Vol. 17 No. 2
July 2005

About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI, The Energy and Resources Institute, was established in 1974 as the Tata Energy Research Institute. While initially, TERI's focus was mainly on documentation and information dissemination, research in the fields of energy, environment, and sustainable development was initiated towards the end of 1982. The genesis of these activities lay in TERI's firm belief that efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

The Biotechnology and Management of Bioresources Division

Focusing on ecological, environmental, and food security issues, the division's activities include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grass-roots level, with village communities. The division functions through two areas—the Centre for Mycorrhizal Research, and Plant Tissue Culture and Molecular Biology. It is actively engaged in mycorrhizal research. The Mycorrhiza Network has specifically been created to help scientists across the globe in carrying out research on mycorrhiza.

The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

Established in April 1988 at TERI, New Delhi, the Mycorrhiza Network first set up the MIC (Mycorrhiza Information Centre) in the same year and the CMCC (Centre for Mycorrhizal Culture Collection) – a national germplasm bank of mycorrhizal fungi – in 1993. The general objectives of the Mycorrhiza Network are to strengthen research, encourage participation, promote information exchange, and publish the quarterly newsletter *Mycorrhiza News*.

The MIC has been primarily responsible for establishing an information network, which facilitates sharing of information among the network members and makes the growing literature on mycorrhiza available to researchers. Comprehensive database on Asian mycorrhizologists and mycorrhizal literature (RIZA) allow information retrieval and supply documents on request.

The main objectives of the CMCC are to procure strains of both ecto and VA mycorrhizal fungi from India and abroad; multiply and maintain these fungi in pure culture; screen, isolate, identify, multiply, and maintain native mycorrhizal fungi; develop a database on cultures maintained; and provide starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction or handling.

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Quantification of living mycorrhizal fungal mass and fine root mass by sterol estimation*

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Evaluation of effectiveness of mycorrhizal isolates in colonizing plant roots and enhancing the seedling performance requires quantification of both intra-radical and extra-radical mycelial mass. Quantification of extra-radical mycelial mass is also necessary to understand the function of specific ectomycorrhizal fungi as the nutrient-uptake capacity of the mycorrhizal fungi is likely to be strongly influenced by the amount of extra-matrical mycelium extending out into the soil from the mycorrhizal roots. Only a few methods are known which allow a precise separation of live fungal mass from the dead ones and quantify the live fungal mass in roots as well as in soil. In recent years, a number of methods have been developed which utilize ergosterol as a specific quantitative marker for live fungal biomass. Ergosterol is a characteristic fungal-specific membrane component formed only in the fungi and not in roots of plants. The ergosterol assay to quantify fungal biomass has successfully proved to be useful in quantifying the live fungal biomass in roots and soil (Antibus and Sinsabaugh 1990).

In addition to ergosterol, many other sterols are found in spores of the mycorrhizal fungi. These sterols have the taxonomic values and may explain some phylogenetic trends in the mycorrhizal fungi (GrandmouginFerjani, Dalpe, Hartmann, *et al*.1997; Weete and Gandhi 1997).

Like ergosterol, plant roots also produce a sterol, sitosterol, which is only produced by plant roots and not by fungal hyphae. Sitosterol is used for estimation of fine root mass in plant roots (Antibus and Sinsabough 1990; Sinsabaugh, Antibus, Jackson, *et al*. 1997).

Procedures for ergosterol estimation

In studies conducted at the Laboratoire de Microbiologie Forestiere, Centre de Recherche Forestieres de Nancy, Institut National de la Recherche Agronomique Champenoux, Seichamps, France, a simplified procedure for estimating fungal ergosterol from a small number of ectomycorrhizae was described. Efficient extraction of free ergosterol is achieved with ethanol. The ergosterol is then quantified by the HPLC ([reverse-phase] high performance liquid chromatography). This procedure was scaled down, allowing the fungal infection to be measured in a few ectomycorrhizae, and was found suitable for estimating ergosterol levels for many fungal isolates. This assay is more sensitive, rapid, and convenient than the chitin assay in measuring the ectomycorrhizal fungal biomass (Martin, Delaruelle, and Hilbert 1990).

