldulensis* roots inoculated with *P. tinctorius* strain K55- both sections showing profusion of globules in epidermal cells and poor elongation of epidermal cells (⟶); (**c**) longitudinal section and (**d**) cross section of *E. camaldulensis* roots inoculated with *P. tinctorius* Vietnam isolate- both sections showing profusion of globules in epidermal cells and poor elongation of epidermal cells (⟶); and (**e**) longitudinal section and (**f**) cross section of *E. globulus* seedlings inoculated with *P. tinctorius* isolate K55- both sections showing few globules in epidermal cells and well-developed Hartig net with elongation of epidermal cells (⟷) and plan view of labyrinthine branching (*). Scale bars = 25 μm.

epidermal cells and the presence of many globules in the epidermal cells (Plate 6.5c,d).

In 1998, mycorrhizal root tips of *E. globulus* and *E. camaldulensis* seedlings from the experiment in 1997 were re-examined using longitudinal and cross sections in order to confirm the presence of structures and also to obtain colour images of the globules and other cell depositions. Examination of *E. globulus* (Plate 6.6e,f) and *E. camaldulensis* (Plate 6.6a,b) mycorrhizas formed by *P. tinctorius* isolate K55, and of *E. camaldulensis* (Plate 6.6c,d) mycorrhizas formed by *P. tinctorius* Vietnam isolate confirmed the observations made previously for them. The colour imaging also showed the pink colour of the globules in the epidermal cells and the brown depositions found in the lower mantle layers.

In 1998, *E. camaldulensis* was again inoculated with *P. tinctorius* isolate K55 in experiment 6, and at the same time *E. globulus* was inoculated with the same isolate. In this case, however, the seedlings were grown for 24 weeks as compared to the seedlings from 1997 which were grown for 18 weeks.

The 24-week-old samples also showed the presence of globules, but they were often larger and more deeply pink-coloured tending to fill the entire epidermal cell (Plate 6.7b,c). Root sections from *E. globulus* inoculated seedlings again showed the features of a typical ectomycorrhiza with good Hartig net development and elongation of epidermal cells with very few pink-coloured globules (Plate 6.7d,e,f). Although brown deposits were found in the lower mantle of most of the mycorrhizas examined during these studies, these deposits were particularly pronounced in the samples from the 24-week-old *E. camaldulensis* seedlings (Plate 6.7b,c).

Samples collected from the dual (AM+EM) inoculation treatment in experiment 6 did not provide any root fragment or root tip where both AM and EM structures could be observed.

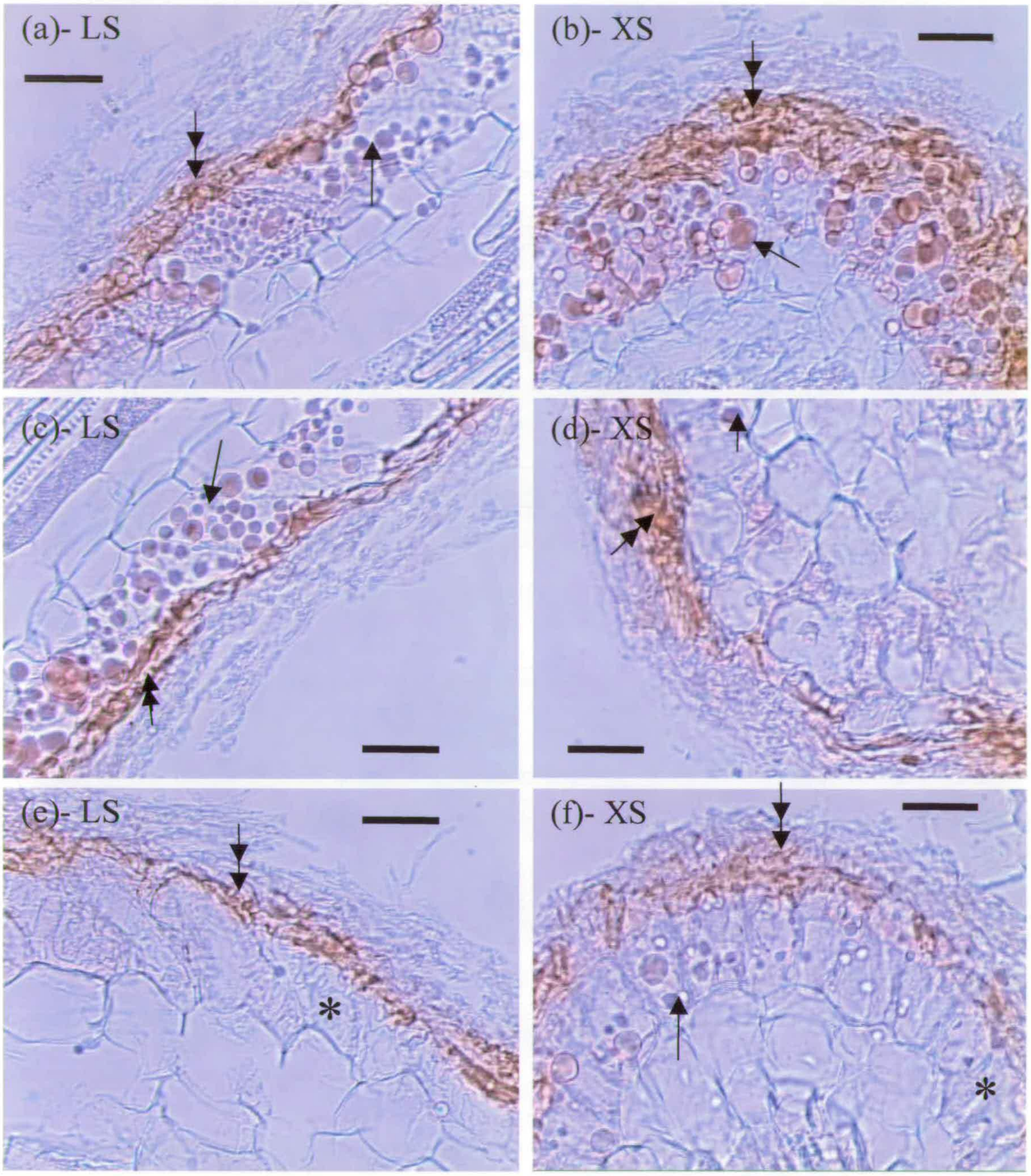


Plate 6.6: (**a**) Longitudinal section and (**b**) cross section of *E. camaldulensis* seedlings inoculated with *P. tinctorius* strain K55 showing pink globules in the epidermal cells (→) and brown deposits in lower mantle (↠); (**c**) longitudinal section and (**d**) cross section of *E. camaldulensis* inoculated with *P. tinctorius* Vietnam isolate showing pink globules in the epidermal cells (→) and brown deposits in lower mantle (↠); and (**e**) longitudinal section and (**f**) cross section of *E. globulus* seedlings inoculated with *P. tinctorius* strain K55 where very few globules (→) and some brown deposits (↠) in the mantle are present but the Hartig net is showing (plan view) labyrinthine branching (*). All seedlings were 18 weeks old. Scale bars = 25 μm.

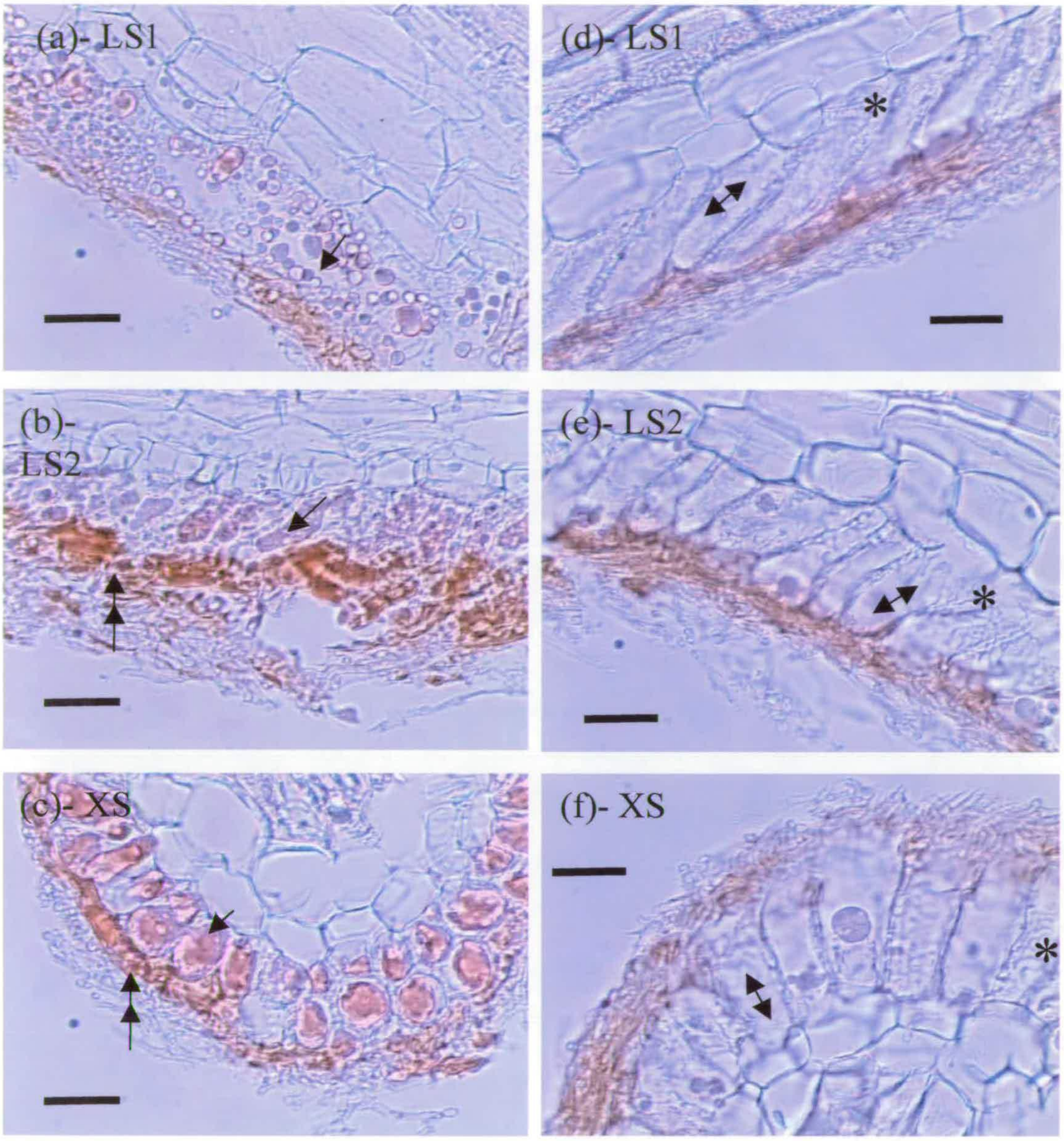


Plate 6.7**:** (**a-c**) Sections of roots of *E. camaldulensis* seedlings inoculated with *P. tinctorius* isolate K55, showing (**a**) pink-coloured globules in young mycorrhiza (→); (**b**) and (**c**) pink-coloured globules occupying the whole epidermal cell (→) and brown deposits in lower mantle in older mycorrhizas (→→). (**d-f**) Sections of roots of *E. globulus* showing development of Hartig net, elongation of epidermal cells (↔) and labyrinthine branching (*) with few globules and less brown deposition in lower mantle. All seedlings were 24 weeks old. Scale bars = 25 μm.

Samples of non-mycorrhizal roots of both *E. camaldulensis* and *E. globulus* seedlings were also examined using longitudinal and cross sections (Plate 6.8). These sections showed that epidermal cells were not elongated, contained no globules but that some possessed brown coloration.

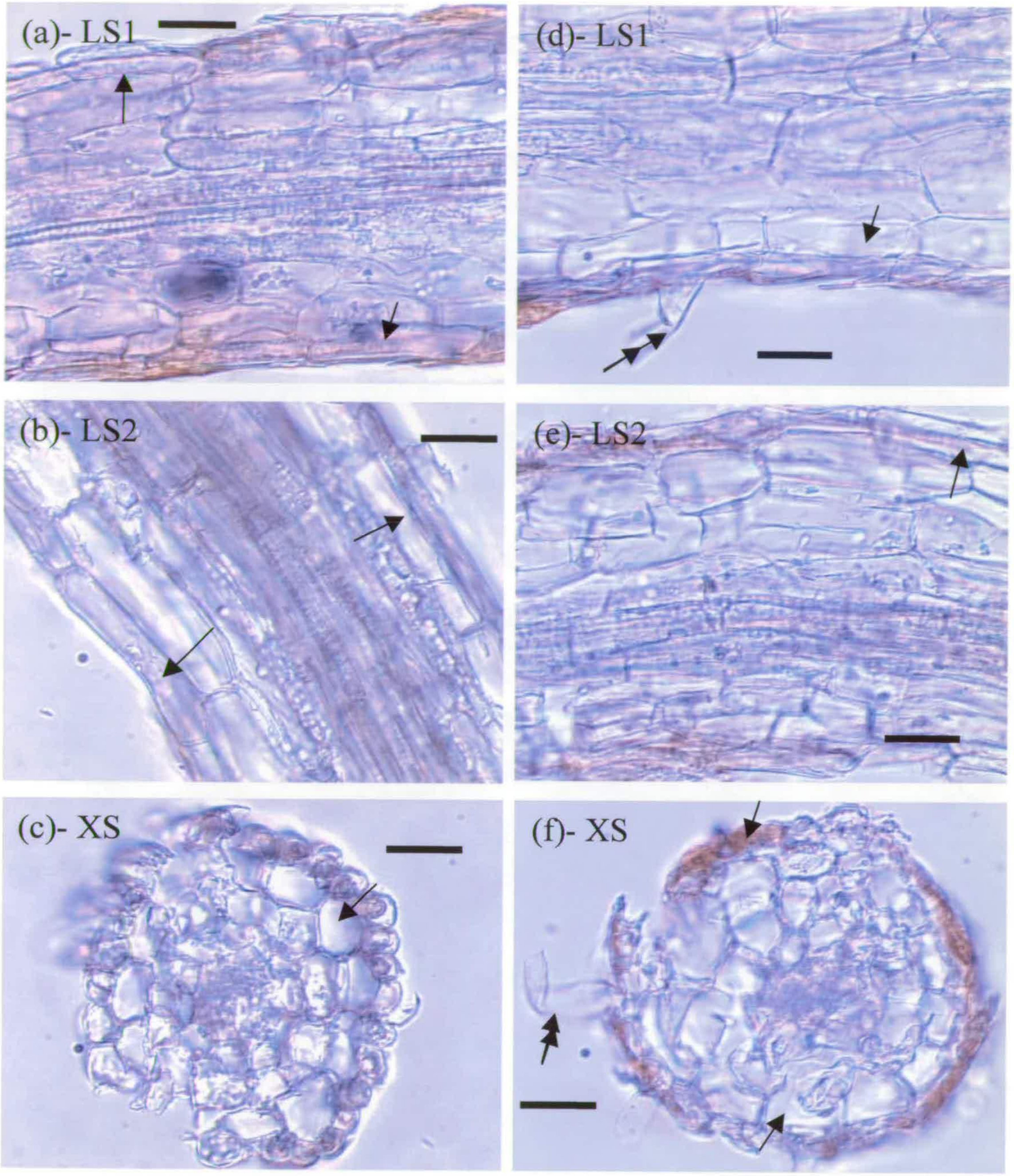


Plate 6.8: Non-mycorrhizal root sections of **(a-c)** *E. camaldulensis* and **(d-f)** *E. globulus*. Epidermal cells (→) are neither disrupted nor elongated and contain no globules, but some brown coloration is present. Root hairs (→→) can be seen on *E. globulus* root sections (Plate 6.8d,f). Scale bars = 25 μm.

6.3 Discussion

6.3.1 *AM Colonisation Structure*

For the establishment of symbiosis, the key events in the life cycle of AM fungi are spore germination and the presymbiotic mycelial growth phase, differential hyphal branching, appressoria formation, root colonisation and arbuscule development (Giovannetti *et al.*, 1994). This sequence does not occur when AM fungi are challenged with non-host roots and thus the life cycle of these obligate symbionts is interrupted (Giovannetti and Sbrana, 1998).

A typical AM fungus usually penetrates a host root through formation of appressoria. Appressoria are morphologically identified as flattened, elliptical hyphal tips that form on the surface of host roots (Garriock *et al.*, 1989). The formation of appressoria is the most significant sign of fungal recognition of a potential host plant (Staples and Macko, 1980). In experiments of this study, appressoria formation accompanied by differential hyphal branching was observed in *G. rosea* FL105-5 inoculated seedlings. This differential branching of hyphae is reported to occur when AM fungi approach host root exudates (Nagahashi *et al*, 1996). Giovannetti *et al.* (1994) suggested that hyphal branching and appressoria formation are the two related morphogenetic events in the symbiosis. Appressoria are formed by AM fungi after recognition of host signals at the root surface, regardless of the outcome of the interaction, that is, whether or not mycorrhizal association will benefit the plant (Giovannetti and Sbrana, 1998). In experiments of this study, no positive growth response due to inoculation could be obtained, and in fact there was a negative growth response (see Chapters 3, 4 and 5).

