IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(P): 2347-4580; ISSN(E): 2321-8851
Vol. 4, Issue 11, Nov 2016, 43-52

EFFICIENCY OF ARBUSCULAR MYCORRHIZAL FUNGI IN PROMOTING GROWTH OF SEEDLINGS OF SOME CALCIFUGE *EUCALYPTUS SPECIES* IN A CALCAREOUS SOIL

DJAMILA ADJOUD-SADADOU & ROSA HALLI-HARGAS

Department of Zoology, University of kalyani, West Bengal, India

ABSTRACT

These paper present informations on the role of soil microbiota with a special relevance to arbuscular mycorrhizal fungi on growth of eucalypt species in calcareous soil. Most species of this genus are calcifuge. Four *Eucalyptus* species were used in this study: three are calcifuge and one calcicole. Seedlings have been grown in soil sterilized or not. Soil was collected around mature trees, in a stand presenting a calcareous soil. Seedling growth (height, stem dry weight, root length and number of secondary roots) and mycorrhization were monitored over 80 days. Results revealed that growth of the calcifuge species was not affected in non sterilized soil but severely hampered in sterilized soil. Seedlings of the calcicole *Eucalyptus* species do not present chlorosis in sterilized soil but its growth was significantly enhanced in non-sterilized soil. All seedlings grown in non sterilized soil were mycorrhizal but rates were higher for calcifuge species. These results suggest that arbuscular mycorrhizal fungi have a beneficial impact on tolerance of calcifuge *Eucalyptus*. Species to soil calcium carbonate and promote their growth.

KEYWORDS: Arbuscular Mycorrhiza, Calcareous Soils, Calcicole Species, Calcifuge Species, *Eucalyptus*

INTRODUCTION

Toxic effects of calcareous soils on calcifuge plants has been attributed to their low capacity to limit calcium absorption (Rossignol et al., 1977, Chevalier & Paris, 1981), and have been related to both calcium ions (Jefferies & Willis, 1964; Clarkson, 1965) and bicarbonate ions (Mengel et al., 1984). The difference in the ability of plants to grow on calcareous soils could be explained by the physiology of their roots in the acquisition of nutrients (Gutschick 1973). In calcareous soils P is fixed by calcium carbonate through adsorption and precipitation that gradually decreases its solubility and consequently its availability (Labidi et al., 2012).

Mycorrhizal fungi can dissolve many toxic metal bearing minerals (Fomina et al., 2004; Gad, 2007). Mycorrhizal symbiosis increases the soil volume explored by the plant roots beyond the phosphorus depletion zone (Kothary et al., 1991; Li et al., 1991). The development of hyphal networks inside and around mycorrhizal roots improve P and N uptake by mycorrhizal plants (Johansen et al., 1993; Smith and Read 2008). Anion and cation absorption by the mycorrhizal plants can alter the rhizosphere pH which may affect the availability of P to plants (Lapeyrie et al 1990). Production of phosphatases by mycorrhizal plants can solubilize the organic P (Taraphdar et al., 1994) and enhance its availability (Marshner et al., 1994). Association of calcifuge woody plant species with ectomycorrhizal fungi induced tolerance to calcareous soils. This has been reported for *Pinus nigra nigricans* (Clement et al., 1977; Le Tacon, 1978) and *Eucalyptus dumosa* (Lapeyrie & Chilvers, 1985).

Most species of the genus *Eucalyptus* are calcifuge species (Pryor, 1976). During a field investigation, we have observed mature trees of *E. camaldulensis* which is known as a calcifuge species, in a patch of calcareous soil. Following this observation, a question arose: what enables this eucalyptus species which is known as a calcifuge one to grow on this calcareous soil? A previous study of the mycorrhizal status of these eucalypts have revealed that they were associated with many arbuscular mycorrhizal fungi (Adjoud-Sadadou and Halli-Hargas, 2000).

In order to understand and deepen the question, in the present experiment the behaviour of some eucalypts, calcicole and calcifuge species, was checked by transplanting them in this calcareous sol taken around eucalypts, thus containing the natural inoculum and in the same soil sterilized (without inocculum).