In studies conducted at the Institute of Plant Genetics, Poznan, Poland, a similar technique was developed for determining the ergosterol content in mycorrhizal pine roots, which also involved the use of HPLC. Analysis of four mycorrhizal fungi in the liquid culture with this method showed that the ergosterol contents differed little between the species. Ergosterol contents of fruit bodies varied between species and differed from the levels estimated in the cultured mycelium. Calculation of fungal biomass in mycorrhizal roots based on the ergosterol readings agreed well with the results obtained by using other methods. Consequently, the technique was considered practical wherever an accurate estimate of the intensity of mycorrhizal

*This paper has been compiled from TERI records in RIZA.

infection was required (Salmanowicz and Nylund 1988).

Estimation and quantification of ergosterol in ectomycorrhizal fungi

In studies conducted at the United States Department of Agriculture, Agricultural Research Service, 215 Johnson Hall, Washington State University, Pullman, WA, USA, analysis of ergosterol and activities of the ADC (arginine decarboxylase) and ODC (ornithine decarboxylase) were used to estimate the fungal biomass and metabolic activity of the fungal hyphae, respectively, in aerial mycelium of *Hebeloma crustuliniform*e. Omission of organic phosphorus from the medium decreased the ergosterol concentration of perimeter mycelium by 15%–30% and increased the ADC and ODC activities up to six-fold. The magnitude of changes with the omission of glucose carbon was similar but the trend was reversed. The ergosterol content and enzyme activities differed among the colony locations. The perimeter mycelia contained up to 1.8 times more ergosterol and up to 3–6 times more ADC and ODC activity per g of mycelia than the mycelia in the core. The ergosterol mass and the colony mass were positively correlated. The equation $y = -5.46 + 0.38\ (x)$, where y = mycelial mass (mg) and x = ergosterol mass (micro g), accounted for 77% of the variance in colony mass. The ratio of ODC : ADC activity was 21 at the perimeter and 30 at the core. This activity ratio may be used to infer the maturation state of the fungal colony. Potential application of the ergosterol content and activities of the ADC and ODC as biochemical indices may be important in mycological research (Johnson and McGill 1990a).

Studies conducted at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden, showed that in some studies, ergosterol contents in mycorrhizal fungi varied due to different growth conditions. The ergosterol values dropped in the centre of the fungal colony where the fungus had started to die. Thus, variations in the ergosterol contents under different growth conditions are likely to be due to the varying amounts of dead hyphae. Different fungi contain different amounts of ergosterol, which means that the ergosterol values cannot be translated to biomass when the fungi analysed are unknown. Different fungi have been reported to contain 1–5 µg ergosterol per mg of mycelium (Wallander, Nylund, and Ekbald 1992).

Factors affecting ergosterol production

Seasonal variation

Studies conducted at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden, showed that there was low variation in the ergosterol content of two mycorrhizal types during the year. Values in the middle of winter when the soil was frozen were approximately the same as in spring, which is the period of active root growth. Samples taken from the mycorrhizal types growing along a bed rock contained the same amount of ergosterol as the samples of the same type collected from the sites with a thick humus layer (Wallander, Nylund, and Ekbald 1992).