Typical appressoria formation by the other two inocula was not observed, but the abundant structures of coiled and septate hyphae along with arbuscule formation in the roots colonised by those inocula suggests that appressoria formation had indeed occurred at the beginning of the colonisation process. The process of appressorium differentiation may result in a successive enlargement of hyphal tips separated by frequent septa which may show up as reminiscent of appressoria (Giovannetti *et al.*, 1993). So the septate hyphae observed in root samples from seedlings inoculated with *G. clarum* BR148-1 and Bangladesh culture may be the remnants of appressoria formed early in the colonisation process. In fact differentiation of appressoria along with hyphal branching is followed rapidly (as early as 48 h after the beginning of the interaction) by the root penetration, colonisation and arbuscule formation (Giovannetti and Citernesi, 1993; Giovannetti, 1997).

After the differentiation of appressoria, AM fungi usually colonise host roots by forming inter- and intra-cellular hyphae and intracellular arbuscules (Giovannetti and Sbrana, 1998). Anderson (1992) suggested that, following hyphal penetration of the epidermal cells, the AM fungus first forms coiled hyphae in the outer cortical cells. Later, highly branched arbuscular structures are formed in the inner cortical cells. These two structures are considered as indicators of a successful AM colonisation (Anderson, 1992; Horton *et al.*, 1998). In my experiments both of these structures can be seen in the mycorrhiza formed by the three inocula. But these structures were observed in only some of the roots, probably because they are ephemeral and can be difficult to observe (Horton *et al.*, 1998).

Presence of external hyphae and external and internal spores (in some mycorrhiza) are all typical of a normal AM (Harley and Smith, 1983). In my experiments, *G. clarum* BR148-1 produced large external and internal spores and vesicles along with larger amounts of external and internal hyphae, while *G. rosea* FL105-5 had both external and internal hyphae but no vesicles. These features are characteristic of root colonisation made by these fungi (Brundrett *et al.*, 1996c). No spores were observed in association with roots of *G. rosea* FL105-5 inoculated seedlings. The completion of life cycle, up to the formation of spores, takes longer for Gigasporaceae than for species of *Glomus* (Boddington and Dodd, 1999). The experimental period in this case (14 weeks) was probably not long enough for *G. rosea* FL105-5 to produce its spores.

So the structures, for example, appressoria, coiled hyphae and arbuscules, produced in experiments of this study by the three inoculant fungi, indicate that all three produced mycorrhizas that were typical of normal AM association.

6.3.2 *EM Colonisation Structure*

When an EM fungus develops a mantle, the hyphae stop growing in bundles or as isolated hyphae and organises a more complicated structure (Bonfante-Fasolo and Scannerini, 1992). However, the structure and morphology of the mantle is largely determined by the fungal species independent of host species (Godbout and Fortin, 1985; Ingleby *et al.*, 1990). Generally, mantles formed by *P. tinctorius* are poorly differentiated with loose hyphae forming the outer mantle which gradually become compacted in the inner mantle (Rose *et al.*, 1981; Massicotte, 1988; Weiss, 1992).

Burgess *et al.* (1996) observed a thickened mantle divided into two layers in their study of compatible *E. grandis-P. tinctorius* mycorrhizas. In an earlier study (1994) they found that less aggressive *P. tinctorius* colonisers of *Eucalyptus* tended to form a superficial association with a loose mantle. Loose mantles were also observed by Jones *et al.* (1998) in their study of *Laccaria bicolor-E. coccifera* mycorrhizas. In this study, differences in mantle thickness or the presence of layering were not observed on mycorrhizas of *E. camaldulensis* or *E. globulus*, due mainly to the difficulty of determining thickness or layering in the loosely structured mantles formed by *P. tinctorius*.

Wong and Fortin (1990) observed hyphal colonisation of root surfaces with incompatible partners and suggested that hyphal envelopes may simply be the result of growth on root exudates without requiring fungus-root attachment, but they still considered hyphal envelopes as specific to EM development. In most samples in this study, *E. camaldulensis-P. tinctorius* K55 association was found to have produced hyphal envelopes but with very poor internal structures such as Hartig net. Structurally, therefore, these hyphal envelopes were specific to EM formation but lacked full Hartig net development. This phenomenon was explained by Martin and Hilbert (1991) who suggested that the array of signals (morphological, biochemical and molecular) between host root and fungal isolate may be inadequate for sustained EM development. Dell *et al.* (1994) suggested that mantle formation may proceed in the absence of compatible recognition signals. Therefore, mantle formation in the associations between *E. camaldulensis* and *P. tinctorius* isolate K55 and between *E. camaldulensis* and *P tinctorius* Vietnam isolate in this study was far from that required for effective EM development.

In the study of *E. camaldulensis-P. tinctorius* K55 mycorrhizas, root apices of some mycorrhizas were found to be growing out from the mantle. A similar phenomenon was observed by Massicotte *et al.* (1999) and Martins *et al.* (1996) in their studies of *Paxillus involutus-Alnus glutinosa* and *Laccaria laccata-Castanea sativa* mycorrhizas respectively. Massicotte *et al.* (1999) called these roots 'transient ectomycorrhiza' and suggested that their presence indicated a certain degree of incompatibility.

In the process of a typical Hartig net formation, hyphae are oriented transversely to the root axis and branch irregularly forming a labyrinthine pattern (Kottke and Oberwinkler, 1986). The epidermal cells of *Eucalyptus* show a rapid response to the presence of EM fungus in the form of a considerable radial elongation (Smith and Read, 1997). Horan *et al.* (1988) proposed that the Hartig net only develops after the fungus has altered the development of epidermal cell walls following contact near the root tip. Hartig net formation in *Eucalyptus-P. tinctorius* mycorrhiza is described as paraepidermal, with hyphae partially enclosing the epidermal cells (Massicotte *et al.*, 1987). In this study, *E. globulus* mycorrhizas were found to have possessed a typical paraepidermal labyrinthine Hartig net formation with a radial elongation of epidermal cells, whereas *E. camaldulensis* mycorrhizas showed little Hartig net development with very poor radial elongation in epidermal cells. The process of Hartig net development requires the modification of cell walls leading to wall loosening thus enabling its mechanical penetration which is facilitated by fungal IAA signals (Gea *et al.*, 1994). It seems therefore that the Hartig net development is tightly controlled in the symbiosis whereas mantle formation, as observed in the *E. camaldulensis-P. tinctorius* mycorrhiza in this study, may have proceeded in the absence of compatible recognition signals (Dell *et al.*, 1994), which resulted in little Hartig net formation and poor elongation of epidermal cells.

Even with structurally compatible associations between *Eucalyptus* and different isolates of *P. tinctorius*, the speed of colonisation initiation may reflect differences in the host-fungus recognition process (Tonkin *et al.*, 1989). Dell *et al.* (1994) suggested that sometimes the specificity in the *Pisolithus-Eucalyptus* system may appear to be related to the rate of development rather than the extent of differentiation of EM structures. In some samples of *Eucalyptus* mycorrhizas observed in these experiments, Hartig net development appeared to be more pronounced than others. Therefore, the rate of development of EM might explain why development of Hartig net in those samples was more pronounced than others.

In a study of EM formation on micropropagated *Eucalyptus* plantlets and seedlings, Tonkin *et al.* (1989) found that one of the two *P. tinctorius* isolates formed EM only on clonal lines from mature trees while the other isolate formed EM on seedlings as well as clonal lines of juvenile and mature trees. This observation suggests that the developmental maturity of host material can also influence compatibility. Therefore, it could be possible that the isolates used here might have had higher affinity for older *E. camaldulensis* seedlings. However, even after 24 weeks of growth, *E. camaldulensis* seedlings inoculated with *P. tinctorius* isolate K55 formed incomplete mycorrhiza with loose mantles, little Hartig net formation and poor elongation of epidermal cells. *E. camaldulensis* seedlings also responded similarly to the *P. tinctorius* Vietnam isolate after 18 weeks of growth. Whereas, *E. globulus* seedlings were found to be colonised as early as 12 weeks by that isolate which resulted in a complete EM development.

Tonkin *et al.* (1989) also found that the *P. tinctorius* isolate which only formed mycorrhizas on clonal lines of mature trees showed a build-up of phenolics in the root epidermal cells of juvenile clones, which is thought to be a sign of incompatibility (Molina *et al.*, 1992). Deposition of phenolic compounds in plant cell walls and vacuoles frequently indicates an incompatible interaction between EM fungi and host roots (Ling-Lee *et al.*, 1975; Nylund and Unestam, 1982; Malajczuk *et al.*, 1984; Duddridge, 1986; Horan *et al.*, 1988; Massicotte *et al.*, 1999). Thus a higher accumulation of phenolics occurring immediately below the hyphal mantle and in vacuoles of the epidermal cells in mycorrhizal *E. camaldulensis* roots in the experiments of this study may also indicate an incompatible association. In spite of the deposition of phenolics in epidermal cell walls and vacuoles, some hyphae were able to penetrate between epidermal cells to form a limited Hartig net which is in agreement with the observations of Massicotte *et al.* (1999) in the *Paxillus involutus*-*Alnus glutinosa* mycorrhiza. However, the Hartig net found in *E. camaldulensis* mycorrhizas was never very extensive and rarely showed the labyrinthine branching observed in *E. globulus* mycorrhizas. These observations are in agreement with those of Molina (1981) who noted similar symptoms in mycorrhizas of *Paxillus involutus* and a number of *Alnus* species.

The environmental conditions such as temperature or pH did not seem to have been responsible for incomplete EM development. Temperature was well above 20°C. Similar temperature has been found to be suitable for EM formation in *Eucalyptus* by *P. tinctorius* in several studies (for example, in the studies of Bougher and Malajczuk, 1990; Burgess *et al.*, 1994; Mason *et al.*, 1999b). The pH in growth medium in these experiments ranged between 5.0-5.5. Similar pH has been reported to be suitable for growth of most EM fungi (Smith and Read, 1997). Although availability of light controls EM formation, it is unclear whether the light intensity used in the experiments (400-800 μmol photons m^{-2} s^{-1}) was responsible for the formation of incomplete EM structures.

Choice of fungal species used in these experiments being inappropriate also can be discounted as *P. tinctorius* has been widely used as an inoculant for nursery seedlings of *Eucalyptus* (see Brundrett *et al.*, 1996c and references therein). Moreover, both the isolates used here were of *Eucalyptus* origin (*E. globulus* and *E. camaldulensis*). *P. tinctorius* isolates of pine origin have been reported to have formed incompatible EM in *E. grandis* in the study of Burgess *et al.* (1994). Therefore, host specificity of the isolates may not have been the reason for an incompatible EM formation in *E. camaldulensis* in this study.

In summary, the occurrence of very limited epidermal cell elongation or an absence of it in most samples, a poor Hartig net development, and presence of phenolics-filled vacuoles or globules in epidermal cells tend to confirm the proposition that the EM associations formed by *E. camaldulensis* and *P. tinctorius* isolates in these studies were incompatible. The possible reasons for the incompatibilty could have been the developmental maturity of the host or a lack of aggressiveness on the part of the fungal isolates used to form EM in *E. camaldulensis*. Alternatively, the growth conditions under which the experiments were undertaken may have been influential.

CHAPTER 7

General Conclusions and Recommendations

CHAPTER 7

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7.0 Introduction

As indicated in Chapter 1, the background of the present study was based on the growth performance of *E. camaldulensis* in Bangladesh plantations. The Forest Department in Bangladesh (Rahman, personal communication) had recently found that this species was not performing well on degraded hill forest areas although it grew well for the first 4-5 years. At the outset of this study, it was hypothesised that the poor growth performance of *E. camaldulensis* in Bangladesh was the result of inadequate mycorrhizal colonisation, or due to presence of inappropriate fungal symbionts or absence of inoculum. The present study set out to test five main hypotheses:

(1) *E. camaldulensis* seedlings can form effective AM associations and they have a positive impact on growth and nutrient uptake;
(2) *E. camaldulensis* seedlings can form effective EM associations in the early period of seedlings growth and they have a positive impact on growth and nutrient uptake;
(3) *E. camaldulensis* seedlings can form both AM and EM in the same root system and they have a positive impact on growth and nutrient uptake;
(4) there is a relationship between plant growth and nutrient uptake, and mycorrhizal colonisation, with respect to variation in nutrient supply; and
(5) the AM and EM structures indicate a compatible and effective association.

Results from different experiments are discussed in light of these hypotheses.

7.1 Summary of Principal Findings

7.1.1. Hypothesis 1: *E. camaldulensis* Can Form Effective AM Associations and They Have a Positive Impact on Seedling Growth and Nutrient Uptake.

AM fungal inoculation resulted in negative growth responses in *E. camaldulensis* seedlings which were observed in experiments 1, 4 and 6 (see Chapters 3, 4 and 5 respectively). In experiment 1, although the extent of colonisation by *G. intraradices* UT143-2 was up to 6% (of total root length) it significantly reduced seedling dry mass compared to the uninoculated control. In experiment 4, extent of colonisation by different inoculant fungi ranged between 40-50% (of total root length), and again colonisation resulted in significantly reduced seedling dry mass compared to the uninoculated control. Experiment 6 was also no exception in this regard.

Seedlings inoculated with *G. clarum* BR148-1 had significantly higher foliar concentrations (per g dry mass of leaves) of N and P irrespective of nutrient regimes in experiments 4 and 6. Inoculation with *G. clarum* BR148-1 also resulted in the highest extent of colonisation (up to 50% in experiment 4 and 40% in experiment 6) and growth depression in seedlings inoculated by this fungus occurred concurrently with a higher foliar P and N concentration. As discussed in Chapter 4, similar observations have also been made in studies with other species (for example, Khaliq and Sanders, 1998). However, when calculated on the basis of total leaf dry mass, foliar N and P contents of seedlings for that treatment in both experiments did not vary significantly from the uninoculated control. Therefore, growth depression in *G. clarum* BR148-1 inoculated seedlings presumably resulted from the fungal carbon drain not being compensated for by the nutrients applied. Several workers [for example, McGonigle *et al.* (1990); Adjoud *et al.* (1996)] have shown that total percentage root length colonised by an AM fungus is not a particularly good predictor of growth-promoting effects by that fungus. The possible reasons for a negative growth response in AM inoculated seedlings may include one or a combination of the following:

1) low irradiance;
2) restricted volume of growth medium for growth of mycorrhizal seedlings;
3) use of inappropriate inoculant fungi; and
4) nutrient limitation

These possible reasons are considered below.

7.1.1.1 *Low irradiance*

Low irradiance can be a major cause of growth depression in mycorrhizal seedlings which occurs due to the carbon drain caused by AM fungi (see Johnson *et al.*, 1997; Olsen *et al.*, 1999). Comparative information from other mycorrhizal experiments of *Eucalyptus* is hard to obtain [for example, Vishwakarma and Singh (1996a,b) did not quantify the amount of light in their experiments]. In experiments of this study irradiance ranged between 400-800 μmol photons m^{-2} s^{-1}. In a study of growth responses in 11 AM-inoculated *Eucalyptus* species in a greenhouse, Adjoud *et al.* (1996) quantified the amount of supplementary light (280 μmol photons m^{-2} s^{-1}) but not the total available light. The actual light in their study might therefore have been much higher. However, Jones *et al.* (1998) reported successful mycorrhiza (both AM and EM) formation and resulting growth responses in *E. coccifera* at 400 μmol photons m^{-2} s^{-1} in a growth-cabinet study.

Eucalyptus species may vary in their light requirements. Although *E. camaldulensis* is reported to occur under a wide range of climatic conditions, it has been found to be mainly associated with arid to semi-arid areas along or near seasonal watercourses, and streams and rivers in Australia (Boland *et al.*, 1984) implying that it favours open forests. These habitat characteristics of this species might suggest that it is probably a highly light-demanding species although there are no comparative studies, for example, between different species of *Eucalyptus* or between *Eucalyptus* and other tropical species. Therefore, inadequate availability of light could have been a major factor for a growth depression in *E. camaldulensis* in this study. This was an unforeseen matter as it was envisaged that the natural light in the glasshouse would be sufficient for a positive growth response in mycorrhizal *E. camaldulensis* seedlings because of results obtained with *E. globulus* (Mason *et al.*, 1999b). As noted in Chapter 2, occasionally there were fluctuations in the availability of natural light due to periods of cloudy weather, and that artificial light from mercury vapour lamps were provided in addition to natural light. However, even with that additional light, the total amount of light was perhaps not adequate for mycorrhizas to have a positive impact on seedling growth. Other people working with other tree species (for example, *Calliandra calothyrsus* Meissner and *Brachystegia spiciformis* Benth.) were able to obtain considerable AM colonisation (>40% of the total root length) in the same glasshouse during the same period (Ingleby and Mason, personal communication) which resulted in positive growth responses in seedlings of those tree species.