MATERIALS AND METHODS

Experimental Design

The soil used in this experiment was collected in a patch of calcareous (127g/Kg $CaCO_3$ total) and P deficient (0,048 mg $g^{-1}P_2O_5$ Dyert) soil supporting *Eucalyptus camaldulensis* species. A lot of soil samples were taken at about 1.5 m from the base of the trunks in about 10 radii around the trees and down to 20 cm depth. Then, they were pooled together, carefully mixed to avoid heterogeneity and sieved (2 mm.). Physico-chemical analysis has been performed. After, the soil obtained was divided into two parts. One was autoclaved three times (1 hour, 120°C.) with a 48 h interval and left for 5 days. Physico-chemical analysis of this sterilized soil has been carried out again in order to check the effect of sterilization on its chemical composition. Values obtained were the same. The part was used as it is. Four *Eucalyptus* species were tested: *Eucalyptus maideni, E. globulus*, *E. camaldulensis* are calcifuge species; *E. gomphocephala* is a calcicole one *(*Pryor, 1976). Seeds of *Eucalyptus maideni, E. globules and E. gomphocephala* were collected from trees of a plantation presenting a non calcareous soil. For *E. camaldulensis*, seeds were collected from the trees of this calcareous stand. After surface disinfecting (H_2O_2 30%) all the seeds were germinated in a sterilized peat-perlite mixture (60-40 v/v). After a month of growth, seedlings were transplanted into 600 cm^3 plastic pots. For each species, 64 pots were prepared. Half of these were filled with sterilized soil. The remaining pots received non sterilized soil. At the beginning of the experiment, 4 seedlings were planted in each pot and then cleared to one per pot 6 days after. The pots were kept in a gently ventilated chamber (day light, 18°C night; 25°C day). They were arranged in a complete randomized block design and redistributed randomly once a week. Blocks were randomly redistributed every two weeks. Seedlings were watered as necessary, avoiding excessive drainage. The experiment was carried out over 80 days.

Determination of Plant Growth

Measurements began 21 days after transplanting. For each species and each condition (soil sterilized or not), 8 plants were harvested at about 15 to 21 days intervals. Total root length was determined using the line intersect method (Tennant, 1975). Number of secondary roots ($\geq 2^{nd}$ order) and plant height were recorded. Stems were oven dryed (24 hours at 70 °C) and weighed.

Mycorrhizal Monitoring

To assess arbuscular mycorrhizal (**AM**) colonization, root systems were cleared in 10% KOH and stained with Trypan blue (Phillips and Hayman, 1970). Then, AM mycorrhizal root lengths were estimated using the grid line intersect method (Giovannetti. and Mosse, 1980).

AM fungi were identified to the genus level on the basis of the morphological characters of the different structures (arbuscules, vesicles, hyphae) and colonization patterns (Abbot and Robson, 1979; Abbot, 1982; Shenck and Perez, 1990).

Data Analysis

Data obtained for growth were analyzed using stat box 6. For mycorrhizal quantification, data were subjected to Anova analyses using STAT ITCF and Newman Keuls test ($P< 0.05$).

RESULTS AND DISCUSSIONS

Results obtained for growth in sterilized and non sterilized soils are reported on figures 1,2, 3, and 4. In non sterilized soil, all seedlings do not show any sign of deficiency. At the end of the experiment, 80 days after transplanting, all the parameters reached values of about twice those measured at the beginning of the bioassay, 21 days after transplanting. In sterilized soil, seedlings of calcifuge species showed leaf chlorosis. These symptoms have been related to toxic effects of calcareous soils on calcifuge plant species. Indeed, these toxic effects are revealed by a chlorosis which is generally accompanied by an iron deficiency (Menguel et al., 1984) and /or by a calcium accumulation in tissues. This is especially dangerous when it relates to cytoplasm and vacuole (Salsac, 1980). In the case of this study the problem is exacerbated by the P soil deficiency. Sensitivity to the calcareous soil of the three calcifuge species differed: *E. maideni* was the most sensitive species; after 42 days; seedlings showed a severe chlorosis and died (Figure 1). For *E. globulus* growth slowed down at 42 days after transplanting and seedlings died at 57 days (Figure 2). *E. camaldulensis* seedlings grew until 57 days after and then died (figure 3). *E. gomphocephala*, the calcicole species, do not show any sign of deficiency in sterilized soil although growth was better and all parameters measured were higher in non sterilized soil. (Figure 4)