In studies conducted at the Forest Sciences Laboratory, Institute of Tree Root Biology, Southern Research Station, United States Department of Agriculture, Forest Service, Green Street, Athens, GA, USA, ergosterol was used to estimate the live mycelial biomass in ectomycorrhizae. Loblolly pine (*Pinus taeda*) seeds were sown in April 1993 and grown with standard nursery practices. Correlations between the total seedlings' ergosterol and visual assessment of mycorrhizal colonization were high during July and August but were low as the ectomycorrhizal development continued into the growing season. Percentages of mycelial dry weight over the lateral roots decreased from over 9% in July to 2.5% in November because the lateral root dry weight of seedlings accumulated faster than the mycelial dry weight. The total ergosterol per seedling increased from July through February. As the lateral root dry weight ceased to increase during the winter months, ectomycorrhizal mycelia became the major carbohydrate sink of the pine seedlings. No distinctive seasonal pattern of the soil ergosterol content was observed. The impact of ectomycorrhizal fungi on carbohydrate source–sink dynamics can be quantitatively estimated with the ergosterol analysis but not with conventional visual determination (Sung, White, Marx, *et al*. 1995).

Studies conducted on seasonal variations of mycorrhizal colonization of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) rootlets in two stands by comparing ergosterol content and very fine root tip counts for a three-year period showed that there were significant correlations and interactions between three environmental factors, namely the year, season, and horizon. It was shown that the root tip counting together with the ergosterol analysis of rootlets helps to follow changes induced by the environment on a long-term basis (Vanpraag, Logany, Carletti, *et al*. 1994).

Effect of macronutrients

Studies were conducted at the Department of Plant Physiology, University of Umea, Sweden, to evaluate the effect of N, P, K, Ca, Mg, and S on the fungal biomass of *Paxillus involutus* colonizing *Pinus sylvestris* and *Alnus incana* (alder). The ergosterol content, used as a measure of fungal biomass, was evaluated in both mycorrhizal roots and extramatrical mycelium to see if the nutrients influenced the allocation of fungal biomass. The nutrient solutions were added with the macronutrient levels varied according to a two-level fractional

factorial design. This also allowed interactions among nutrients. A total of 200 seedlings, each of *A. incana* and *P. sylvestris*, were co-grown in pots in coarse sand in a growth chamber for two growing seasons. Roots were separated by a 20-µm-mesh nylon filter. Half of the plants of both species were inoculated with *P. involutus* and all the alder plants were also inoculated with Frankia. Some major findings for inoculated seedlings were that the soil ergosterol content (µg per g dry sand) was negatively affected by P and NP in the pine half of the pot and by NP in the alder half. The ergosterol content in sand was on average 1.58-times higher in the alder half of the pot than in the pine half. This difference was independent of the nutrient treatment. In contrast, there was on an average, four-times-higher ergosterol content (µg per g) in pine roots than in alder roots. The results suggested that changes in root-bound fungal biomass might not reflect changes in the extramatrical fungal biomass (Ekblad and Wallander 1992).

Studies were further continued at the Department of Forest Ecology, Swedish University of Agricultural Sciences, Forest Soils, Umea, Sweden, on the effects of macronutrients on production and distribution of fungal biomass and plant biomass in ectomycorrhizal (*Paxillus involutus*) and non-mycorrhizal *P. sylvestris* and *Alnus incana*. Fungal biomass was measured by estimating ergosterol content in roots and extramatrical mycelium. *A. incana* was nodulated with Frankia. All six macronutrients (N, P, K, Ca, Mg, and S) were varied according to a two-level fractional factorial design as in the above experiment. The levels of N, P, and sometimes K, and interactions between them had highly significant effects whereas Ca, Mg, and S had no significant effects on the fungal biomass. Production of extramatrical mycelium peaked when P was low and the other nutrients were high. This investment in the extramatrical mycelium resulted in a 660% higher fungal biomass in mycorrhizal compared to non-mycorrhizal *P. sylvestris* at that nutrient regime. The proportion of fungal biomass in roots was stable in *P. sylvestris* but more variable in *A. incana*. *A. incana* grew less when it was mycorrhizal than when it was non-mycorrhizal. The growth response to mycorrhiza and to different nutrient treatments was evident at the end of the first growing period. Non-mycorrhizal *P. sylvestris* did not respond to P limitation by a production of proportionately new roots. This might be a reflection of an obligate dependency on mycorrhiza for effective P uptake. By contrast, the root / shoot ratio in both mycorrhizal and non-mycorrhizal *P. sylvestris* decreased strongly in response to the increased N. The opposite root / shoot response was found in *A. incana* where the ratio decreased strongly in response to the increased P and increased in response to the increased N (Ekblad, Wallander, Carlson, *et al*. 1995).