7.1.1.2 *Restricted volume of growth medium for growth of mycorrhizal seedlings*

In nature, external hyphae of mycorrhizas have the ability to explore soil beyond normal nutrient depletion zones for the uptake of nutrients. In experiments of this study, vigorous growth of the *E. camaldulensis* root system was observed at higher nutrient regimes (10 mg l^{-1} P and 20 mg l^{-1} P) even without inoculation. Although colonisation by *G. clarum* was the highest at 10 mg l^{-1} P nutrient regime, high density of root system in that nutrient treatment being coupled with the restricted volume of growth medium and a resulting reduced nutrient depletion zone, could therefore have contributed to the inefficiency of mycorrhizas. Although nutrient solution was supplied as exponentially increasing dosages it might have had a rôle in reducing the nutrient depletion zone which on its own could be detrimental to mycorrhizal effectiveness. Growth depression may sometimes occur in pot-grown mycorrhizal seedlings because of their increased root density in restricted volume of growth media (Khaliq and Sanders, 1998). In the three preliminary experiments, pots of one litre capacity were used for raising seedlings, but for the subsequent experiments pots of two litre capacity were used. However, in none of these experiments was growth promotion resulting from AM inoculation observed. The length of external hyphae produced by a mycorrhizal fungus can be a good predictor of its relative ability to take up P (Jones *et al.*, 1990), but in other cases, it is the average distance of extension from the root which is more important (Jakobsen, Abbott and Robson, 1992a,b). Estimation of external hyphae was beyond the scope of this study. However, this may not be a good indicator if the volume of growth medium is restricted as occurs in pot experiments.

7.1.1.3 ***Use of inappropriate inoculant fungi***

Inoculation with inappropriate fungi could also result in growth depression in mycorrhizal seedlings (see Boddington and Dodd, 1998). The single cultures of AM inoculants used in this study (*G. clarum* BR148-1 and *G. rosea* FL150-5) have been reported to have formed mycorrhiza in *Gliricidia sepium* (Jacq.) Walp seedlings where *G. clarum* BR148-1 contributed to highest growth rate of seedlings in the study of Twum-Ampofo (1995). However, it is difficult to establish if they were unsuitable for *E. camaldulensis* in this study, as other factors such as light or high root density of seedlings in pots are more likely to be the causes for a negative growth responses in seedlings. Vishwakarma and Singh (1996a) inoculated *E. camaldulensis* with seven different AM fungi (*Glomus etunicatum*, *G. fasciculatum*, *G. mosseae*, *G. versiforme*, *Gigaspora margarita*, *Acaulospora laevis* and *Sclerocystis rubiformis*) where some of the fungi contributed to enhanced growth but none resulted in any growth depression. However, growth depression in seedlings of a number of *Eucalyptus* species (other than *E. camaldulensis*) resulting from AM inoculation has been observed while *P. tinctorius* inoculation contributed to a significantly higher dry mass in seedlings (Oliveira *et al.*, 1995). In this study, all the fungal inoculants resulted in growth depression in *E. camaldulensis* seedlings. It is, therefore, possible that these fungal inoculants tested were inappropriate for inoculation of *E. camaldulensis*; however the fact that growth responses were similar regardless of the fungal species, suggests that some other factor may account for the growth depression observed.

7.1.1.4 *Nutrient limitation*

N limitation has been found to be a cause for carbon drain in AM seedlings (see Attiwill and Adams, 1993). However, availability of nitrogen at 10 mg l^{-1} P (62.5 mg l^{-1} N) in experiments of this study, where most AM colonisation occurred, was higher than the rate (43 mg l^{-1} N) of Adjoud *et al.* (1996) for similar P concentration. They observed positive growth responses and increased leaf P concentration from inoculation of seedlings of most of the 11 *Eucalyptus* species tested, although *E. camaldulensis* was not included. Results from this study indicated that *G. clarum* BR148-1 inoculated seedlings had a higher foliar N (and P) concentration than uninoculated seedlings, indicating that N limitation is unlikely to be responsible for the growth depression observed. However, although K concentration between *G. clarum* BR148-1 inoculated seedlings and uninoculated controls did not vary significantly in experiments 4 and 6, total leaf K content for the former was significantly lower than the latter. K concentration for 10 mg l^{-1} N where most colonisation occurred was 35 mg l^{-1} which was slightly lower than what (38 mg l^{-1}) Adjoud *et al.* (1996) used in their AM experiment with *Eucalyptus* species. It is therefore possible that mycorrhizal seedlings in these experiments were K deficient. The role of K in transferring mobile polyphosphate through the hyphae into host root is recognised (see Bucking and Heyser, 1999). Therefore, K deficiency would be expected to have a negative effect on foliar P uptake in plants. However, P concentrations in AM seedlings significantly increased compared to uninoculated controls. Therefore, this suggests that K deficiency might not have affected mycorrhizal effectiveness in these experiments.

Results from the experiments 4 and 6 indicate that *E. camaldulensis* can form AM but their effectiveness may depend on availability of light and volume of growth media. However, as supported by the results from other studies, it appears that *E. camaldulensis* may form AM but not necessarily benefit from such associations at least in terms of enhanced growth.

7.1.2 Hypothesis 2: *E. camaldulensis* Can Form Effective EM Associations in the Early Period of Seedling Growth and They Have a Positive Impact on Seedling Growth and Nutrient Uptake.

Several isolates of *P. tinctorius* and one isolate of *Hydnangium carneum* were used for EM experiments in this study. Only one isolate of *P. tinctorius* (isolate K55) was found to have resulted in a considerable colonisation (up to 27% of the root tips) in *E. camaldulensis* seedlings which was found in only one experiment (experiment 5). Other isolates tested were either dead by the end of experiment (experiment 3) or resulted in a very little colonisation (1% of the root tips being colonised) in experiment 5. The inability of some *P. tinctorius* isolates to form mycorrhizas in *Eucalyptus* has been reported in several studies (see Lei *et al.* 1990; Tonkin *et al.*, 1989; Burgess *et al.*, 1994). Casual observations of inoculated seedlings (see Chapter 4) indicated that colonisation by the effective isolate of *P. tinctorius* (isolate K55) occurred more than 10 weeks later (16 weeks after inoculation) than that by AM fungi (6 weeks after inoculation) in experiments 5 and 6. This is in agreement with what Chilvers *et al.* (1987) and Lapeyrie and Chilvers, (1985) hypothesised in terms of AM-EM succession in *Eucalyptus*. Therefore, EM fungi may not have affinity for young *E. camaldulensis*.

EM inoculation resulted in negative growth response in shoot dry mass of *E. camaldulensis* seedlings in experiment 5. However, foliar N, P and K status of seedlings were not affected by EM inoculation. This negative growth response, again, probably was due to either one or a combination of the following:

1) low irradiance;
2) nutrient availability;
3) restricted volume of growth medium for growth of mycorrhizal seedlings
4) use of inappropriate inoculant fungal isolates

These are considered further below.

7.1.2.1 *Low irradiance*

Low irradiance can be a major cause of growth depression in mycorrhizal seedlings which occurs due to the carbon drain caused by the EM fungi (see Eltrop and Marschner, 1996). Comparative information from other EM experiments of *Eucalyptus* is hard to obtain [for example, Bougher *et al.* (1990); Burgess *et al.* (1994) carried out their experiments with natural light in glasshouse and they did not quantify the amount of light in their experiments]. Mason *et al.* (1999a,b,c) reported a light intensity of 1800 μmol photons m^{-2} s^{-1} in their EM experiment with *E. globulus* which appeared to be much higher compared to that (400 μmol photons m^{-2} s^{-1}) reported by Jones *et al.* (1998) in their EM experiment with *E. coccifera*, and also it is difficult to judge from their (Mason *et al.*, 1999a,b,c) work whether such a high availability of light was maintained throughout the experimental period or not. However, although successful EM formation occurred in their experiments they did not study growth responses in mycorrhizal *E. globulus* seedlings as compared to the uninoculated controls.

In experiments of this study, irradiance ranged between 400-800 μmol photons m^{-2} s^{-1}. Successful EM formation with a resulting growth response in seedlings of two tree species (*Afzelia quanzensis* Welw. and *Brachystegia spiciformis* Benth.) from the African Miombo woodland was observed (data unpublished) in the same period and in the same glasshouse where the experiments of this study were carried out. Therefore, availability of light in this range was probably adequate for an effective EM symbiosis for those tree species, even though it may not have been for *E. camaldulensis*. Seedlings of *E. camaldulensis* may have received insufficient light to produce adequate carbohydrate required for fungal growth in addition to maintaining their own growth. It is to be noted here that inoculation of *E. globulus* with the same EM fungal isolate in the same glasshouse also did not result in a positive growth response in seedlings; in fact growth rate was depressed. Mycorrhizal *E. globulus* seedlings in the study of Mason *et al.* (1999b) were supplied with a constantly higher amount of light (see above), and the seedlings in their study were >70% mycorrhizal compared to 40% in this case. Therefore, availability of light may have been a reason for poor EM formation in *E. camaldulensis* (only 27% root tips mycorrhizal), yet it resulted in a growth depression (in terms of shoot dry mass) in seedlings presumably reflecting carbon drain by the fungus.

7.1.2.2 *Nutrient availability*

Wallander (1995) has postulated that more host carbohydrate becomes available for production of fungal mycelium and fruit bodies under low N availability than under high N supply. As noted in section 2.8 of Chapter 2, one of the objectives of this study was to assess EM formation and resulting growth responses at varying concentrations of all nutrient elements. In order to be consistent with that objective, N concentrations in the low P nutrient treatments were proportionally lowered. In experiments of this study, highest EM colonisation was observed in the 2.5 mg l^{-1} P treatment where N availability was low (18.75 mg per pot), which is in agreement with what Wallander (1995) postulated. However, EM colonisation has resulted in a significant reduction in shoot growth at that nutrient regime. This growth depression resulting from inoculation recorded here may therefore be indirectly attributed to N availability via an intermediate effect on the extent of mycorrhizal colonisation. However, a positive growth response in *P. tinctorius* inoculated *E. grandis* seedlings has been recorded at a higher N availability (150 mg per pot) for a similar P regime in the study of Burgess *et al.* (1994).

7.1.2.3 *Restricted volume of growth medium for growth of mycorrhizal seedlings*

Growth depression in *E. camaldulensis* seedlings associated with the highest EM colonisation was recorded at 2.5 mg l^{-1} P nutrient regime where both root and shoot growth of the seedlings were not vigorous. Therefore, unlike the AM experiment where vigorous growth of root was observed at 10 mg l^{-1} P, restricted volume of growth medium is unlikely to have been a factor for a lack of mycorrhizal effectiveness in *E. camaldulensis* seedlings in the EM experiment.

7.1.2.4 *Use of inappropriate inoculant fungal isolates*

Results from a number of studies (for example, studies of Burgess *et al.*, 1994; Aggangan *et al.*, 1996; and Reddy and Satyanarayana, 1998) indicate that different isolates of *P. tinctorius* may have different effects on *Eucalyptus* (for example, *E. grandis*, *E. urophylla* and *E. tereticornis* respectively) seedlings. However, in all those studies, seedlings inoculated with any *P. tinctorius* isolate (except pine isolates in the study of Burgess *et al.*, 1994) had significantly higher biomass than uninoculated controls. In experiments of this study, no pine isolate of *P. tinctorius* was used; all isolates came from under *Eucalyptus* plantations including two from *E. camaldulensis*. Therefore, the isolates used here cannot be taken as ineffective at least relative to those which have been used for the studies mentioned above. However, effectiveness of fungal isolates may decline with several years of repeated subculturing on agar media (Laiho, 1970; Marx and Daniel, 1976; Thomson *et al.*, 1993). This could have been true for isolates PT3 and PT8 which had gone through repeated subculturing since their isolation from glasshouse experiments at ITE in 1993 [these isolates resulted in a very low colonisation (<1% of the root tips)]. Other isolates of *P. tinctorius*, for example, isolate PTE (1990) and the Vietnam isolate (1997) were isolated from *E. camaldulensis* plantations in the Philippines and Vietnam respectively. These isolates were not subjected to repeated subculturing. However, inoculation of *E. camaldulensis* with these isolates again resulted in a very low colonisation (<1% of the root tips) with no significant effect on growth of seedlings. On the contrary, the isolate K55 which was isolated in 1993 from a *E. globulus* plantation at Obidos in Portugal had not gone through repeated subculturing. This isolate, therefore, may not be taken as an ineffective isolate from either subculturing or colonising ability point of view.

Inoculation of *E. camaldulensis* seedlings with *P. tinctorius* has been reported to have resulted in significant increases in growth of seedlings (Abouelkhair *et al.*, 1986), and also it has been demonstrated that *P. tinctorius* colonisation can increase growth rate of *E. camaldulensis* more than other EM fungi [for example, inoculation with *P. tinctorius* has resulted in increased growth rate in *E. camaldulensis* compared to that with *Thelephora terrestris* in the study of Dixon and Hiol-Hiol (1992)].

Therefore, results from the experiments 5 and 6 indicate that *E. camaldulensis* can form EM at a relatively late stage of seedling development but their effectiveness may depend on adequate supply of both light and N which is essential for production of sufficient carbohydrate for growth of both the host and the mycobiont.

7.1.3 Hypothesis 3: *E. camaldulensis* Can Form Both AM and EM in the Same Root System and They Have a Positive Impact on Seedling Growth and Nutrient Uptake.

At the final harvest (24-week harvest) of experiment 6, while colonisation by *G. clarum* BR148-1 was >30% irrespective of nutrient treatments, *P. tinctorius* K55 resulted in <10% colonisation, most of which occurred at 2.5 mg l^{-1} P nutrient treatment. When AM or EM colonisation in the dual inoculation treatment was compared with those in the single fungal treatments, they were not found to be affected by each other in that treatment. This indicated that AM and EM fungi did not antagonise each other while adequate inoculum of each type was available to colonise roots. However, the lower extent of EM colonisation (<10%) as compared to that of AM (>30%) indicated that AM symbiosis was favoured by young *E. camaldulensis* seedlings. A similar trend was observed when time of emergence of mycorrhiza and extent of colonisation by AM and EM fungi in experiments 4 and 5 respectively were assessed (see Chapter 4).

Effect of dual (AM-EM) inoculation treatment resulted in a significantly higher dry mass of *E. camaldulensis* seedlings compared to the AM inoculated seedlings, however, both of these resulted in a significantly lower dry mass compared to either the EM or the uninoculated control. This result indicated that there was an interaction by the AM and EM fungi in the dual inoculation treatment which had some kind of a beneficial effect on *E. camaldulensis* seedlings. However, this beneficial effect did not result in a significantly higher dry mass in the dual inoculation treatment compared to the uninoculated control or the EM treatment which contradicts the results obtained in the study of Vishwakarma and Singh (1996b) who observed 137.8% increased biomass in dual inoculated seedlings compared to the controls. Carbon drain by the fungi was, again, probably the major cause for this unexpected result. The rate of consumption of carbohydrate for the sustenance of both types of fungi in the dual inoculated seedlings could probably not be offset by their beneficial impact on seedling growth. The reasons, again, may include one or a combination of the following- poor availability of light, restricted volume of growth medium for growth of mycorrhizal seedlings, or N limitation. These factors have been discussed in the preceding sections.

Therefore, it appeared that *E. camaldulensis* seedlings can form both AM and EM in their roots. However, efficiency in terms of a positive growth response in seedlings may depend on the growth conditions of seedlings such as adequate availability of light (for both AM and EM formation), adequate N (particularly for EM) and sufficient volume of growth media in pot experiments so that mycorrhizal seedlings can explore large volume of soil which is a prerequisite for the symbiosis to be effective in their functioning.

7.1.4 Hypothesis 4: There is a Relationship Between Plant Growth and Nutrient Uptake, and Mycorrhizal Colonisation, with Respect to Variation in Nutrient Supply.

7.1.4.1 *AM experiments*

As expected, different AM inoculants had different colonisation potential. Colonisation by *G. rosea* FL105-5 showed no significant difference along the nutrient concentration gradient (from 2.5 mg l^{-1} to 20 mg l^{-1} P) used in experiment 4. However, colonisation by *G. clarum* BR148-1 and Bangladesh culture in this experiment varied significantly along that nutrient concentration gradient with both 2.5 mg l^{-1} P (the lowest) to 20 mg l^{-1} P (the highest) nutrient treatments contributing to significantly lower extent of colonisation compared to either 5.0 mg l^{-1} P or 10 mg l^{-1} P. Hence, there was no significant relationship between extent of colonisation and nutrient availability. Different responses of different AM fungi/fungal isolates to various soil fertilities have been demonstrated for many tropical plant species [see Sieverding (1991) and references therein]. These differences arise because of the association of different fungi with habitats of various soil types and fertilities (Janos, 1980a,b).