Anatomical structures of AM, mainly of the *Arum* type with typical arbuscules, were found within roots of all the species used when grown in non sterilized soil. The most frequently observed AM fungi were identified to genus level as several *Glomus* species according to the morphological descriptions of the different structures (arbuscules, vesicles, hyphae and spores) and colonization patterns (Abbot, 1982; Shenck and Perez, 1990). They were the same ones as those observed on mature *E*. trees (Adjoud-Sadadou and Halli-Hargas, 2000; Adjoud-Sadadou, 2004) in the stand where soil used for the experiment was taken. These results suggest that mycorrhizal fungi have conferred calcicole characteristics to the calcifuge *E*. species tested. This ability has been reported for other plants as *Pinus nigra nigricans* (Le Tacon,1978), *Helianthemum* **(**Kianmehr, 1978**)** and *E. dumosa* (Lapeyrie and Chilvers, 1985). Such species has been qualified as "symbiocalcicole" species by Lapeyrie (1987). Nevertheless, not all mycorrhizal fungi can confer this character to calcifuge plants (Lapeyrie 1987).

Arbuscular mycorrhizal root lengths values increased significantly over all the duration of the bio assay and that, for all the species transplanted in non sterilized soil (Figure 5). When comparing mycorrhizal colonization of the calcifuge species, results obtained are noteworthy: the most sensitive species (*E maideni* and *E. globulus*) were the most mycorrhizal. At all sampling times, the calcicole species, *E. gomphocephala*, showed the lowest values of root colonization. This is in accordance with results obtained in our previous study where mycorrhizal dependency of 11 *Eucalyptus* species have been studied (Adjoud et al., 1996). Results obtained revealed that *E. gomphocephala* was not AM dependent, so it did not really need to be associated with AM fungi to achieve its growth. However it grew up better in non

sterilized soil where the natural inoculum was present.

CONCLUSIONS

Arbuscular mycorrhizal fungi had a beneficial impact on tolerance of calcifuge *Eucalyptus*. Species to soil calcium carbonate and promoted their growth. The 3 calcifuge species revealed differences in their susceptibility to calcium carbonate. For *E. camaldulensis*, although seeds have been collected on trees in a calcareous soil the seedlings revealed a susceptibility to calcium carbonate. Consequently, it would be very interesting to identify precisely the different *Glomus* strains involved in this experiment and check them for efficiency.

REFERENCES

1. Abbott, L.K. 1982. Comparative Anatomy of Vesicular-Arbuscular Mycorrhizas formed on Subterranean *Clover. Austraian. Journal. of Botany*. *30*: 485-499. doi:10.1071/BT9820485.

1. Adjoud-Sadadou D (2004) La Symbiose mycorhizienne chez l'*Eucalyptus*. Interactions multiples et Dynamique de la Symbiose en relation avec l'environnement Thèse de *Doctorat d'état Biologie et Physiologie végétale. Université Mouloud Mammeri. Tizi ouzou.*. *200p*.

2. Adjoud-Sadadou D & Halli-Hargas R (2000) Occurrence of arbuscular mycorrhiza on aged *Eucalyptus. Mycorrhiza*. **9**: 287-290.

3. Adjoud-Sadadou D, Plenchette C., Halli-Hargas R & Lapeyrie F (1996) Response of 11 *Eucalyptus* species to inoculation with three arbuscular mycorhizal fungi. *Mycorrhiza*. 6 : 129-135.