In studies conducted at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden, soil samples were taken in November 1991 and 1992 from a 25-year-old Norway spruce stand which was a second-generation coniferous forest with the highest levels of atmospheric deposition and with soil being poor in base cations. This stand was fertilized with vitality fertilizers containing 48, 43, 218, 46, and 75 kg of P, K, Ca, Mg, and S, respectively, as two single doses in four plots in a randomized block design experiment. The annual input of atmospheric nitrogen was approximately 15–20 kg N per year. Mycorrhizal-type frequency and mycorrhizal biomass were estimated on fine roots (<1.5 mm) in the humus layer (LFH [litter, fermented, humus layers] horizon). In total, 13 and 18 types were found in 1991 and 1992, respectively. No consistent differences as regards the total number or composition of types were found between vitality-fertilized and control plots. Mycorrhizal biomass was estimated in 1991 using the ergosterol method. The ergosterol concentration was 55.1 (SE±7.3) µg per g of fine root dry weight. Assuming an average ergosterol concentration of 0.3% in the fungal symbiont, approximately, 2% of the fine root biomass was mycorrhizal, excluding extramatrical mycelia or sclerotia. No significant differences were found between control and vitality fertilized plots. In the last sampling, a treatment with ammonium sulphate was included. Preliminary results of ammonium sulphate treatment showed that there was a decrease and a dramatic change in the composition of mycorrhizal types in vitality-fertilized plots as compared to control plots (Ola, Nylund, Hogerg, *et al*. 1993).

Effect of phosphorus

In studies conducted at the United States Department of Agriculture, Agricultural Research Service, Washington State University, Pullman, WA, USA, influences of soil P fertilization on temporal changes in ergosterol content and ODC activity were monitored in rhizosphere soil, non-rhizosphere soil, and *Pinus contorta* roots ectomycorrhized with *Hebeloma crustuliniforme* grown in loamy sand. With addition of mycorrhizal inoculum to loamy sand, the ODC activity per mg of root increased between 10- and two-fold within 21 weeks of planting. Inoculation also decreased the root mass per seedling. Inoculation increased the mycelial mass per root mass by up to two-folds but no differences were observed for the total seedling mass until 35 weeks after planting. Intramatrical mycelia were detrimental to early plant growth but inoculated seedlings had 1.7-times more root mass and 1.3-times more shoot mass at 35 weeks. Rhizosphere soil contained up to five-times more mycelia and up to six-times greater ODC activity than non rhizosphere soil. Inoculation increased the rhizospheric metabolic activity and intramatrical mycelial mass. Their sensitivity to fungal inoculation, P fertilization, and temporal trends may

make the method useful in studies of rhizosphere ecology and root microbe relationships (Johnson and McGill 1990b).

Studies conducted at the Katholieke University, Leuven, Dev Biol Laboratory, Kardinaal Merciarlaan, Louvain, Belgium, showed that external mycelia of the ectomycorrhizal fungi, *Thelephora terrestris* and *Suillus luteus* associated with *P. sylvestris* roots exhibited a substantial extracellular acid phosphatase activity. The activity was positively correlated with ergosterol concentration in the growth substratum and decreased with an increasing P nutrition (Colpaert, VanLaere, VanTichelen, *et al.*1997).