Negative growth responses were only observed in three nutrient regimes (2.5, 5.0 and 10 mg l^{-1}). At 10 mg l^{-1} P, colonisation by *G. clarum* was the highest and it resulted in a significantly lower total dry mass of the seedlings as compared to the uninoculated control. At 20 mg l^{-1} P, there was no difference between the three inoculants and the uninoculated control in terms of their effect on seedling growth. 20 mg l^{-1} P nutrient treatment appeared to be high for *G. clarum* BR148-1 and Bangladesh culture where colonisation by these two inoculants were the lowest. However, AM (by *Glomus macrocarpum*, *Gigaspora pellucida* and *Glomus etunicatum*) formation has been reported to have resulted in 60-80% colonisation with a positive growth response in green ash (*Fraxinus pennsylvanica* Marsh.) at higher (148 ppm P) nutrient applications (Lamar and Davey, 1988) where solid sources of P were used. Similarly, Davis *et al.* (1984) identified a number of AM fungi which were capable of eliciting growth enhancement in plants under conditions of high soil fertility (up to 244 ppm Bray extractable P) of which *G. clarum* was one. However, the isolate of *G. clarum* used here was not tolerant of high nutrient concentration and neither was the Bangladesh culture.

7.1.4.2 *EM experiments*

Most of the EM colonisation occurred at the 2.5 mg l^{-1} P nutrient treatment (up to 27%). Mason *et al.* (1999b) observed highest colonisation (77.3% of total root tips) in *E. globulus* seedlings by *P. tinctorius* at a similar P concentration. In the study of Burgess *et al.* (1994) colonisation at this P concentration also resulted in significantly higher dry mass in seedlings compared to the uninoculated control. As noted in Chapter 4 and in section 7.1.2, colonisation of *E. camaldulensis* seedlings by *P. tinctorius* K55 at this P concentration resulted in a significantly reduced shoot dry mass compared to the uninoculated control.

Colonisation also occurred at other nutrient concentrations, for example at 5 mg l^{-1} P and 10 mg l^{-1} P, it was >10 % but at 20 mg l^{-1} P, it was <10 % in experiment 5. There was a significant relationship between nutrient concentration and extent of colonisation by *P. tinctorius* K55 with a progressively higher nutrient concentration contributing to decreasing extent of colonisation in *E. camaldulensis* seedlings. This observation is in agreement with what Bougher *et al.* (1990) found in their EM experiment with *E. diversicolor* and different EM fungi.

In none of the AM or EM experiments was there a relationship between foliar concentration of N, P or K and extent of colonisation. The fact that sometimes there is no relationship between extent of colonisation and growth promotion of host seedlings in mycorrhizal experiments (see McGonigle *et al.*, 1990; Adjoud *et al.*, 1996; Jones *et al.*, 1998) could perhaps be equally true for the relationships between extent of colonisation and foliar N, P or K concentration in case of all but *G. clarum*

BR148-1 inoculants in experiments of this study. Only *G. clarum* BR148-1 had significantly higher foliar N and P concentration as compared to the uninoculated control suggesting an opposite phenomenon to what other workers described as 'decreased shoot nutrient concentrations resulting from a dilution effect', where mycorrhizal inoculation resulted in enhanced growth in plants grown in controlled environments (Lamar and Davey, 1988; Strandberg and Johansson, 1999).

Therefore, variation in nutrient availability can affect both AM and EM colonisation in *E. camaldulensis* seedlings although different fungi may have different responses to them. Also this variation in extent of colonisation as affected by nutrient concentration can have significant effects on growth of *E. camaldulensis* seedlings.

7.1.5 Hypothesis 5: The AM and EM Structures Indicate a Compatible and Effective Association.

7.1.5.1 *AM structures and functionality of the mycorrhiza*

The AM structures produced in the experiments (for example, inter- and intra-radical hyphae, coiled hyphae and arbuscules) were all typical of normal mycorrhizas (Gianinazzi-Pearson *et al.*, 1996). However, these typical structures do not always imply a functional mycorrhiza in terms of its effect on growth responses in seedlings. Depending on environmental conditions and stage of growth of mycorrhizal seedlings, a normal AM with typical mycorrhizal structures could sometimes have a parasitic effect on hosts (Johnson *et al.*, 1997). Therefore, fungal structures do not indicate the functionality of AM in the experiments of this study.

7.1.5.2 *EM structures and functionality of the mycorrhiza*

A few studies (for example, studies of Abouelkhair *et al.*, 1986; Vishwakarma and Singh, 1996b) have shown positive growth responses in the association between *E. camaldulensis* and *P. tinctorius*. However, no structural analyses of the association have been presented in those studies. The EM structures (poor Hartig net development with little elongation of epidermal cells) produced in *E. camaldulensis* seedlings in experiments of this study appeared to be incomplete. Accumulation of phenolics in root epidermal cells may have indicated a tendency of *E. camaldulensis* to resist EM colonisation at a young age. It is unclear whether this host reaction reflects some form of genetic incompatibility with the fungal isolates used, or was the result of the environmental conditions under which the seedlings were grown (for example, light availability).

With compatible fungal isolates, EM colonisation can be expected to produce complete fungal structures such as a full Hartig net development with elongation in epidermal cells. Complete EM development has been previously observed in *E. camaldulensis* with compatible fungi (see Malajczuk *et al.*, 1984). Incomplete mycorrhizal development, however, may not necessarily be linked to a reduction in growth of host seedlings, for example, in the study of Burgess *et al.* (1994), the isolates of *P. tinctorius* which produced incomplete mycorrhizal structures did not result in negative growth responses in *E. grandis* seedlings.

Therefore, the mycorrhizal structures produced in these experiments may be useful in assessing compatibility and incompatibility but not necessarily in assessing growth responses in seedlings.

7.2 Key Issues of Mycorrhiza Development and Functioning in *E. camaldulensis* and Implication of the Results

The results obtained from various experiments in this study have given rise to some issues such as EM development in young *E. camaldulensis*, mycorrhiza and carbon economy, mycorrhiza and plant fitness, mycorrhizal fungal host specificity in the symbiosis and AM-EM succession. These issues are discussed in light of results obtained and implications of the findings are indicated.

7.2.1 EM Development in Young *E. camaldulensis*

While it was found that *E. camaldulensis* seedlings formed AM as early as 6 weeks of growth, there were indications that *E. camaldulensis* seedlings tended to be ectomycorrhizal after 16 weeks of growth in glasshouse, although even after 16 weeks EM colonisation was significantly lower compared to AM colonisation. A similar trend has been observed in a Brazilian *Eucalyptus* plantation (for example, in *E. viminalis* plantation by Bellei *et al.*, 1992) where AM predominated up to 7-8 months after plantation establishment before being taken over by EM. Therefore, a later development of EM in *E. camaldulensis* seedlings in this study was similar to what other workers have found in other *Eucalyptus* species belonging to the same sub-genus *Symphyomyrtus*.

7.2.2 Mycorrhiza and Carbon Economy

The presence of mycorrhizas on plant root systems can be correlated with higher net photosynthetic rates (Reid *et al.*, 1983; Nylund and Wallander, 1989). Improved nutrient status of mycorrhizal plants, especially P and N nutrition, is a well known phenomenon (Bougher *et al.*, 1990; Jones *et al*, 1991; Finlay *et al.*, 1996). According to Wallander and Nylund (1992), P-deficiency increases the carbohydrate pool in plants, which in non-mycorrhizal plants should increase chloroplast starch and thus down-regulate photosynthesis. Consequently, in mycorrhizal plants, where P increases and there is drain of carbon compounds to the fungus, photosynthesis should increase since there is no carbohydrate accumulation to inhibit photosynthesis. However, resource limitation such as N limitation may hinder the photosynthetic process (Marschner, 1995). Therefore, although it did not appear that N limitation was a possible reason for carbon drain in the AM experiments (see section 7.1.1.4), it perhaps occurred in the case of EM experiments with *E. camaldulensis* (see section 7.1.2.2). Therefore, an increased N regime may be suitable for both AM and EM formation. Adequate N regimes are necessary for formation of external mycelia for enhanced nutrient uptake in AM (Hawkins and

George, 1999), and for balanced allocation of carbon to host roots for effective EM formation (Mason *et al.*, 1999b).

7.2.3 Mycorrhiza and Plant Fitness

The significance of mycorrhizas to plants is generally based on improved survival of individuals following transplantation into an exotic environment, biomass increases in experimental systems, or altered physiology that can be perceived as an improvement (for example, increased nutrient uptake or increased drought tolerance).

Johnson *et al.* (1997) has given a good explanation of mycorrhizal systems. In natural systems, plant fitness is typically measured by survival and fecundity, and biomass changes might or might not be a good indicator of reproductive success. By contrast, biomass is usually a good variable to measure in agricultural systems where seed or biomass yields are the currency of agricultural success. Fitness is measured at the scale of an individual. But in the real world, individuals are not isolated in experimental pots. Interactions at the scale of populations, communities, and ecosystems, mediate the actual fitness of individuals. In experiments in this study, though negative growth response due to mycorrhizal inoculation was obtained, it can be said at least in case of AM that as the mycorrhizal structures produced were of typical nature, they would probably be appropriate for the seedlings when they are planted in the field.

Resource limitation is a key component of cost:benefit analysis of mycorrhizal effects on plant fitness (Eissenstat *et al.*, 1993). Carbon allocated to a fungus is only a cost if it could otherwise have been allocated to increase plant fitness, and resource gained through the activities of a fungal symbiont are only beneficial if those resources are in limiting supply. Although the reciprocal exchange of limiting resources is the most obvious (and traditional) choice for cost:benefit analysis, in many natural systems, other (often subtle) mycorrhiza-induced changes might ultimately be more important to plant fitness. For example, plant morphology, allometry, phenology, and chemistry are affected by the presence of mycorrhizal fungi (Johnson *et al.*, 1997 and references therein). Some of these changes will complicate the cost:benefit analysis.

Put into a community or ecosystem context, mycorrhizal symbioses can substantially impact plant fitness both directly and indirectly through altered relationships with other components of the system (see Johnson *et al.*, 1997 and references therein). Such impact can be in terms of a greater disease resistance in mycorrhizal plants against harmful organisms in the ecosystem or dealing with environmental stresses such as drought. Complexity at community and ecosystem scales means that mycorrhiza-induced changes in plant allocation patterns might have unpredictable effects on plant fitness (Johnson *et al.*, 1997).

Therefore, a negative growth response in mycorrhizal seedlings in a controlled environment may discourage mycorrhizal inoculation of seedlings in the nursery stage but the ultimate benefit of inoculation should be judged in terms of long-term survival and growth of seedlings after transplanting in the field. It is also important to consider whether the negative growth responses recorded in this investigation also occur in the field; if so, plant fitness could be affected.

7.2.4 Mycorrhizal Fungi and Host Specificity

7.2.4.1 *AM Host Specificity*

AM fungi are reported to have broad host range (Smith and Read, 1997). However, there are reports that some AM fungi can be more efficient than others in mycorrhizal formation and in their effect on plant growth (for example, in case of *Eucalyptus* AM see Adjoud *et al.*, 1996; Vishwakarma and Singh, 1996a cited in Chapter 4). Evidence is accumulating from recent molecular studies that AM fungi too can be host specific (Douds *et al.*, 1998; Sequeira *et al.*, 1991). From the host-specificity point of view, it is difficult to say if the AM fungi used in this experiment were efficient or not because all of them produced mycorrhizal structures of typical AM.

7.2.4.2 *EM Host Specificity*

Eucalyptus is reported to be a genus associated with many species of genus-specific fungi, particularly of hypogeous Basidiomycetes. Recent data from mycological expeditions in Australia (Trappe, Castellano, Bougher, and Malajczuk, unpublished, as cited in Molina *et al.*, 1992) indicate that *Eucalyptus* may have the richest flora of genus-specific EM fungi in the world, reflecting the diversity of *Eucalyptus* species and their dominance on an isolated continent. EM formed with *E. camaldulensis* seedlings in this study showed symptoms of incomplete or incompatible mycorrhiza. *P. tinctorius* K55 was isolated from under a *E. globulus* plantation in Portugal while *P. tinctorius* Vietnam isolate was isolated from under a *E. camaldulensis* plantation in Vietnam. It would be tempting to attribute the cause of the incomplete mycorrhiza formation between *E. camaldulensis* and the *P. tinctorius* isolate K55 in terms of differential specificity of the isolate between two different hosts under the same genus *Eucalyptus*. However, a similar explanation would not be plausible for *E. camaldulensis-P. tinctorius* Vietnam-isolate mycorrhiza where, even though the isolate came from a *E. camaldulensis* plantation, inoculation of *E. camaldulensis* seedlings with this isolate did not result in complete EM. Moreover, another isolate of *P. tinctorius* (isolate PTE) which was isolated from under a *E. camaldulensis* plantation in the Philippines also did not form adequate mycorrhiza (<1%) in this study (in experiments 1, 3 and 5). Molina *et al.* (1992) argued that there is no known example of gene-for-gene level of host-fungus specificity. Therefore, host specificity of the isolates used here, particularly that of K55 between *E. camaldulensis* and *E. globulus*, probably was not a factor in EM development.

7.2.5 AM-EM Succession in *E. camaldulensis*

In this study, young seedlings of *E. camaldulensis* appeared to have been readily colonised by AM fungi. When seedlings were 16 weeks or older, they tended to be readily colonised by EM although extent of colonisation was still low compared to that of AM. Many members of the sub-genus *Symphyomyrtus* (to which *E. camaldulensis* belongs) have been reported to form both AM and EM [for example, in *E. saligna, E. urophylla* and *E. grandis* by Oliveira *et al.* (1998), and in *E. camaldulensis* and *E. tereticornis* by Vishwakarma and Singh (1996b) and Jamaluddin and Chandra (1997)]. Also AM-EM succession has been reported for other members of this sub-genus (Bellei *et al.*, 1992), for example, in a *E. viminalis* plantation in southern Brazil AM predominated in young stands (7-8 months) while EM predominated as the stands aged. A similar succession could also be possible in *E. camaldulensis* which is discernible from the responsiveness of seedlings to mycorrhizal inoculation in different experiments of this study where AM was prevalent on young (6 weeks old) seedlings while EM tended to associate with relatively old (16 weeks old) seedlings.

7.3 General Conclusions and Implications of the Findings

As survival and performance of *E. camaldulensis* may partly depend on mycorrhizal status, it was considered important to explore the growth strategy of this species in relation to various mycorrhizal associations. The principal work undertaken here involved the study of the effects of AM, EM and dual (AM and EM) inoculation on growth and nutrient uptake in *E. camaldulensis* seedlings. This study has indicated that *E. camaldulensis* can form both AM and EM but their effectiveness may depend on environmental conditions (probably light being the most important factor), nutrient regimes and perhaps choice of fungal inoculants. However, the key finding was that both mycorrhizal types may result in a negative growth response under the environmental conditions tested. The results from this study, therefore, provide no support for mycorrhizal inoculation under the set of conditions used here as a technique for improving the growth of *E. camaldulensis* established in plantations. However, observation of positive growth responses in *E. camaldulensis* seedlings from both AM and EM inoculation has been reported in other studies (see Abouelkhair *et al.*, 1986; Dixon and Hiol-Hiol, 1992; Vishwakarma and Singh, 1996a,b). Therefore, the relationship between environmental variation (for example, light availability) and mycorrhizal colonisation would appear to merit further investigation.

In this study, there was an indication of AM-EM succession in *E. camaldulensis* seedlings as they developed. This aspect need to be considered during transplanting seedlings from nursery to the field. In the field, availability of inoculum and competition with indigenous fungi and other microbial organisms may largely control the efficacy and persistence of mycorrhizal symbionts. For example, succession from AM to EM may become delayed if availability of suitable EM inoculum is sparse or absent. If EM can ensure a higher survival and growth rates of *E. camaldulensis* as plantations mature, inoculation of seedlings with effective EM fungi (which can outcompete indigenous fungi) should be carried out in the nursery stage. However, this clearly needs to be tested in the field. It is conceivable that although initial growth responses may be negative, mycorrhizal inoculation may result in positive effects on growth and survival in the long term. Therefore, complementary studies including both indigenous and effective exotic fungal isolates should be carried out before embarking on a large-scale nursery inoculation. This kind of study can be best done in the field, the result of which may readily be available for use in nursery inoculation programmes.

7.4 Evaluation of the Experimental Approach

As described at the outset of this chapter, there were five hypotheses which have been tested in this study. The means through which these issues were addressed were by:

1) confirmation of the mycorrhizal status of *E. camaldulensis* and mycorrhizal fungi and their compatibility;
2) comparison of mycorrhizal and non-mycorrhizal plant growth responses to mycorrhizal inoculation at different nutrient availabilities.

As noted in Chapter 1, experimental objectives of this kind can only be fulfilled by conducting experiments in controlled environments (see Brundrett *et al.*, 1996c). Therefore, it was decided to carry out pot-experiments in a glasshouse. Throughout the study, liquid fertiliser instead of solid fertiliser was supplied to the experimental seedlings. Solid sources of fertiliser (for example, slow-release osmocote by Mason *et al.* 1999b, or rock phosphate by McGreevy, 1996) have been used for raising mycorrhizal seedlings. However, the major disadvantage of using solid fertilisers in mycorrhizal experiments in a controlled environment is that it is difficult to demonstrate the relationship between mycorrhizal formation and nutrient availability. The other disadvantage is that as the solid fertilisers need to be applied as a basal dressing or in mixture with the growth medium the actual amount of nutrients released to the medium is difficult to determine. Therefore, in this series of experiments liquid nutrient solution was used. This was done to carefully control the amount of nutrients supplied to the experimental seedlings (see Brundrett *et al.*, 1996c) and to establish the relationship between mycorrhizal formation and nutrient availability. This has an implication in nursery inoculation of seedlings (grown in field soils) where application of liquid fertilisers is more convenient than that of solid fertilisers.