4. Chevalier S & Paris N (1981) Absorption et fixation du calcium par les chloroplasts de lupin jaune (*Lupinus luteus* L.) calcifuge et de féverole (*Vicia faba* L.) calcicole. *Physiologie. Végétale*. *19*: 23-31.

5. Clarkson D T (1965) Calcium uptake by calcicole and calcifuge species in the genus *Agrostis*. *Journalof Ecology. 46*: 749-757.

6. Clement A, Garbaye G, & Le Tacon F (1977) Importance des ectomycorhizes dans la résistance au calcaire du pin noir (*Pinus nigra* Arn.ssp. *nigricans* Host). *Ecologia Plantarum 12* : 111-131.

7. Fomina MA, Alexander IJ, Hillier S & Gad GM (2004) Zinc phosphate and pyromorphyte solubilization by soil plant symbiotic fungi. *Geomicrobiology journal 21*: 351-366

8. Gad G M (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research 111*: 3-49.

9. Giovannetti M & Mosse B (1980) an evaluation of techniques for measuring VA mycorrhizal infection in roots. *New Phytologist. 84*: 489-500.

10. Gutschick V.P (1993) Nutrient limited growth rates: roles of nutrient use efficiency and of adaptation to increase uptake rates. *Journal of Experimental Botany 44*,, 41-52

11. Jefferies R L & Willis A J (1964). Studies on the calcicole-calcifuge habit. II. The influence of calcium on the growth and establishment of four species in soil and sand cultures. *Journal of Ecology*: *52*: 691-707.

12. Johansen A Jakobsen I Jensen ES (1993) External hyphae of vesicular arbuscular mycorrhizal fungi associated with Trifolium subterraneum L: 3. Hyphal transport of $^{3\,2}$P and ^{15}N. *New Phytologist 124*, 61-68.
13. Kianmehr H., (1978). The response of *Helianthemum chamaecistus* Mill. to mycorrhizal infection in two different types of soil. *Plant soil 50*: 719-722.
14. Labidi S, Ben Jeddi F, Tisserand B., Debiane D., Rezgui S., Grandmougin-Ferjani A., Lounes- Hadj Sahraoui A (2012). Role of arbuscular mycorrhizal symbiosis in root mineral uptake under $CaCO_3$ stress. *Mycorrhiza 22*, 337-345.
15. Lapeyrie F. (1987) Les mycorhizes de l'*Eucalyptus* en conditions d'excès de carbonate de calcium.
16. Approche écologique et physiologie des associés ectomycorhiziens. *Thèse de Doctorat d'Etat ès sciences*. Université Claude Bernard. Lyon. 198 p.
17. Lapeyrie F F & Chilvers GA (1985) An endomycorrhiza-ectomycorrhiza succession associated with enhancedgrowth of *Eucalyptus dumosa*seedlings planted in a calcareous soil. *New Phytologist 100*: 93-104.
18. Le Tacon F (1978) La présence de calcaire dans le sol. Influence sur le comportement de l'épicéa commun (*Picea excelsa* Link) et du pin noir d'Autriche (*Pinus nigra* Arn.ssp. *nigricans* Host). *Annales des sciences Forestières 35*: 165-174
19. Li XL George E Marshner H 1991 Extention of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil; *Plant and soil 136* 41-48.
20. Marshner H and Dell B 1994 Nutrient uptake in mycorrhizal symbiosis *Plant and Soil 159* 89-102
21. Mengel K, Breininger M TH & Bübl W (1984) Bicarbonate, the most important factor inducing iron chlorosis in vine grapes on calcareous soil. *Plant and Soil*. *81*: 333-344.
22. Phillips J.M. & Hayman DS (1970) Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assesment of infection. *Trans. British. Mycoologicall. Society. 55*: 158-161.
23. Pryor LD (1976) *Biology of eucalypts*. The institute of Biology's studies in Biology. **(61).** Edward Arnold, 82 p.
24. Rossignol M, Lamant D, Salsac L & Heller R (1977) Calcium fixation by the roots of calcicole and calcifuge plants: the importance of membrane systems and their lipid composition. In: *Transmembrane ionic exchange in plants* (Ed by M Thellier, A. Monnier, M Demarty & Dainty J. (CNRS ed.) 483-490.
25. Salsac L (1980) L'absorption du calcium par les raciness des plantes calcicoles ou calcifuges. Sciences du sol. *Bulletin de l'AFES.1* : 45-77.
26. Shenck, N.C., and Perez Y. 1990. Manual for the identification of VA mycorrhizal fungi. Second edition. International Culture Collection of VA Mycorrhizal Fungi. Gainesville, Florida. USA
27. Smith, S.E., and Read, D. 2008. *Mycorrhizal symbiosis*. Academic Press-Elsevier. Third ed.
28. Tarafdar JC and H. Mashner 1994 Phosphatase activity in the rhizosphere and hyposphere of VAof mycorrhizal