Effect of nitrogen

Studies were conducted at the School of Biological Sciences, University of Manchester, Oxford Road, Manchester, UK, to find the effects of repeated N fertilizer application for three years (1989–92) on mycorrhizal infection in *Colluna vulgaris* growing in peat soils in North Wales. Solutions of NH_4NO_3 were added at regular intervals (10–20 times annually) to provide 0, 40, 80, or 120 kg N per ha per year above the background deposition. An estimate of mycorrhizal biomass in washed roots from soil cores sampled in May and July 1992 was obtained by determining the concentrations of ergosterol in ethanol extracts. The concentrations of ergosterol (per mg fresh weight) were significantly greater in fine hair roots than in thicker roots and also higher in surface horizon than deeper in the soil core. In May, the only significant effect of the N application was found in the fine roots in the surface (0–15 mm) soil. In this fraction, ergosterol was significantly higher in plots, which received 80 kg N per ha per year than in all other treatments. However, in July, the ergosterol concentration in the fine surface root fraction was not changed by the N additions. No changes were observed in soil nutrients (total N and P extractable base cations) or surface pH but N fertilizers did stimulate shoot growth, flowering, and litter production. The N concentration in living roots and litter was raised as a result of the N inputs while the levels of other main nutrients P and K were not altered. Given the relatively small changes measured in the amount of mycorrhizal infection, it was suggested that this measurement may be a poor indicator of the excess atmospheric N deposition in heathland soils (Caporn, Song, Read, *et al*. 1995).

Effect of heavy metals

In studies conducted at the Department of Pure and Applied Chemistry, University of Strathclyde, Lanark, Scotland, distribution of caesium and silver in the soils was measured and correlated with ergosterol as a measure of fungal biomass. Soil beneath the individual fruit bodies and fairy rings was sampled from a clay loam grassland site (Hogarth Park) in south-west Scotland, a sandy loam site (Troon, south-west Scotland), and a peaty soil (Glensaugh, north-west Scotland). Considerable variation in the ability of the fungi to translocate and accumulate the metals was observed. *Cortinarius* and *Mycena* spp. and *Suillus grevillei* accumulated caesium and silver whereas additionally, *Coprinus comatus, Entoloma* sp., *Hygrocybe psittacina,* and *Psathyrella* sp. accumulated silver. *Clitocybe* and *Inocybe* spp, Lycoperdaceae, and *Marasmius oreades* formed the fairy rings. Along the diametric transects of each ring in most cases, there were maxima in the ergosterol contents of soil corresponding with the enhanced vegetative growth. There were also maxima in the caesium contents of soil, most of which corresponded to those of ergosterol. Site selection was an important factor in determination of the relationship between ergosterol and caesium. At a single-site level of analysis, a quantitative relationship between ergosterol and caesium was apparent only for one site but overall, the enhancement was significantly linearly correlated implying that translocation by the fungi had occurred. No correlation between ergosterol and silver contents was observed (Anderson, Davidson, Littlejohn, *et al.*1997).

Effect of fungicides

In studies conducted at the Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen, UK, the effect of two monthly additions of an aqueous suspension of captan on the changes in a peaty podzol soil at two sites, contaminated two or three years earlier by the injection of ^{134}Cs, was quantified. The less acid soil (pH 5.0) was improved by lime and fertilizer and re-seeded with grass and clover. The more acid soil (pH [subwater] > 3.8) was under hill grasses, herbs, and heather. On both sites, addition of fungicides did not alter the amount or concentration of radio caesium in plant material sampled monthly or the depth distribution of radio caesium in soil profile. Concentration of the fungal constituent ergosterol in the soil measured monthly was unaffected by the fungicide treatment but evidence was obtained from a pot experiment to show that the ergosterol decomposed slowly in cold, wet soils. On the more acid soil, two weeks after the last application of fungicide, there was a decline in the active fungi as measured by fluorescine diacetate staining. Radiocaesium in seven different fungi grown in pure culture was found to be almost entirely extractable (more than 95%) with 1-M ammonium acetate. *Amanita rubescens* showed some retention and 88% of it was extractable. These findings do not preclude the fungal biomass as an important soil component controlling plant availability of the radiocaesium from acid, organic soil by maintaining radiocaesium in a biological cycle but make it unlikely that any fixation by the fungi in a chemical sense is involved (Shand, Cheshire, Smith, *et al*. 1995).