In these experiments, the possible deleterious effects of application of liquid fertilisers was kept under control by exponentially increasing fertiliser additions which, as noted in Chapter 2, were reported to have stimulated mycorrhizal development compared with conventional constant-rate fertiliser addition (Ingestad *et al.*, 1986; Quoreshi and Timmer, 1998). A balanced nutrient solution was used where the proportions of different nutrient elements were kept constant for all the nutrient treatments. *E. camaldulensis* has been reported to be able to grow in soils of a wide range of fertilities. Therefore, use of various concentrations of nutrient elements enabled to assess how mycorrhizal inoculation of this species affected its growth and nutrient uptake under different fertilities. However, where the rate of release of nutrients from the solid sources of fertilisers (such as rock phosphate or osmocote) could be established, there should be no problem using them for mycorrhizal experiments in controlled environments or in the field. They could as well be used for raising mycorrhizal inoculated seedlings in the nursery.

The fact that results of pot-based investigations of mycorrhizal colonisation may have limited applicability to field situations has been recognised previously (St John and Coleman, 1983); however, field based experiments suffer from technical difficulties and high degree of variation, which may obscure treatment effects (Newton, 1989). An attempt has been made in this study to provide an insight into some of the issues relevant to mycorrhiza formation and its effect on growth such as fungus-nutrient interactions and AM-EM interactions which were thought to be best investigated in controlled conditions such as that used in this case.

A quantitative assessment of mycorrhizal structures such as internal hyphae and arbuscules and/or vesicles in the AM colonised roots or fungal mycelium or mantle and Hartig net hyphae in the EM colonised roots, and of external hyphae in the rhizosphere could have provided answers to the questions with respect to the carbon drain which was observed in most experiments here. However, this was beyond the scope of this study.

7.5 Suggestions for Further Work

Availability of light appeared to have been a cause for growth depression in mycorrhizal seedlings in this study. The relationship between light availability and mycorrhizal functioning, therefore, deserves further attention; this aspect has been little studied in the past.

This study could be extended to evaluate a broader range of nutrient regimes including different combinations of N and P in terms of their effect to mycorrhiza formation and their effectivity. By doing so, issues such as nutrient limitation (for example, N in the case of EM formation) could be addressed with respect to mycorrhizal formation and their effects on growth of *E. camaldulensis* seedlings.

A broader range of both AM and EM fungal isolates should be tested. In the tropics, and in Bangladesh in particular, while indigenous AM fungi might be readily available for testing them for their efficiency, young and aggressive isolates of suitable and compatible EM fungi may need to be provided from elsewhere (for example, from Australia for *E. camaldulensis*) for testing their efficiency. This way suitable AM and EM fungal inoculants can be chosen for inoculating seedlings.

There were indications in this study that a dual inoculation of *E. camaldulensis* seedlings may result in a higher growth rate in seedlings than AM inoculated alone. As there are different nutrient requirements for AM and EM fungi, an optimisation is therefore necessary to determine suitable nutrient concentration for the promotion of growth of both types of fungi in the same root in the initial period of seedling growth. On a commercial scale, this would have implications on the performance of the seedlings after they are transplanted in the field.

Once suitable AM and EM fungi are selected in terms of their performance in controlled conditions, experiments could be extended to the field for a long term study. The results from field experiments would generate valuable information with regard to efficacy of mycorrhizal fungi and their role in determining fungal succession which may be important for ecological performance of *E. camaldulensis* in the field.

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APPENDICES

APPENDIX A

Ingestad's Nutrient Solution

<u>Solution B</u>	g litre^{-1}
NH_4NO_3	140.2
KNO_3	37.2
KH_2PO_4	41.3
K_2SO_4	14.0

<u>Solution C</u>	
HNO_3	1.6
H_3BO_3	0.57
$Fe_2(SO_4)_3$	2.5
$Ca(NO_3)_2 . 4H_2O$	20.58
$Mg(NO_3)_2 . 6H_2O$	44.92
$MnSO_4 . 4H_2O$	0.81
$CuCl_2 . 2H_2O$	0.043
$ZnSO_4 . 7H_2O$	0.064
$NaMoO_4 . 2H_2O$	0.008

Note: 2 litres of Solution B (all amounts x2) and 1 litre of Solution C were made up first. Solutions were mixed in proportion of 1.7B : 1C when making up the nutrient solution. Solutions B and C were kept in separate bottles until making up appropriate solution and mixed in water.

APPENDIX B

Table 1: Analysis of variance of stem diameter (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.9019	0.2255	1.95	
Substrate	1	0.0058	0.0058	0.05	0.825
Nutrient	1	9.8804	9.8804	85.27	<0.001
Inoculation	1	0.4709	0.4709	4.06	0.053
Substrat.Nutrient	1	0.0325	0.0325	0.28	0.601
Substrat.Inoculation	1	0.7840	0.7840	6.77	0.015
Nutrient.Inoculation	1	0.3920	0.3920	3.38	0.076
Substrat.Nutrient.Inoculation	1	0.1145	0.1145	0.99	0.329
Residual	28	3.2443	0.1159		
Total	39	15.8263			

Table 2: Analysis of variance of height (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.6968	71.46	2.41	
Substrate	1	32.76	32.76	1.11	0.302
Nutrient	1	714.02	714.02	24.08	<0.001
Inoculation	1	43.68	43.68	1.47	0.235
Substrat.Nutrient	1	96.72	96.72	3.26	0.082
Substrat.Inoculation	1	133.22	133.22	4.49	0.043
Nutrient.Inoculation	1	0.3920	0.3920	3.38	0.076
Substrat.Nutrient.Inoculation	1	48.84	48.84	1.65	0.210
Residual	28	830.14	29.65		
Total	39	2185.21			

Table 3: Analysis of variance of leaf number (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks. Log transformations were made on leaf number.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.51878	0.12969	2.59	
Substrate	1	0.4709	0.4709	4.06	0.053
Nutrient	1	1.28399	1.28399	25.67	<0.001
Inoculation	1	0.56735	0.56735	11.34	0.002
Substrat.Nutrient	1	0.15755	0.15755	5.15	0.087
Substrat.Inoculation	1	0.25752	0.25752	3.15	0.031
Nutrient.Inoculation	1	0.01243	0.01243	0.25	0.622
Substrat.Nutrient.Inoculation	1	0.01414	0.01414	0.28	0.599
Residual	28	1.40039	0.05001		
Total	39	4.71102			

Table 4: Analysis of variance of leaf area (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks. Log transformations were made on leaf area.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.16968	0.04242	0.44	
Substrate	1	0.49532	0.49532	5.12	0.032
Nutrient	1	5.98859	5.98859	61.88	<0.001
Inoculation	1	0.39234	0.39234	4.05	0.054
Substrat.Nutrient	1	0.06500	0.06500	0.67	0.419
Substrat.Inoculation	1	0.32035	0.32035	3.31	0.080
Nutrient.Inoculation	1	0.01803	0.01803	0.19	0.669
Substrat.Nutrient.Inoculation	1	0.01469	0.01469	0.15	0.700
Residual	28	2.70957	0.09677		
Total	39	10.17357			

Table 5: Analysis of variance of average leaf area (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	20.796	5.199	0.69	
Substrate	1	0.551	0.551	0.07	0.788
Nutrient	1	115.434	115.434	15.37	0.001
Fungus	1	0.795	0.795	0.11	0.747
Subtrate.Nutrient	1	1.198	1.198	0.16	0.693
Substrate.Inoculation	1	0.013	0.013	0.00	0.967
Nutrient.Inoculation	1	0.158	0.158	0.02	0.886
Substrate.Nutrient.Inoculation	1	0.437	0.437	0.06	0.811
Residual	28	210.255	7.509		
Total	39	349.637			

Table 6: Analysis of variance of shoot dry mass (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks. Log transformations were made on shoot dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.5903	0.1476	1.08	
Substrate	1	0.4103	0.4103	3.00	0.094
Nutrient	1	5.4011	5.4011	39.46	<0.001
Inoculation	1	0.3806	0.3806	2.78	0.107
Substrat.Nutrient	1	0.0475	0.0475	0.35	0.561
Substrat.Inoculation	1	0.5916	0.5916	4.32	0.047
Nutrient.Inoculation	1	0.0202	0.0202	0.15	0.703
Substrat.Nutrient.Inoculation	1	0.0345	0.0345	0.25	0.619
Residual	28	3.8325	0.1369		
Total	39	11.3085			

Table 7: Analysis of variance of root dry mass (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks. Log transformations were made on root dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	4.0775	1.0194	3.65	
Substrate	1	0.0080	0.0080	0.03	0.866
Nutrient	1	1.4380	1.4380	5.15	0.031
Inoculation	1	0.5104	0.5104	1.83	0.187
Substrat.Nutrient	1	0.0626	0.0626	0.35	0.561
Substrat.Inoculation	1	0.1730	0.1730	0.22	0.640
Nutrient.Inoculation	1	0.2900	0.2900	1.04	0.317
Substrat.Nutrient.Inoculation	1	0.0042	0.0042	0.01	0.904
Residual	28	7.8256	0.2795		
Total	39	14.3893			

Table 8: Analysis of variance of total dry mass (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks. Log transformations were made on total dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.52159	0.13040	2.28	
Substrate	1	0.58782	0.58782	10.30	0.003
Nutrient	1	4.23838	4.23838	74.25	<0.001
Inoculation	1	0.00524	0.00524	0.09	0.764
Substrat.Nutrient	1	0.04089	0.04089	0.72	0.405
Substrat.Inoculation	1	0.26248	0.26248	4.60	0.041
Nutrient.Inoculation	1	0.06730	0.06730	1.18	0.287
Substrat.Nutrient.Inoculation	1	0.01786	0.01786	0.31	0.580
Residual	28	1.59830	0.05708		
Total	39	7.33987			

Table 9: Analysis of variance of root:shoot ratio (for Table 3.1.1) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at six weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	2.6297	0.6574	1.50	
Substrate	1	1.3323	1.3323	3.04	0.092
Nutrient	1	0.3034	0.3034	0.69	0.413
Inoculation	1	0.3313	0.3313	0.76	0.392
Substrat.Nutrient	1	0.1307	0.1307	0.30	0.589
Substrat.Inoculation	1	0.5276	0.5276	1.20	0.282
Nutrient.Inoculation	1	0.0214	0.0214	0.05	0.827
Substrat.Nutrient.Inoculation	1	0.0092	0.0092	0.02	0.886
Residual	28	12.2743	0.4384		
Total	39	17.5598			

Table 10: Analysis of variance of stem diameter (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	5.49688	1.37422	14.30	
Substrate	1	2.98662	2.98662	31.08	<0.001
Nutrient	1	14.22056	14.22056	147.96	<0.001
Inoculation	1	0.49506	0.49506	5.15	0.031
Substrat.Nutrient	1	0.09312	0.09312	0.97	0.333
Substrat.Inoculation	1	0.00506	0.00506	0.05	0.820
Nutrient.Inoculation	1	0.22052	0.22052	2.29	0.141
Substrat.Nutrient.Inoculation	1	0.26732	0.26732	2.78	0.107
Residual	28	2.69104	0.09611		
Total	39	26.47620			

Table 11: Analysis of variance of height (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	134.34	33.58	0.42	
Substrate	1	275.62	275.62	3.48	0.072
Nutrient	1	2146.23	2146.23	27.13	<0.001
Inoculation	1	176.40	176.40	2.23	0.147
Substrat.Nutrient	1	119.03	119.03	1.50	0.230
Substrat.Inoculation	1	0.00	0.00	0.00	1.000
Nutrient.Inoculation	1	25.60	25.60	0.32	0.574
Substrat.Nutrient.Inoculation	1	8.10	8.10	0.10	0.751
Residual	28	2214.96	9.11		
Total	39	5100.27			

Table 12: Analysis of variance of leaf number (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks. Log transformation was made on leaf number.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.7878	0.04470	0.55	
Substrate	1	0.40818	0.40818	5.00	0.033
Nutrient	1	1.63101	1.63101	20.00	<0.001
Inoculation	1	0.03479	0.03479	0.43	0.519
Substrat.Nutrient	1	0.12748	0.12748	1.56	0.222
Substrat.Inoculation	1	0.11399	0.11399	1.40	0.247
Nutrient.Inoculation	1	0.30401	0.30401	3.73	0.064
Substrat.Nutrient.Inoculation	1	0.01315	0.01315	0.16	0.691
Residual	28	2.28369	0.08156		
Total	39	5.09508			

Table 13: Analysis of variance of leaf area (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks. Log transformation was made on leaf area.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.116765	0.029191	3.64	
Substrate	1	0.802281	0.802281	99.93	<0.001
Nutrient	1	4.027423	4.027423	501.67	<0.001
Inoculation	1	0.017424	0.017424	2.17	0.152
Substrat.Nutrient	1	0.000254	0.000254	0.03	0.860
Substrat.Inoculation	1	0.009824	0.009824	1.22	0.278
Nutrient.Inoculation	1	0.000823	0.000823	0.10	0.751
Substrat.Nutrient.Inoculation	1	0.003773	0.003773	0.47	0.499
Residual	28	0.224787	0.008028		
Total	39	5.203354			

Table 14: Analysis of variance of average leaf area (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	117.61	29.40	0.96	
Substrate	1	9.02	9.02	0.29	0.591
Nutrient	1	162.02	162.02	5.30	0.029
Inoculation	1	7.99	7.99	0.26	0.613
Substrate.Nutrient	1	16.21	16.21	0.53	0.473
Substrate.Inoculation	1	16.69	16.69	0.55	0.466
Nutrient.Inoculation	1	96.63	96.63	3.16	0.086
Substrate.Nutrient.Inoculation	1	16.24	16.24	0.53	0.472
Residual	28	856.43	30.59		
Total	39	1298.83			

Table 15: Analysis of variance of shoot dry mass (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	2.5927	0.6482	1.70	
Substrate	1	31.0641	31.0641	81.50	<0.001
Nutrient	1	138.4956	138.4956	363.35	<0.001
Inoculation	1	2.2705	2.2705	5.96	0.021
Substrat.Nutrient	1	2.7510	2.7510	7.22	0.012
Substrat.Inoculation	1	0.3441	0.3441	0.90	0.350
Nutrient.Inoculation	1	0.0000	0.0000	0.00	0.994
Substrat.Nutrient.Inoculation	1	0.0555	0.0555	0.15	0.706
Residual	28	10.6727	0.3812		
Total	39	188.2463			

Table 16: Analysis of variance of root dry mass (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.3025	0.0756	0.42	
Substrate	1	1.4470	1.4470	8.03	0.008
Nutrient	1	11.2339	11.2339	62.35	<0.001
Inoculation	1	0.8880	0.8880	4.93	0.035
Substrat.Nutrient	1	0.2541	0.2541	1.41	0.245
Substrat.Inoculation	1	0.1270	0.1270	0.70	0.408
Nutrient.Inoculation	1	1.0654	1.0654	5.91	0.022
Substrat.Nutrient.Inoculation	1	0.1109	0.1109	0.62	0.439
Residual	28	5.0445	0.1802		
Total	39	20.4733			

Table 17: Analysis of variance of total dry mass (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks. Log transformation was made on total dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.025972	0.006493	0.79	
Substrate	1	0.920427	0.920427	112.21	<0.001
Nutrient	1	4.880957	4.880957	595.06	<0.001
Inoculation	1	0.096487	0.096487	11.76	0.002
Substrat.Nutrient	1	0.00513	0.00513	0.06	0.245
Substrat.Inoculation	1	0.008203	0.008203	1.0	0.804
Nutrient.Inoculation	1	0.001679	0.001679	0.20	0.654
Substrat.Nutrient.Inoculation	1	0.002166	0.002166	0.26	0.611
Residual	28	0.229669	0.008202		
Total	39	6.166073			

Table 18: Analysis of variance of root:shoot ratio (for Table 3.1.4) of AM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 12 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.026168	0.006542	0.73	
Substrate	1	0.000371	0.000371	0.04	0.840
Nutrient	1	0.061172	0.061172	6.82	0.014
Inoculation	1	0.038573	0.038573	4.30	0.047
Substrat.Nutrient	1	0.042416	0.042416	4.73	0.038
Substrat.Inoculation	1	0.000082	0.000082	0.01	0.925
Nutrient.Inoculation	1	0.004301	0.004301	0.48	0.494
Substrat.Nutrient.Inoculation	1	0.004101	0.004101	0.46	0.505
Residual	28	0.251204	0.008972		
Total	39	0.428388			

APPENDIX C

Table 1: Substrate-nutrient interaction on shoot dry mass at the 12-week harvest of experiment 1 with *E. camaldulensis*. SP= sand-perlite; VP= vermiculite-peat; low P= 10 mg l^{-1} P and high P= 30 mg l^{-1} P. Values for each variable followed by different letters differ significantly at *P*≤0.05 (ANOVA).