wheat supplied with inorganic phosphorus. *Soil Biology and Biochemistry*.26 387-395.

29. Tennant D (1975) A test of modified line intersect method of estimating root length. *Journal. of Ecology*. *63*: 995-1001

APPENDICES

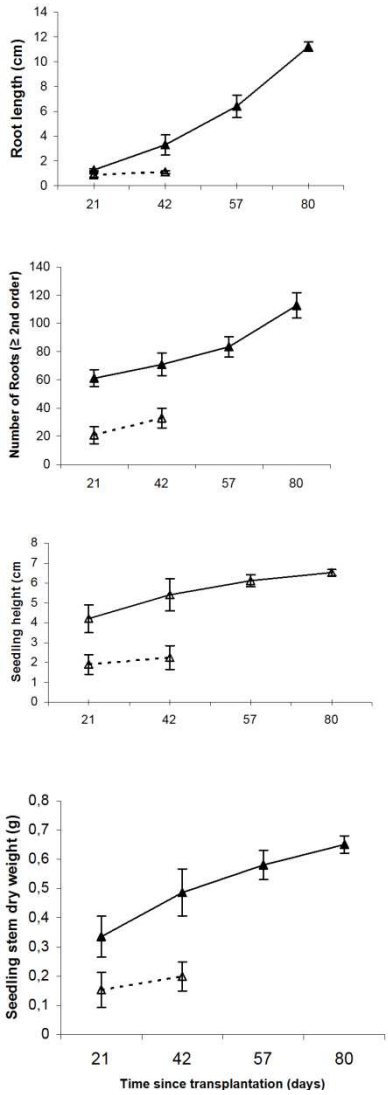

Figure 1: Growth of *Eucalyptus Maideni* in a Calcareous Soil, Either Non-Sterilized or Sterilized, Followed Over 80 Days after Transplanting Unbroken Graph Lines and Solid Symbols Refer to Non-Sterilized Soil. Broken Graph Lines and Open Symbols Refer to Sterilized Soil. For Each Parameter, Values are the Means of Eight Replicates. Vertical Bars Represent Confidence Intervals (P = 0.05)