Studies at the University of Kuopio, Department of Ecology and Environment Science, Kuopio,

Finland, in a two-year experiment in an open-air ozone fumigation field on the effect of copper oxychloride and propiconazol fungicides, single or combined, on fine root and mycorrhizal condition of Scots pine (*P. sylvestris*) showed that propiconazol reduced the mycorrhizal infection while copper oxychloride treatment and ozone exposure slightly stimulated it after the first year. After the second year, ectendomycorrhiza disappeared in the propiconazol treatment, while in control treatment, ectendomycorrhiza formed the majority of the light–brown morphotypes. The root biomass was not affected by the fungicide treatment but ozone exposure increased the total amount of short roots and fresh weight of propiconazol-treated roots. No significant differences in the concentrations of ergosterol, starch, and total phenolics in pine roots between treatments were found. However, ergosterol concentration correlated positively with the mycorrhizal infection level (Manninen, Laatikainen, and Holopinen 1998).

Estimation and quantification of ergosterol in vesicular arbuscular mycorrhiza fungi

In studies conducted at the Swiss Federal Research Station, Wadenswil, Switzerland, ergosterol (ergosta-5, 7, 22-tri-3-enol) was identified by gas chromatography: mass spectrometry in roots of berseem (*Trifolium alexandrinum)* and maize (*Zea mays* cv. Honeycomb) infected with *Glomus intraradices.* The fungal-derived compound ergosterol was determined quantitatively in root extracts using reverse-phase HPLC. The concentration of ergosterol in VAM-infected roots reached 72 micro g per g dry material in berseem and 52 micro g per g in maize after 80 days of growth whereas concentration in non-infected roots remained below 8 micro g per g dry weight. Ergosterol, as a characteristic fungal substance, is proposed as an indicator of fungal biomass in the early stage of VAM infection (Frey, Buser, and Schuepp 1992).

Simultaneous estimation of ectomycorrhizal fungal and fine root biomass

Studies were conducted at the Department of Biology, Clarkson University, Potsdam, New York, on simultaneous measurement of live fungal and root biomass by examining ergosterol (a specific fungal component) and sitosterol (a specific plant root component) contents in pure cultures of ectomycorrhizal fungi and re-synthesized, and naturally occurring ectomycorrhizae in 11 strains of nine ectomycorrhizal fungi to determine the range in variation of the ergosterol content. The fungi, which represented a range of host species were grown at different temperatures and inorganic P and phytic acid. The ergosterol was extracted with methanol, separated, and quantified by a reverse phase HPLC system with the attached integrator. Values obtained for the fungi examined generally fell within 3–10 µg ergosterol per mg of dry mass. Ergosterol contents of ectomycorrhizal fungi were similar to those of wood-decaying basidiomycetes. Phosphorus source, inorganic P, or phytic acid did not significantly influence the ergosterol content whereas temperature affected the ergosterol values in some strains. Ergosterol analysis was performed on field-collected grey birch ectomycorrhizae and red maple VAM. Percentage of root dry mass contributed by mycorrhizal fungi was calculated using a conversion factor derived from pure cultures. Ectomycorrhizal roots contained from 20% – 40% fungal mass whereas VAM contained approximately, 4% fungal mass. Sitosterol, based on co-elution with purified standards, was not detected in ectomycorrhizal fungi but was present in tree roots. Preliminary studies indicated that sterol ratios can be obtained simultaneously from samples and may offer a sensitive and precise means for evaluating the extent of mycorrhizal infection in root samples (Antibus and Sinsabaugh 1990).