Substrate	Nutrient	
	Low P	High P
SP	2.707*a*	5.904*c*
VP	3.945*b*	8.191*d*

Table 2: Means of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g) and root shoot ratio (RSR) of *E. camaldulensis* seedlings under two nutrient regimes: low P (10 mg l^{-1} P) and high P (30 mg l^{-1} P) at the 12-week harvest of experiment 1. Values for each variable followed by different letters differ significantly at *P*≤0.05 (ANOVA).

Variable	Nutrient	
	Low P	High P
DIAM	3.779*a*	4.972*b*
HT	49.10*a*	63.70*a*
LFNO	28.40*a*	43.80*b*
LFAR	332.4*a*	630.0*b*
SDM	3.326*a*	7.047*b*
RDM	1.395*a*	2.455*b*
TDM	4.720*a*	9.500*b*
RSR	0.433*b*	0.355*a*

APPENDIX D

Table 1: Analysis of variance of stem diameter (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks. Log transformations were made on stem diameter.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.042184	0.010546	1.77	
Substrate	1	0.156931	0.156931	26.35	<0.001
Nutrient	1	0.294929	0.294929	49.51	<0.001
Inoculation	1	0.000621	0.0000621	0.10	0.749
Substrat.Nutrient	1	0.000067	0.000067	0.01	0.917
Substrat.Inoculation	1	0.010765	0.010765	1.81	0.190
Nutrient.Inoculation	1	0.020356	0.020356	3.42	0.075
Substrat.Nutrient.Inoculation	1	0.035851	0.035851	6.02	0.021
Residual	28	0.166780	0.005956		
Total	39	0.728483			

Table 2: Analysis of variance of height (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks. Log transformations were made on height.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.06109	0.01527	0.61	
Substrate	1	0.00965	0.00965	0.38	0.540
Nutrient	1	0.22240	0.22240	8.85	0.006
Inoculation	1	0.03500	0.03500	1.39	0.248
Substrat.Nutrient	1	0.01528	0.01528	0.61	0.442
Substrat.Inoculation	1	0.01276	0.01276	0.51	0.482
Nutrient.Inoculation	1	0.00042	0.00042	0.02	0.898
Substrat.Nutrient.Inoculation	1	0.0090	0.0090	0.04	0.851
Residual	28	0.70356	0.02513		
Total	39	1.06107			

Table 3: Analysis of variance of leaf number (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks. Log transformations were made on leaf number.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.18264	0.04566	1.25	
Substrate	1	0.28764	0.28764	7.85	0.009
Nutrient	1	0.12431	0.12431	3.39	0.076
Inoculation	1	0.04155	0.04155	1.13	0.296
Substrat.Nutrient	1	0.00776	0.00776	0.21	0.649
Substrat.Inoculation	1	0.01505	0.01505	0.41	0.527
Nutrient.Inoculation	1	0.03486	0.03486	0.95	0.338
Substrat.Nutrient.Inoculation	1	0.00008	0.00008	0.00	0.963
Residual	28	1.02587	0.03664		
Total	39	1.71977			

Table 4: Analysis of variance of leaf area (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	9255	2314	1.47	
Substrate	1	38925	38925	24.76	<0.001
Nutrient	1	147477.	147477	93.80	<0.001
Inoculation	1	220	220	0.14	0.711
Substrat.Nutrient	1	12	12	0.01	0.931
Substrat.Inoculation	1	582.	582	0.37	0.548
Nutrient.Inoculation	1	246	246	0.16	0.695
Substrat.Nutrient.Inoculation	1	1503	1503	0.96	0.337
Residual	28	44022	1572		
Total	39	242242			

Table 5: Analysis of variance of average leaf area (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	49.619	12.405	1.37	
Substrate	1	21.691	21.691	2.40	0.133
Nutrient	1	261.667	261.667	28.91	<0.001
Inoculation	1	5.615	5.615	0.62	0.438
Substration.Nutrient	1	3.610	3.610	0.40	0.533
Substrate.Inoculation	1	0.883	0.883	0.10	0.757
Nutrient.Inoculation	1	5.137	5.137	0.57	0.458
Substrate.Nutrient.Inoculation	1	5.834	5.834	0.64	0.429
Residual	28	253.447	9.052		
Total	39	607.503			

Table 6: Analysis of variance of shoot dry mass (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks. Log transformations were made on shoot dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.05359	0.01340	0.61	
Substrate	1	0.78774	0.78774	35.95	<0.001
Nutrient	1	2.52272	2.52272	115.13	<0.001
Inoculation	1	0.00785	0.00785	0.36	0.554
Substrat.Nutrient	1	0.04069	0.04069	1.86	0.184
Substrat.Inoculation	1	0.01020	0.01020	0.47	0.501
Nutrient.Inoculation	1	0.00140	0.00140	0.06	0.802
Substrat.Nutrient.Inoculation	1	0.01828	0.01828	0.83	0.369
Residual	28	0.61353	0.02191		
Total	39	4.05600			

Table 7: Analysis of variance of root dry mass (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.20558	0.05139	3.89	
Substrate	1	0.01862	0.01862	1.41	0.245
Nutrient	1	0.09168	0.09168	6.94	0.014
Inoculation	1	0.00770	0.00770	0.58	0.452
Substrat.Nutrient	1	0.02102	0.02102	1.59	0.218
Substrat.Inoculation	1	0.00203	0.00203	0.15	0.698
Nutrient.Inoculation	1	0.00140	0.00140	0.11	0.747
Substrat.Nutrient.Inoculation	1	0.02416	0.02416	1.83	0.187
Residual	28	0.37004	0.01322		
Total	39	0.74223			

Table 8: Analysis of variance of total dry mass (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.2914	0.0728	0.70	
Substrate	1	1.9154	1.9154	18.43	<0.001
Nutrient	1	6.8932	6.8932	66.33	<0.001
Inoculation	1	0.0014	0.0014	0.01	0.909
Substrat.Nutrient	1	0.0285	0.0285	0.27	0.605
Substrat.Inoculation	1	0.0196	0.0196	0.19	0.668
Nutrient.Inoculation	1	0.0098	0.0098	0.09	0.761
Substrat.Nutrient.Inoculation	1	0.1629	0.1629	1.57	0.221
Residual	28	2.9097	0.1039		
Total	39	12.2317			

Table 9: Analysis of variance of root:shoot ratio (for Table 3.2.1) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at eight weeks. Square root transformations were made on root:shoot ratio.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.083722	0.020930	5.93	
Substrate	1	0.021195	0.021195	6.01	0.021
Nutrient	1	0.044350	0.044350	12.57	0.001
Inoculation	1	0.008461	0.008461	2.40	0.133
Substrat.Nutrient	1	0.000670	0.000670	0.19	0.666
Substrat.Inoculation	1	0.006013	0.006013	1.70	0.202
Nutrient.Inoculation	1	0.001574	0.001574	0.45	0.510
Substrat.Nutrient.Inoculation	1	0.007140	0.007140	2.02	0.166
Residual	28	0.098798	0.003528		
Total	39	0.271923			

Table 10: Analysis of variance of stem diameter (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks. Log transformations were made on stem diameter.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.016593	0.004148	0.70	
Substrate	1	0.219291	0.219291	37.08	<0.001
Nutrient	1	0.249584	0.249584	42.21	<0.001
Inoculation	1	0.000684	0.000684	0.12	0.736
Substrat.Nutrient	1	0.020800	0.020800	3.52	0.071
Substrat.Inoculation	1	0.000022	0.000022	0.00	0.952
Nutrient.Inoculation	1	0.009877	0.009877	1.67	0.207
Substrat.Nutrient.Inoculation	1	0.007906	0.007906	1.34	0.257
Residual	28	0.165578	0.005913		
Total	39	0.690334			

Table 11: Analysis of variance of height (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks. Log transformations were made on height.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.13010	0.03252	0.42	
Substrate	1	0.68476	0.68476	18.20	<0.001
Nutrient	1	0.67499	0.67499	17.94	<0.001
Inoculation	1	0.03041	0.03041	0.81	0.376
Substrat.Nutrient	1	0.01094	0.01094	0.29	0.594
Substrat.Inoculation	1	0.11605	0.11605	3.08	0.090
Nutrient.Inoculation	1	0.00945	0.00945	0.25	0.620
Substrat.Nutrient.Inoculation	1	0.00726	0.00726	0.19	0.664
Residual	28	1.05350	0.03763		
Total	39	2.71745			

Table 12: Analysis of variance of leaf number (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	282.15	70.54	1.23	
Substrate	1	756.90	756.90	13.23	<0.001
Nutrient	1	336.40	336.40	5.88	0.022
Inoculation	1	40.00	40.00	0.70	0.410
Substrat.Nutrient	1	108.90	108.90	1.90	0.179
Substrat.Inoculation	1	4.90	4.90	0.09	0.772
Nutrient.Inoculation	1	0.40	0.40	0.01	0.934
Substrat.Nutrient.Inoculation	1	0.90	0.90	0.02	0.901
Residual	28	1601.85	57.21		
Total	39	3132.40			

Table 13: Analysis of variance of leaf area (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	31610	7903	2.52	
Substrate	1	317036	317036	101.30	<0.001
Nutrient	1	407132	407132	130.08	<0.001
Inoculation	1	7659	7659	2.45	0.129
Substrat.Nutrient	1	29079	29079	9.29	0.005
Substrat.Inoculation	1	1415	1415	0.45	0.507
Nutrient.Inoculation	1	2075	2075	0.66	0.422
Substrat.Nutrient.Inoculation	1	1	1	0.00	0.986
Residual	28	87635	3130		
Total	39	883642			

Table 14: Analysis of variance of average leaf area (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	176.07	44.02	2.96	
Substrate	1	65.34	65.34	4.39	0.045
Nutrient	1	226.92	226.92	15.24	0.001
Inoculation	1	8.42	8.42	0.57	0.458
Substrate.Nutrient	1	10.99	10.99	0.74	0.398
Substrate.Inoculation	1	5.03	5.03	0.34	0.566
Nutrient.Inoculation	1	1.35	1.35	0.09	0.766
Substrate.Nutrient.Inoculation	1	1.63	1.63	0.11	0.743
Residual	28	416.90	14.89		
Total	39	912.65			

Table 15: Analysis of variance of shoot dry mass (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks. Log transformations were made on height.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.09555	0.02389	1.69	
Substrate	1	2.57689	2.57689	182.54	<0.001
Nutrient	1	2.56902	2.56902	181.98	<0.001
Inoculation	1	0.00148	0.00148	0.10	0.749
Substrat.Nutrient	1	0.00638	0.00638	0.45	0.507
Substrat.Inoculation	1	0.03926	0.03926	2.78	0.107
Nutrient.Inoculation	1	0.00013	0.00013	0.01	0.923
Substrat.Nutrient.Inoculation	1	0.00001	0.00001	0.00	0.976
Residual	28	0.39527	0.01412		
Total	39	5.68399			

Table 16: Analysis of variance of root dry mass (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.8191	0.2048	1.74	
Substrate	1	0.9275	0.9275	7.87	0.009
Nutrient	1	1.1652	1.1652	9.89	0.004
Inoculation	1	0.0062	0.0062	0.05	0.821
Substrat.Nutrient	1	0.0143	0.0143	0.12	0.730
Substrat.Inoculation	1	0.0006	0.0006	0.01	0.943
Nutrient.Inoculation	1	0.0907	0.0907	0.77	0.388
Substrat.Nutrient.Inoculation	1	0.0577	0.0577	0.49	0.490
Residual	28	3.2993	0.1178		
Total	39	6.3807			

Table 17: Analysis of variance of total dry mass (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	3.9350	0.9837	2.12	
Substrate	1	48.1386	48.1386	103.57	<0.001
Nutrient	1	50.0797	50.0797	107.75	<0.001
Inoculation	1	0.0751	0.0751	0.16	0.691
Substrat.Nutrient	1	1.2156	1.2156	2.62	0.117
Substrat.Inoculation	1	0.6034	0.6034	1.30	0.264
Nutrient.Inoculation	1	0.0559	0.0559	0.12	0.731
Substrat.Nutrient.Inoculation	1	0.1549	0.1549	0.33	0.568
Residual	28	13.0142	0.4648		
Total	39	117.2723			

Table 18: Analysis of variance of root:shoot ratio (for Table 3.2.4) of EM inoculated and uninoculated *E. camaldulensis* seedlings grown in two substrates: SP and VP and two nutrient regimes: 10 mg l^{-1} P and 20 mgl^{-1} P, harvested at 16 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.056645	0.014161	1.77	
Substrate	1	0.000035	0.000035	0.00	0.948
Nutrient	1	0.175061	0.175061	21.91	0.001
Inoculation	1	0.192945	0.192945	24.15	<0.001
Substrat.Nutrient	1	0.010656	0.010656	1.33	0.258
Substrat.Inoculation	1	0.015713	0.015713	1.97	0.172
Nutrient.Inoculation	1	0.001267	0.001267	0.16	0.694
Substrat.Nutrient.Inoculation	1	0.010586	0.010586	1.32	0.259
Residual	28	0.223700	0.007989		
Total	39	0.686608			

APPENDIX E

Table 1: Substrate-nutrient interaction on leaf area at the 16-week harvest of experiment 2 with *E. camaldulensis*. SP= sand-perlite; VP= vermiculite-peat; low P= 10 mg l^{-1} P and high P= 30 mg l^{-1} P. Values for each variable followed by different letters differ significantly at *P*≤0.05 (ANOVA).

Substrate	Nutrient	
	Low P	High P
SP	223.3*a*	371.2*b*
VP	347.5*b*	603.2*c*

Table 2: Means of stem diameter (DIAM, mm), height (HT, cm), leaf number (LFNO), leaf area (LFAR, cm^2), shoot dry mass (SDM, g), root dry mass (RDM, g), total dry mass (TDM, g) and root shoot ratio (RSR) of *E. camaldulensis* seedlings under two nutrient regimes: low P (10 mg l^{-1} P) and high P (30 mg l^{-1} P) at the 16-week harvest of experiment 2. Values for each variable followed by different letters differ significantly at *P*≤0.05 (ANOVA).

Variable	Nutrient	
	Low P	High P
DIAM	3.388*a*	3.984*b*
HT	45.30*a*	59.30*a*
LFNO	21.40*a*	27.20*b*
LFAR	285.4*a*	487.2*b*
SDM	2.901*a*	4.797*b*
RDM	1.460*a*	1.801*b*
TDM	4.360*a*	6.600*b*
RSR	0.525*b*	0.393*a*

APPENDIX F

Table 1: Analysis of variance of stem diameter (for Table 3.3.1) of *E. camaldulensis* seedlings under 11 fungal inoculation treatments and the uninoculated control (Experiment 3).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	5	0.09723	0.01945	0.66	
Inoculation	11	0.19923	0.01811	0.61	0.809
Residual	55	1.62241	0.02950		
Total	71	1.91886			

Table 2: Analysis of variance of height (for Table 3.3.1) of *E. camaldulensis* seedlings under 11 fungal inoculation treatments and the uninoculated control (Experiment 3).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	5	116.56	23.31	1.07	
Inoculation	11	213.93	19.45	0.90	0.550
Residual	55	1194.50	21.72		
Total	71	1524.99			

Table 3: Analysis of variance of leaf number (for Table 3.3.1) of *E. camaldulensis* seedlings under 11 fungal inoculation treatments and the uninoculated control (Experiment 3).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	5	84.403	16.881	2.00	
Inoculation	11	109.819	9.984	1.18	0.320
Residual	55	464.097	8.438		
Total	71	658.319			

Table 4: Analysis of variance of leaf area (for Table 3.3.1) of *E. camaldulensis* seedlings under 11 fungal inoculation treatments and the uninoculated control (Experiment 3).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	5	331.9	66.4	0.22	
Inoculation	11	3140.1	285.5	0.95	0.502
Residual	55	16535.5	300.6		
Total	71	20007.5			

Table 5: Analysis of variance of total dry mass (for Table 3.3.1) of *E. camaldulensis* seedlings under 11 fungal inoculation treatments and the uninoculated control (Experiment 3).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	5	0.70844	0.14169	6.68	
Inoculation	11	0.41120	0.03738	1.76	0.087
Residual	49(6)	1.03937	0.02121		
Total	65(6)	2.14827			

APPENDIX G

Table 1: Analysis of variance of extent of colonisation (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	938.90	234.73	4.21	
Inoculation	3	29944.93	9981.64	179.15	<0.001
Nutrient	3	837.51	279.17	5.01	0.004
Inoculation.Nutrient	9	1151.52	127.95	2.30	0.028
Residual	59(1)	3287.25	55.72		
Total	78(1)	3 5708.07			