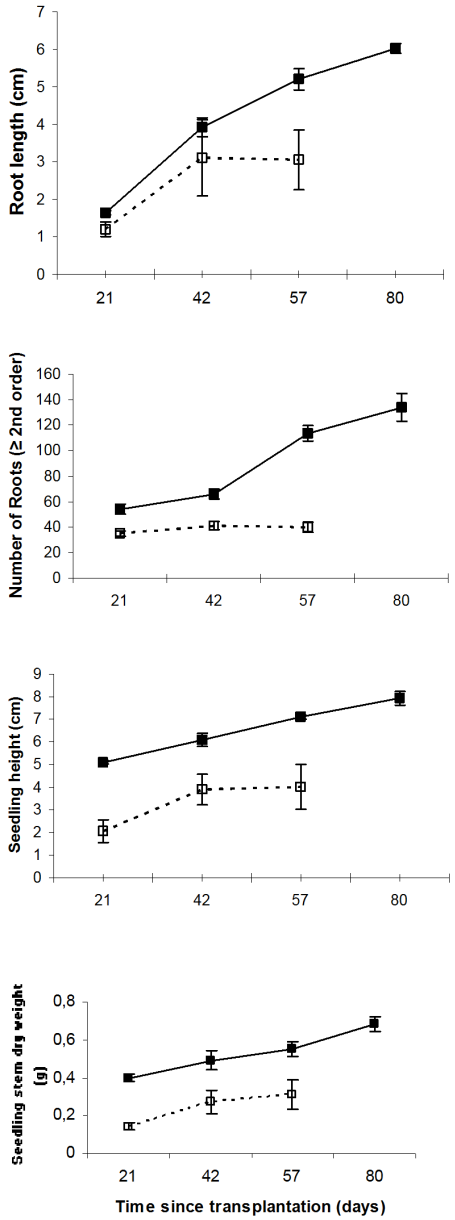

Figure 2: Growth of *Eucalyptus Globulus* in a Calcareous Soil, Either Non-Sterilized or Sterilized, Followed Over 80 Days after Transplanting Unbroken Graph Lines and Solid Symbols Refer to Non-Sterilized Soil. Broken Graph Lines and Open Symbols Refer to Sterilized Soil. For Each Parameter, Values are the Means of Eight Replicates. Vertical Bars Represent Confidence Intervals (P = 0.05)

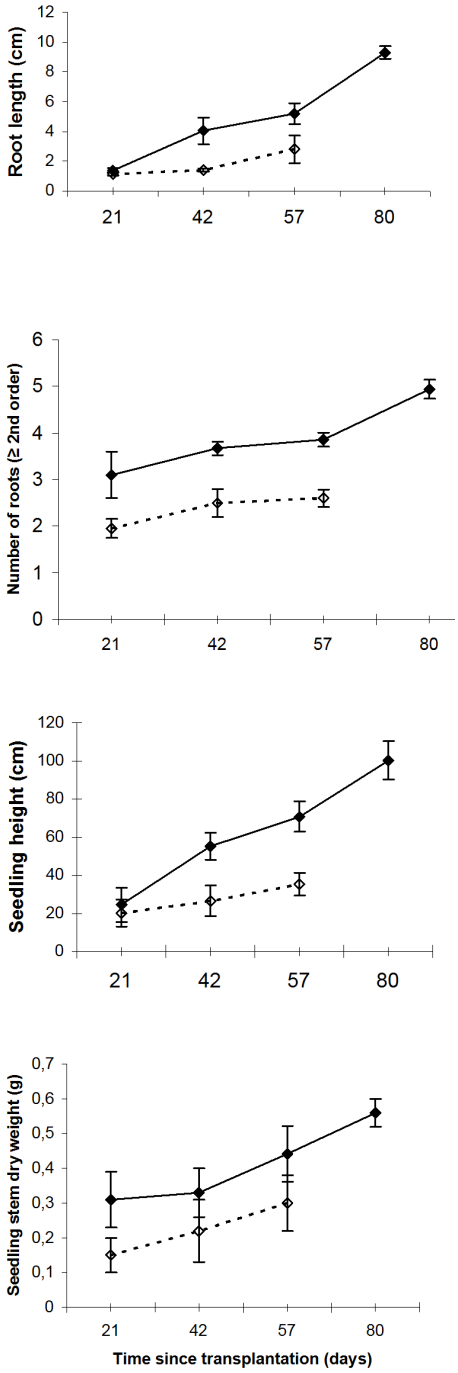

Figure 3: Growth of *Eucalyptus Camaldulensis* in a Calcareous Soil, either Non-Sterilized or Sterilized, Followed over 80 Days after Transplanting Unbroken Graph Lines and Solid Symbols Refer to Non-Sterilized Soil. Broken Graph Lines and Open Symbols Refer to Sterilized Soil. For Each Parameter, Values Are The Means Of Eight Replicates. Vertical Bars Represent Confidence Intervals (P = 0.05)