In further studies conducted at the above university, ergosterol was not detected in roots of uninoculated *Betula populifolia* and sitosterol was not detected in an ectomycorrhizal fungal isolate but was present in birch roots. Ergosterol was produced in all isolates examined and the values ranged from 3 to 18 µg per mg dry mass. These values agreed well with reported values for other mycorrhizal and decomposer fungi. Hyphal ergosterol was the same during growth on phytic acid and KH_2PO_4. Reduction of growth temperature from 25 ºC to 15 ºC had little effect on the ergosterol content of cultures harvested at similar growth stages. Ergosterol and sitosterol were detected in field-collected ectomycorrhizae of *B. populifolia* and *P. sylvestris,* and VA mycorrhizae of *Acer rubrum* and *Plantago major*. Both, the ergosterol content and ergosterol to sitosterol ratios were significantly lower in the VA mycorrhizae than ectomycorrhizae. Calculations of viable fungal biomass associated with field-collected roots were in agreement with those reported by others using the method on re-synthesized ectomycorrhizae. Estimates of the total mass could be obtained for field-collected *B. populifolia* roots by simultaneously using ergosterol to estimate fungal biomass and sitosterol to estimate root mass (Antibus and Sinsabaugh1993).

In studies conducted at the Department of Biology, University of Toledo, Toledo, OH, USA, betasitosterol, a component of plant membranes and an indicator of phytomass in soils, was extracted from soil, root, and litter samples with hot methanol, following the procedure used for fungal ergosterol and quantified by the HPLC. Concentrations of betasitosterol in fine roots (less than 2 mm diameter) of 16 herbaceous and woody plants was 0.2–2.0 µg per mg organic mass (mean 1.0, SD = 0.5). The concentrations of betasitosterol in 2 cm diameter x 15-cm-deep soil cores collected

under old field deciduous hardwood and pine canopies was 2.4–26.5 µg per g of soil dry mass, equivalent to fine root standing crops of 5–60 mg per ha. Microcosm studies indicated that in the early stages of decomposition, betasitosterol degrades at about the same rate as bulk litter (Sinsabaugh, Antibus, Jackson, *et al*. 1997).

Estimation of ergosterol in organic matter and in soil

Studies were conducted at the Faculty of Forestry, Joensuu University, Joensuu, Finland, to determine the spatial structure of the organic layer fungal biomass estimated from the soil ergosterol content of 1 ha plot in a mature Scots pine (*P. sylvestris)* forest from 181 samples and to examine it in relation to the organic layer properties (thickness; pH; total C, N, K, Mg, and Ca) and two above-ground factors, namely gap fraction that is, proportion of the sky not shaded by trees directly above the sampling point and through fall. Ergosterol content of the organic layer had a clear spatial dependence over the studied area and the spatially structured autocorrelation variance accounted for up to 90% of the total sample variance in a 4-metre range. Most measured properties of the organic layer and the above-ground environmental factors were also spatially dependent, although to varying degrees. The spatial pattern of ergosterol was most strongly related to the pH, organic layer thickness, and the total C content. The analysis did not show any direct influence of the gap fraction and through fall on ergosterol. However, the gap fraction and through fall were related to the organic layer thickness, indicating the importance of the associated factors (moisture, light, temperature, and quality of litter) on forest litter decomposition (Mottonen, Jarvinin, Hokkanen, *et al*. 1999).

Studies conducted at the Department of Soil Biology, University of Kassel, Nordbahnhofstr IA, Witzenhausen, Germany, on fungal and microbial biomass determined by ergosterol and fumigation-extraction respectively in bulk grassland soil (less than 2 mm), rhizosphere, and root material showed that in the bulk soil, the average concentration was 3.37 µg per g for ergosterol, 800 µg per g for microbial biomass, and 30.4 mg per g for organic carbon. In the rhizosphere material, the corresponding concentrations exceeded those of the bulk soil by 80%, 80%, and 50%, respectively. The large average ergosterol concentration of 74.2 mg per g root revealed a strong fungal colonization of the root material. About 75% of the total ergosterol was found in the bulk soil fraction, 11% in rhizosphere, and 14% in root material. In one type of soil, nearly half the total ergosterol was found in the root material. However, the average ergosterol-to-biomass ratio in the root material was less than a third of the bulk soil or the rhizosphere soil, indicating that approximately two-thirds of the $CHCl_3$-labile C is presumably root-derived (Jorgensen 2000).