Table 2: Analysis of variance of stem diameter (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	4.4620	1.1155	6.11	
Inoculation	3	2.6503	0.8834	4.84	0.004
Nutrient	3	19.2591	6.4197	35.14	<0.001
Inoculation.Nutrient	9	1.2485	0.1387	0.76	0.654
Residual	59(1)	10.7786	0.1827		
Total	78(1)	38.1337			

Table 3: Analysis of variance of height (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	344.80	86.20	2.11	
Inoculation	3	101.16	33.72	0.82	0.486
Nutrient	3	8179.56	2726.52	66.64	<0.001
Inoculation.Nutrient	9	576.73	64.08	1.57	0.147
Residual	59(1)	2414.09	40.92		
Total	78(1)	11445.56			

Table 4: Analysis of variance of leaf number (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	104.92	26.23	0.71	
Inoculation	3	74.78	24.93	0.67	0.571
Nutrient	3	2099.96	699.99	18.94	<0.001
Inoculation.Nutrient	9	171.84	19.09	0.52	0.857
Residual	59(1)	2180.23	36.95		
Total	78(1)	4614.76			

Table 5: Analysis of variance of leaf area (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks. Log transformation was made on leaf area.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.07835	0.01959	1.28	
Inoculation	3	0.48568	0.16189	10.62	<0.001
Nutrient	3	13.22037	4.40679	289.06	<0.001
Inoculation.Nutrient	9	0.25690	0.02854	1.87	0.074
Residual	59(1)	0.89947	0.01525		
Total	78(1)	14.87292-			

Table 6: Analysis of variance of shoot dry mass (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks. Log transformation was made on shoot dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.04235	0.01059	0.65	
Inoculation	3	0.52243	0.17414	10.69	<0.001
Nutrient	3	14.56958	4.85653	298.23	<0.001
Inoculation.Nutrient	9	0.41936	0.04660	2.86	0.007
Residual	59(1)	0.96079	0.01628		
Total	78(1)	16.43600			

Table 7: Analysis of variance of root dry mass (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks. Log transformation was made on root dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.08796	0.02199	0.40	
Inoculation	3	0.65834	0.21945	3.99	0.012
Nutrient	3	12.90995	4.30332	78.21	<0.001
Inoculation.Nutrient	9	0.45608	0.05068	0.92	0.514
Residual	59(1)	3.24639	0.05502		
Total	78(1)	17.28197			

Table 8: Analysis of variance of total dry mass (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks. Log transformation was made on total dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.01902	0.00475	0.39	
Inoculation	3	0.56674	0.18891	15.60	<0.001
Nutrient	3	14.03468	4.67823	386.39	<0.001
Inoculation.Nutrient	9	0.35055	0.03895	3.22	0.003
Residual	59(1)	0.71434	0.01211		
Total	78(1)	15.60949			

Table 9: Analysis of variance of RGR (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.198E-05	0.495E-06	0.39	
Inoculation	3	0.590E-04	0.197E-04	15.60	<0.001
Nutrient	3	0.146E-02	0.487E-03	386.39	<0.001
Inoculation.Nutrient	9	0.365E-04	0.406E-05	3.22	0.003
Residual	59(1)	0.744E-04	0.126E-05		
Total	78(1)	0.163E-02			

Table 10: Analysis of variance of NAR (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.07246	0.01812	1.23	
Inoculation	3	0.22677	0.07559	5.14	0.003
Nutrient	3	0.89783	0.29928	20.33	<0.001
Inoculation.Nutrient	9	0.09982	0.01109	0.75	0.659
Residual	59(1)	0.86852	0.01472		
Total	78(1)	2.15853			

Table 11: Analysis of variance of LAR (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks. Log transformation was made on LAR.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.046991	0.011748	1.30	
Inoculation	3	1.20355	0.040118	4.45	0.007
Nutrient	3	0.80838	0.026946	2.99	0.038
Inoculation.Nutrient	9	0.055862	0.006207	0.69	0.717
Residual	59(1)	0.532144	0.009019		
Total	78(1)	0.835970			

Table 12: Analysis of variance of SLA (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	676.4	169.1	1.45	
Inoculation	3	1269.5	423.2	3.64	0.018
Nutrient	3	0.80838	0.026946	2.99	0.038
Inoculation.Nutrient	3	270.5	90.2	0.77	0.513
Residual	59(1)	6865.6	116.4		
Total	78(1)	10748.0			

Table 13: Analysis of variance of LMR (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.012320	0.003080	0.94	
Inoculation	3	0.001366	0.000455	0.14	0.936
Nutrient	3	0.008642	0.002881	0.88	0.456
Inoculation.Nutrient	9	0.016545	0.001838	0.56	0.822
Residual	59(1)	0.192639	0.003265		
Total	78(1)	0.230141			

Table 14: Analysis of variance of RMR (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.006287	0.001572	0.47	
Inoculation	3	0.001477	0.000492	0.15	0.931
Nutrient	3	0.008567	0.002856	0.85	0.471
Inoculation.Nutrient	9	0.016780	0.001864	0.56	0.827
Residual	59(1)	0.197834	0.003353		
Total	78(1)	0.230938			

Table 15: Analysis of variance of RSR (for Table 4.1.1) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.02385	0.00596	0.37	
Inoculation	3	0.00835	0.00278	0.17	0.913
Nutrient	3	0.04798	0.01599	1.00	0.397
Inoculation.Nutrient	9	0.06903	0.00767	0.48	0.881
Residual	59(1)	0.93979	0.01593		
Total	78(1)	1.08893			

Table 16: Analysis of variance of foliar N concentration (for Table 4.1.6) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks. Log transformation was made on foliar N concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	5.60705	1.40176	63.66	
Inoculation	3	0.34421	0.11474	5.21	0.003
Nutrient	3	0.86950	0.28983	13.16	<0.001
Inoculation.Nutrient	9	0.09711	0.01079	0.49	0.876
Residual	59(1)	1.29920	0.02202		
Total	78(1)	8.19522			

Table 17: Analysis of variance of foliar P concentration (for Table 4.1.6) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.0065353	0.0016338	5.13	
Inoculation	3	0.0062019	0.0057983	18.22	<0.001
Nutrient	3	0.0173949	0.28983	13.16	<0.001
Inoculation.Nutrient	9	0.0009210	0.0001023	0.32	0.965
Residual	59(1)	0.0187738	0.0003182		
Total	78(1)	0.0498230			

Table 18: Analysis of variance of foliar K concentration (for Table 4.1.6) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.72615	0.43154	6.15	
Inoculation	3	0.10020	0.03340	0.48	0.700
Nutrient	3	1.25300	0.41767	5.96	0.001
Inoculation.Nutrient	9	0.80398	0.08933	1.27	0.270
Residual	59(1)	4.13700	0.07012		
Total	78(1)	7.87002			

Table 19: Analysis of variance of foliar N content (for Table 4.1.6) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks. Log transformation was made on foliar N concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	32.6486	8.1621	37.16	
Inoculation	3	1.4401 1	0.4800	2.19	0.099
Nutrient	3	172.0475	57.3492	261.07	<0.001
Inoculation.Nutrient	9	2.5595	0.2844	1.29	0.259
Residual	59(1)	12.9607	0.2197		
Total	78(1)	220.0871			

Table 20: Analysis of variance of foliar P content (for Table 4.1.6) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.068346	0.017087	4.75	
Inoculation	3	0.018959	0.006320	1.76	0.165
Nutrient	3	2.653295	0.884432	246.06	<0.001
Inoculation.Nutrient	9	0.025305	0.002812	0.78	0.633
Residual	59(1)	0.212071	0.003594		
Total	78(1)	2.967578			

Table 21: Analysis of variance of foliar K content (for Table 4.1.6) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *G. rosea* FL 105-5, a mixed culture from Bangladesh and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	11.5722	2.8930	3.55	
Inoculation	3	9.4056	3.1352	3.85	0.014
Nutrient	3	306.4852	102.1617	125.52	<0.001
Inoculation.Nutrient	9	6.5921	0.7325	0.90	0.531
Residual	59(1)	48.0188	0.8139		
Total	78(1)	376.9897			

APPENDIX H

Table 1: Analysis of variance of extent of colonisation (for section 4.2.2.1) of *E. camaldulensis* seedlings by *P. tinctorius* K55 under four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	461.30	115.32	2.08	
Nutrient	3	1110.80	370.27	6.68	0.007
Residual	12	664.70	55.39		
Total	19	2236.80			

Table 2: Analysis of variance of stem diameter (for Table 4.2.1) of *E. camaldulensis* seedlings under six inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	18.1905	4.5476	21.75	
Inoculation	5	2.8828	0.5766	2.76	0.023
Nutrient	3	84.1070	28.0357	134.11	<0.001
Inoculation.Nutrient	15	2.2577	0.1505	0.72	0.759
Residual	92	19.2323	0.2090		
Total	119	126.6704			

Table 3: Analysis of variance of height (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on height.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.18904	0.04726	1.79	
Inoculation	5	0.06241	0.01248	0.47	0.796
Nutrient	3	6.60482	2.20161	83.38	<0.001
Inoculation.Nutrient	15	0.39653	0.02644	1.00	0.461
Residual	92	2.42913	0.02640		
Total	119	9.68194			

Table 4: Analysis of variance of leaf number (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on leaf number.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.04984	0.01246	0.31	
Inoculation	5	0.11815	0.02363	0.58	0.713
Nutrient	3	5.42645	1.80882	44.67	<0.001
Inoculation.Nutrient	15	0.71547	0.04770	1.18	0.303
Residual	92	3.72568	0.04050		
Total	119	10.03558			

Table 5: Analysis of variance of leaf area (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on leaf area.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.06312	0.01578	0.68	
Inoculation	5	0.08117	0.01623	0.70	0.626
Nutrient	3	22.20166	7.40055	318.33	<0.001
Inoculation.Nutrient	15	0.46898	0.03127	1.34	0.192
Residual	92	2.13885	0.02325		
Total	119	24.95378			

Table 6: Analysis of variance of shoot dry mass (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P .tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on shoot dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.25550	0.06388	2.15	
Inoculation	5	0.25145	0.05029	1.69	0.144
Nutrient	3	23.67891	7.889297	265.94	<0.001
Inoculation.Nutrient	15	0.81098	0.05407	1.82	0.043
Residual	92	2.73050	0.02968		
Total	119	27.7234			

Table 7: Analysis of variance of root dry mass (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on root dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.07791	0.01948	0.57	
Inoculation	5	0.34431	0.06886	2.01	0.085
Nutrient	3	23.11686	7.70562	224.88	<0.001
Inoculation.Nutrient	15	0.72516	0.04834	1.41	0.159
Residual	92	3.15243	0.03427		
Total	119	27.41668			

Table 8: Analysis of variance of total dry mass (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on total dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.09009	0.02252	1.14	
Inoculation	5	0.11416	0.02283	1.16	0.335
Nutrient	3	23.38200	7.79400	396.13	<0.001
Inoculation.Nutrient	15	0.39971	0.02665	1.35	0.187
Residual	92	1.81014	0.01968		
Total	119	25.79610			

Table 9: Analysis of variance of RGR (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.567E-05	0.142E-05	1.14	
Inoculation	5	0.719E-05	0.144E-05	1.16	0.335
Nutrient	3	0.147E-02	0.491E-03	396.13	<0.001
Inoculation.Nutrient	15	0.252E-04	0.168E-05	1.35	0.187
Residual	92	0.114E-03	0.124E-05		
Total	119	0.162E-02			

Table 10: Analysis of variance of NAR (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.08767	0.02192	0.98	
Inoculation	5	0.03012	0.00602	0.27	0.929
Nutrient	3	1.49083	0.49694	22.20	<0.001
Inoculation .Nutrient	15	0.33417	0.02228	1.00	0.467
Residual	92	2.05945	0.02239		
Total	119	4.00224			

Table 11: Analysis of variance of LAR (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	67.48	16.87	0.76	
Inoculation	5	25.00	5.00	0.22	0.951
Nutrient	3	270.61	90.20	4.04	0.010
Inoculation.Nutrient	15	359.50	23.97	1.07	0.392
Residual	92	2055.30	22.34		
Total	119	2777.89			

Table 12: Analysis of variance of SLA (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on SLA.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.03776	0.00944	0.64	
Inoculation	5	0.16773	0.03355	2.26	0.055
Nutrient	3	0.20939	0.06980	4.69	0.004
Inoculation.Nutrient	15	0.31690	0.02113	1.42	0.154
Residual	92	1.36777	0.01487		
Total	119	2.09955			

Table 13: Analysis of variance of LMR (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.009547	0.002387	1.30	
Inoculation	5	0.018565	0.003713	2.02	0.083
Nutrient	3	0.013965	0.004655	2.54	0.062
Inoculation.Nutrient	15	0.045245	0.003016	1.64	0.077
Residual	92	0.168859	0.001835		
Total	119	0.256181			

Table 14: Analysis of variance of RMR (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.021262	0.005316	1.87	
Inoculation	5	0.042629	0.008526	3.01	0.015
Nutrient	3	0.003352	0.001117	0.39	0.758
Inoculation Nutrient	15	0.088026	0.005868	2.07	0.018
Residual	92	0.260943	0.002836		
Total	119	0.416212			

Table 15: Analysis of variance of RSR (for Table 4.2.1) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Square root transformation was made on RSR.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.067959	0.016990	1.89	
Inoculation	5	0.135058	0.027012	3.00	0.015
Nutrient	3	0.011561	0.003854	0.43	0.733
Inoculation.Nutrient	15	0.278276	0.018552	2.06	0.019
Residual	92	0.828177	0.009002		
Total	119	1.321032			

Table 16: Analysis of variance of foliar N concentration (for Table 4.2.6) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on foliar N concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	13.75877	3.43969	139.02	
Inoculation	5	0.02565	0.00513	0.21	0.959
Nutrient	3	1.67968	0.55989	22.63	<0.001
Inoculation.Nutrient	15	0.36579	0.02439	0.99	0.477
Residual	92	2.27622	0.02474		
Total	119	18.10611			

Table 17: Analysis of variance of foliar P concentration (for Table 4.2.6) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on foliar P concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.5373	0.1343	1.25	
Inoculation	5	0.4171	0.0834	0.78	0.559
Nutrient	3	3.2028	1.0676	9.95	<0.001
Inoculation.Nutrient	15	0.7794	0.0520	0.48	0.965
Residual	92	9.8750	0.1073		
Total	119	14.8116			

Table 18: Analysis of variance of foliar K concentration (for Table 4.2.6) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on foliar K concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.15236	0.03809	0.65	
Inoculation	5	0.34341	0.06868	1.17	0.328
Nutrient	3	0.29823	0.09941	1.70	0.172
Inoculation.Nutrient	15	0.56050	0.03737	0.64	0.835
Residual	92	5.37770	0.05845		
Total	119	6.73221			

Table 19: Analysis of variance of foliar N content (for Table 4.2.6) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.07957	0.01989	0.49	
Inoculation	5	0.19532	0.03906	0.97	0.440
Nutrient	3	5.16264	1.72088	42.74	<0.001
Inoculation .Nutrient	15	0.44331	0.02955	0.73	0.744
Residual	92	3.70385	0.04026		
Total	119	9.58469			

Table 20: Analysis of variance of foliar P content (for Table 4.2.6) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on foliar P concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	88.461	22.115	61.40	
Inoculation	5	1.510	0.302	0.84	0.526
Nutrient	3	297.895	99.298	275.68	<0.001
Inoculation .Nutrient	15	2.305	0.154	0.43	0.967
Residual	92	33.137	0.360		
Total	119	423.307			

Table 21: Analysis of variance of foliar K content (for Table 4.2.6) of *E. camaldulensis* seedlings under four inoculation treatments: five different isolates of *P. tinctorius* (K55, PTE, PT3, PT7 and PT8) and the uninoculated control, and four nutrient treatments: 2.5 mg l^{-1} P, 5.0 mg l^{-1} P, 10 mg l^{-1} P and 20 mgl^{-1} P; harvested at 18 weeks. Log transformation was made on foliar K concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.645	0.411	0.09	
Inoculation	5	29.802	5.960	1.28	0.281
Nutrient	3	378.057	126.019	26.99	<0.001
Inoculation .Nutrient	15	78.410	5.227	1.12	0.351
Residual	92	429.597	4.670		
Total	119	917.511			

APPENDIX I

Table 1: Analysis of variance of extent of colonisation (for Table 5.1) of *E. camaldulensis* seedlings by the AM fungus *G. clarum* BR 148-1 under two inoculation treatments: AM alone (*G. clarum* BR 148-1) and the dual inoculation (*G. clarum* BR 148-1 with *P. tinctorius* K55), *P. tinctorius* K55), and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	275.01	68.75	1.91	
Inoculation	1	66.50	66.50	1.85	0.189
Nutrient	2	2328.67	1164.34	32.42	<0.001
Inoculation.Nutrient	2	136.94	68.47	1.91	0.175
Residual	20	718.36	35.92		
Total	29	3525.50			