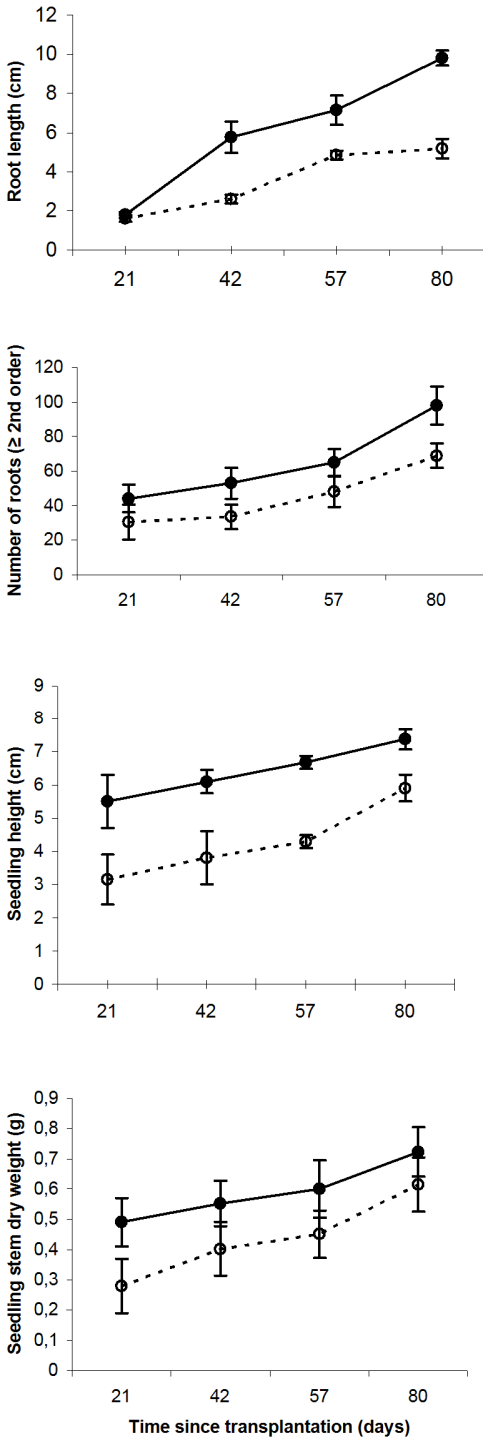

Figure 4: Growth of *Eucalyptus Gomphocephala* in a Calcareous Soil, either Non-Sterilized or Sterilized, Followed Over 80 Days after Transplanting Unbroken Graph Lines and Solid Symbols Refer to Non-Sterilized Soil. Broken Graph Lines And Open Symbols Refer to Sterilized Soil. For Each Parameter, Values are the Means of Eight Replicates. Vertical Bars Represent Confidence Intervals (P = 0.05)

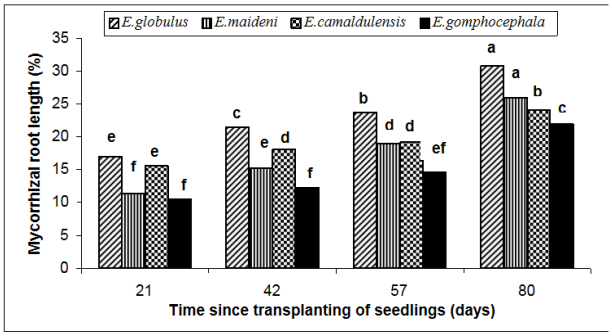

Figure 5: Monitoring of Mycorrhization of Four *Eucalyptus* Species (Arbuscular Mycorrhizal Root Length. % of Total Root Length) Over 80 Days after Transplanting in Calcareous Soil. Calcifuge Species: *Eucalyptus Globulus, E. Maideni, E. Camaldulensis*. Calcicole Species: *E. Gomphocephala*. Values are the Means of Eight Replicates. Columns Labelled with Different Letters Differ Significantly (Newman Keuls Test, P<0.05)