Table 2: Analysis of variance of extent of colonisation (for Table 5.1) of *E. camaldulensis* seedlings by the AM fungus *G. clarum* BR 148-1 under two inoculation treatments: AM alone (*G. clarum* BR 148-1) and the dual inoculation (*G. clarum* BR 148-1 with *P. tinctorius* K55), *P. tinctorius* K55), and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	820.06	205.01	3.64	
Inoculation	1	83.33	83.33	1.48	0.238
Nutrient	2	76.05	38.03	0.68	0.520
Inoculation.Nutrient	2	14.87	7.43	0.13	0.877
Residual	20	1125.23	56.26		
Total	29	2119.54			

Table 3: Analysis of variance of extent of colonisation (for Table 5.1) of *E. camaldulensis* seedlings by the EM fungus *P. tinctorius* K55 under two inoculation treatments: EM alone (*P. tinctorius* K55) and the dual inoculation (*P. tinctorius* K55 with *G. clarum* BR 148-1), and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	2.4667	0.6167	0.64	
Inoculation	1	0.0000	0.0000	0.00	1.000
Nutrient	2	80.2667	40.1333	41.95	<0.001
Inoculation.Nutrient	2	5.6000	2.8000	2.93	0.077
Residual	20	19.1333	0.9567		
Total	29	107.4667			

Table 4: Analysis of variance of extent of colonisation (for Table 5.1) of *E. camaldulensis* seedlings by the EM fungus *P. tinctorius* K55 under two inoculation treatments: EM alone (*P. tinctorius* K55) and the dual inoculation (*P. tinctorius* K55 with *G. clarum* BR 148-1), and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	9.533	2.383	1.18	
Inoculation	1	1.633	1.633	0.81	0.38
Nutrient	2	416.867	208.433	103.01	<0.001
Inoculation.Nutrient	2	10.867	5.433	2.69	0.093
Residual	20	40.467	2.023		
Total	29	479.367			

Table 5: Analysis of variance of stem diameter (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	6.5897	1.6474	13.51	
Inoculation	3	3.7764	1.2588	10.32	<0.001
Nutrient	2	12.8970	6.4485	52.87	<0.001
Inoculation.Nutrient	6	0.7054	0.1176	0.96	0.461
Residual	44	5.3665	0.1220		
Total	59	29.3351			

Table 6: Analysis of variance of height (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks. Log transformation was made on height.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.15619	0.03905	1.84	
Inoculation	3	0.38897	0.12966	6.12	0.001
Nutrient	2	4.20521	2.10261	99.29	<0.001
Inoculation.Nutrient	6	0.11931	0.01989	0.94	0.477
Residual	44	0.93177	0.02118		
Total	59	5.80146			

Table 7: Analysis of variance of leaf number (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	11.900	2.975	0.34	
Inoculation	3	37.383	12.461	1.43	0.246
Nutrient	2	569.233	284.617	32.74	<0.001
Inoculation.Nutrient	6	67.967	11.328	1.30	0.276
Residual	44	382.500	8.693		
Total	59	1068.983			

Table 8: Analysis of variance of leaf area (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks. Log transformation was made on leaf area.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.49447	0.12362	4.15	
Inoculation	3	1.35055	0.45018	15.13	<0.001
Nutrient	2	9.39574	4.69787	157.88	<0.001
Inoculation.Nutrient	6	0.17457	0.02910	0.98	0.452
Residual	44	1.30925	0.02976		
Total	59	12.72459			

Table 9: Analysis of variance of shoot dry mass (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks. Log transformation was made on shoot dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.44394	0.11099	4.06	
Inoculation	3	3.15531	1.05177	38.47	<0.001
Nutrient	2	8.181728	4.09086	149.63	<0.001
Inoculation.Nutrient	6	0.40505	0.06751	2.47	0.038
Residual	44	1.20292	0.02734		
Total	59	13.38894			

Table 10: Analysis of variance of root dry mass (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks. Log transformation was made on root dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.8915	0.4729	3.98	
Inoculation	3	3.3137	1.1046	9.30	<0.001
Nutrient	2	7.0606	3.5303	29.72	<0.001
Inoculation.Nutrient	6	0.8423	0.14048	1.18	0.333
Residual	44	5.2261	0.1188		
Total	59	18.3342			

Table 11: Analysis of variance of total dry mass (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks. Log transformation was made on total dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.49953	0.12488	5.49	
Inoculation	3	2.85635	0.95212	41.87	<0.001
Nutrient	2	8.19607	4.09804	180.20	<0.001
Inoculation.Nutrient	6	0.25887	0.04314	1.90	0.103
Residual	44	1.00061	0.02274		
Total	59	12.81143			

Table 12: Analysis of variance of RGR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.520E-04	0.130E-04	5.49	
Inoculation	3	0.297E-03	0.991E-04	41.87	<0.001
Nutrient	2	0.853E-03	0.427E-03	180.20	<0.001
Inoculation.Nutrient	6	0.270E-04	0.449E-05	1.90	0.103
Residual	44	0.104E-03	0.237E-05		
Total	59	0.133E-02			

Table 13: Analysis of variance of NAR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.15469	0.03867	3.27	
Inoculation	3	0.70477	0.23492	19.88	<0.001
Nutrient	2	0.10673	0.05337	4.52	0.016
Inoculation.Nutrient	6	0.04901	0.00817	0.69	0.658
Residual	44	0.51999	0.01182		
Total	59	1.53520			

Table 14: Analysis of variance of LAR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	519.66	129.92	2.26	
Inoculation	3	1262.90	420.97	7.33	<0.001
Nutrient	2	117.26	58.63	1.02	0.368
Inoculation.Nutrient	6	209.78	34.96	0.61	0.722
Residual	44	2525.36	57.39		
Total	59	4634.96			

Table 15: Analysis of variance of SLA (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1517.1	379.3	1.48	
Inoculation	3	5321.3	1773.8	6.93	<0.001
Nutrient	2	792.3	396.2	1.55	0.224
Inoculation.Nutrient	6	756.5	126.1	0.49	0.810
Residual	44	11265.3	256.0		
Total	59	19652.5			

Table 16: Analysis of variance (of LMR for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.020805	0.005201	1.62	
Inoculation	3	0.026055	0.008685	2.71	0.057
Nutrient	2	0.008708	0.004354	1.36	0.268
Inoculation.Nutrient	6	0.026629	0.004438	1.38	0.243
Residual	44	0.141153	0.003208		
Total	59	0.223350			

Table 17: Analysis of variance of RMR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.045915	0.011479	3.44	
Inoculation	3	0.017006	0.005669	1.70	0.181
Nutrient	2	0.000264	0.000132	0.04	0.961
Inoculation.Nutrient	6	0.043750	0.007292	2.19	0.062
Residual	44	0.146619	0.003332		
Total	59	0.253554			

Table 18: Analysis of variance of RSR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.19663	0.04916	3.43	
Inoculation	3	0.08861	0.02954	2.06	0.119
Nutrient	2	0.00129	0.00065	0.05	0.956
Inoculation.Nutrient	6	0.18211	0.03035	2.12	0.070
Residual	44	0.63090	0.01434		
Total	59	1.09954			

Table 19: Analysis of variance of foliar N concentration (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks. Log transformation was made on foliar N concentration.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.270848	0.067712	6.90	
Inoculation	3	0.108487	0.036162	3.69	<0.001
Nutrient	2	0.657720	0.328860	35.53	<0.001
Inoculation.Nutrient	6	0.056198	0.009366	0.95	0.467
Residual	44	0.431594	0.009809		
Total	59	1.524846			

Table 20: Analysis of variance of foliar P concentration (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.007893	0.001973	1.48	
Inoculation	3	0.030116	0.010039	7.55	<0.001
Nutrient	2	0.004356	0.002178	1.64	0.206
Inoculation.Nutrient	6	0.003344	0.000557	0.42	0.862
Residual	44	0.058532	0.001330		
Total	59	0.104240			

Table 21: Analysis of variance of foliar K concentration (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.06467	0.01617	0.94	
Inoculation	3	0.15981	0.05327	3.09	0.037
Nutrient	2	0.04300	0.02150	1.25	0.297
Inoculation.Nutrient	6	0.37808	0.06301	3.66	0.005
Residual	44	0.75814	0.01723		
Total	59	1.40370			

Table 22: Analysis of variance of foliar N content (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	2.1588	0.5397	4.99	
Inoculation	3	2.5883	0.8628	7.98	<0.001
Nutrient	2	28.4993	14.2496	131.78	<0.001
Inoculation.Nutrient	6	0.6797	0.1133	1.05	0.408
Residual	44	4.7578	0.1081		
Total	59	38.6839			

Table 23: Analysis of variance of foliar P content (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.024118	0.006029	1.26	
Inoculation	3	0.017065	0.005688	1.19	0.325
Nutrient	2	0.312771	0.156386	32.65	<0.001
Inoculation.Nutrient	6	0.021702	0.003617	0.76	0.609
Residual	44	0.210769	0.004790		
Total	59	0.586426			

Table 24: Analysis of variance of foliar K content (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 14 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.7233	0.4308	2.21	
Inoculation	3	6.5576	2.1859	11.20	<0.001
Nutrient	2	27.3216	13.6608	69.99	<0.001
Inoculation.Nutrient	6	2.9699	0.4950	2.54	0.034
Residual	44	8.5876	0.1952		
Total	59	47.1601			

Table 25: Analysis of variance of stem diameter (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks. Log transformation was made on stem diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.062568	0.015642	1.95	
Inoculation	3	0.256332	0.085444	10.68	<0.001
Nutrient	2	2.292485	1.146242	143.23	<0.001
Inoculation.Nutrient	6	0.012192	0.002032	0.25	0.955
Residual	44	0.352113	0.008003		
Total	59	2.975691			

Table 26: Analysis of variance of height (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks. Log transformation was made on height.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.21907	0.05477	1.23	
Inoculation	3	0.32873	0.10958	2.47	0.075
Nutrient	2	8.24444	4.12222	92.82	<0.001
Inoculation.Nutrient	6	0.26496	0.04416	0.99	0.441
Residual	44	1.95409	0.04441		
Total	59	11.01129			

Table 27: Analysis of variance of leaf number (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	90.57	22.64	1.02	
Inoculation	3	27.78	9.26	0.42	0.741
Nutrient	2	3630.90	1815.45	81.89	<0.001
Inoculation.Nutrient	6	50.97	8.49	0.38	0.886
Residual	44	975.43	22.17		
Total	59	4775.65			

Table 28: Analysis of variance of leaf area (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks. Log transformation was made on leaf area.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.9544	0.02386	0.99	
Inoculation	3	0.12673	0.04224	1.76	0.170
Nutrient	2	23.19608	11.59804	481.92	<0.001
Inoculation.Nutrient	6	0.05495	0.00916	0.38	0.887
Residual	44	1.05893	0.02407		
Total	59	24.53213			

Table 29: Analysis of variance of shoot dry mass (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks. Log transformation was made on shoot dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.30831	0.07708	2.79	
Inoculation	3	0.89760	0.29920	10.84	<0.001
Nutrient	2	21.62619	10.81310	391.90	<0.001
Inoculation.Nutrient	6	0.08959	0.01493	0.54	0.774
Residual	44	1.21403	0.02759		
Total	59	24.13572			

Table 30: Analysis of variance of root dry mass (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks. Log transformation was made on root dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.29817	0.07454	1.40	
Inoculation	3	1.89661	0.63220	11.85	<0.001
Nutrient	2	14.04339	7.02170	131.57	<0.001
Inoculation.Nutrient	6	0.08181	0.01364	0.26	0.954
Residual	44	2.34814	0.05337		
Total	59	18.66812			

Table 31: Analysis of variance of total dry mass (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks. Log transformation was made on total dry mass.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.15121	0.03780	1.86	
Inoculation	3	1.20572	0.40191	19.78	<0.001
Nutrient	2	17.78659	8.89330	437.77	<0.001
Inoculation.Nutrient	6	0.04809	0.00801	0.39	0.879
Residual	44	0.89385	0.02031		
Total	59	20.08546			

Table 32: Analysis of variance of RGR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks. Log transformation was made on RGR.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.0028744	0.0007186	1.85	
Inoculation	3	0.0215743	0.00719144	18.55	<0.001
Nutrient	2	0.3005680	0.15028403	387.61	<0.001
Inoculation.Nutrient	6	0.0013666	0.0002278	0.59	0.738
Residual	44	0.0170598	0.0003877		
Total	59	0.3434431			

Table 33: Analysis of variance of NAR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.093244	0.023311	2.77	
Inoculation	3	0.613065	0.204355	24.24	<0.001
Nutrient	2	0.057234	0.028617	3.39	0.043
Inoculation.Nutrient	6	0.030824	0.005137	0.61	0.721
Residual	44	0.370906	0.008430		
Total	59	1.165273			

Table 34: Analysis of variance of LAR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	169.00	42.25	1.66	
Inoculation	3	1057.99	352.66	13.90	<0.001
Nutrient	2	400.84	200.42	7.90	0.001
Inoculation.Nutrient	6	134.32	22.39	0.88	0.516
Residual	44	1116.62	25.38		
Total	59	2878.77			

Table 35: Analysis of variance of SLA (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1889.9	472.5	3.43	
Inoculation	3	1997.1	665.7	4.84	0.005
Nutrient	2	254.0	127.0	0.92	0.405
Inoculation.Nutrient	6	292.7	48.8	0.35	0.903
Residual	44	6052.4	137.6		
Total	59	10486.0			

Table 36: Analysis of variance of LMR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.033310	0.008327	2.24	
Inoculation	3	0.023563	0.007854	2.12	0.112
Nutrient	2	0.010439	0.005219	1.41	0.256
Inoculation.Nutrient	6	0.016815	0.002803	0.75	0.609
Residual	44	0.163372	0.003713		
Total	59	0.247499			

Table 37: Analysis of variance of RMR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.035334	0.008833	2.13	
Inoculation	3	0.022747	0.007582	1.83	0.156
Nutrient	2	0.054829	0.027415	6.62	0.003
Inoculation.Nutrient	6	0.007543	0.001257	0.30	0.932
Residual	44	0.182308	0.004143		
Total	59	0.302761			

Table 38: Analysis of variance of RSR (for Table 5.2) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.41356	0.10339	2.05	
Inoculation	3	0.20984	0.06995	1.39	0.259
Nutrient	2	0.72824	0.36412	7.22	0.002
Inoculation.Nutrient	6	0.04236	0.00706	0.14	0.990
Residual	44	2.21878	0.05043		
Total	59	3.61278			

Table 39: Analysis of variance of foliar N concentration (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.27608	0.06908	2.05	
Inoculation	3	0.37632	0.12544	3.73	0.018
Nutrient	2	1.67843	0.83921	24.95	<0.001
Inoculation.Nutrient	6	0.06255	0.01043	0.31	0.928
Residual	44	1.48005	0.03364		
Total	59	3.87342			

Table 40: Analysis of variance of foliar P concentration (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.0120526	0.0030131	4.08	
Inoculation	3	0.0191035	0.0063678	8.63	<0.001
Nutrient	2	0.004356	0.002178	23.64	<0.001
Inoculation.Nutrient	6	0.0027340	0.0004557	0.62	0.715
Residual	44	0.0324787	0.0007382		
Total	59	0.1012675			

Table 41: Analysis of variance of foliar K concentration (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.03926	0.00982	0.25	
Inoculation	3	0.26025	0.08675	2.20	0.102
Nutrient	2	2.46024	1.23012	31.15	<0.001
Inoculation.Nutrient	6	0.33751	0.05625	1.42	0.227
Residual	44	1.73772	0.03949		
Total	59	4.83498			

Table 42: Analysis of variance of foliar N content (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	1.297	0.324	1.13	
Inoculation	3	0.107	0.036	0.13	0.945
Nutrient	2	192.072	96.036	336.07	<0.001
Inoculation.Nutrient	6	0.966	0.161	0.56	0.757
Residual	44	12.573	0.286		
Total	59	207.015			

Table 43: Analysis of variance of foliar P content (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.07398	0.01849	2.27	
Inoculation	3	0.03247	0.01082	1.33	0.277
Nutrient	2	2.65703	1.32851	163.20	<0.001
Inoculation.Nutrient	6	0.02366	0.00394	0.48	0.816
Residual	44	0.35819	0.00814		
Total	59	3.14532			

Table 44: Analysis of variance of foliar K content (for Table 5.8) of *E. camaldulensis* seedlings under four inoculation treatments: *G. clarum* BR 148-1, *P. tinctorius* K55, *G. clarum* BR 148-1 with *P. tinctorius* K55 and the uninoculated control, and three nutrient treatments: 0.5 mg l^{-1} P, 2.5 mg l^{-1} P and 10 mg l^{-1} P; harvested at 24 weeks.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	4	0.182	0.045	0.10	
Inoculation	3	1.878	0.626	1.37	0.263
Nutrient	2	272.042	136.021	298.40	<0.001
Inoculation.Nutrient	6	3.612	0.602	1.32	0.268
Residual	44	20.056	0.456		
Total	59	297.77			