Other sterols produced by vesicular arbuscular mycorrhiza fungi

In studies conducted at the Leach Science Centre, Department of Botany and Microbiology, Alabama Agricultural Experimental Station, Auburn University, Auburn, AL, USA, the sterol composition of 42 fungal species representing six of the eight orders of Zygomycota was determined using gas–liquid chromatography–mass spectrometry to assess whether the distribution of major sterols in this phylum has taxonomic or phylogenetic relevance. Ergosterol was the major sterol of the Dimargaritales, Zoopagales, and 13 of the 14 Mucrales families included in this study. Desmosterol appeared to be the characteristic sterol of the Martierellaceae (Mucrales). The 24-methyl cholesterol was the major sterol of the Entomophthorales genera Entomophthora, Conidiobolus, and Basidiobolus but cholesterol was the sole sterol detected in *Delacroixia coronatus*. The Kickxellales species analysed in this study were characterized by 22-dihydroergosterol as the major sterol. These studies suggest that some orders of Zygomycota may be distinguished on the basis of major sterols (Weete and Gandhi 1997).

Studies conducted at the Mycology Laboratory, University of Littoral Cote d' opale, Bleriot, Colais, France, on analysis of sterol and fatty acid contents of spores of 16 sp of the AM fungi showed that ergosterol, the most prominent sterol in fungi, was absent. The major sterols found in the AM spores belonged to the Delta 5 - sterols, mainly cholesterol, 24-methyl cholesterol, and 24-ethyl cholesterol. One of the major sporal fatty acid was an unusual monounsaturated fatty acid Delta 11 - hexadecenoic acid never previously found in fungal material. It occurred in all the taxa studied. Comparative analysis of spore sterols and fatty acids from different species may help to clarify some aspects of systematics and evolution of the AM fungi as their classification has been based almost entirely on the spore morphology (GrandmouginFerjani, Dalpe, Hartmann, *et al*. 1997).

Further studies conducted at the above university on the sterol composition of spores of 16 species of the AM fungi belonging to the order Glomales, examined by gas chromatography–mass spectrometery, showed that the major compound was found to be 24-ethyl cholesterol (up to 85%) followed by cholesterol (up to 15%). Several other sterols such as 24-methyl cholesterol, Delta 5-avena sterol, 24-ethylcholesta-5, and 22 dien-3 beta-ol were also detected. Significant amounts of alpha-amyrin, a common vascular plant triterpene, were present in spores of all fungal species analysed. Ergosterol, a classical fungal sterol, was however absent in spores of the AM fungi studied (GrandmouginFerjani, Dalpe, Hartmann, *et al.*1999).

In further studies conducted at the above university, Ri-TDNA-transformed carrot roots were used for

investigating sterol metabolism by the AM fungus *Glomus intraradices* under three distinct experimental conditions. These were (1) a symbiotic stage (fungus still attached to the host roots), (2) a detached stage (fungus physically separated from roots), and (3) a germinating stage (germinating spores). In all three stages, *G. intraradices* was found to contain a mixture of 24-alkylated sterols, with 24-methyl and 24-ethyl cholesterols as the main compounds but with no ergosterol (the prominent sterol in most fungi) found. Feeding experiment with sodium acetate (1-C-14) was performed to check the ability of fungus to synthesize sterols. Whatever the experimental conditions, *G. intraradices* was able to actively take up exogenous acetate and incorporate it into sterols and their precursors. The data provided first evidence for de novo sterol synthesis by an AM fungus (Fontaine, GrandmouginFerjani, Hartmann, *et al*. 2001).

References

Anderson P, Davidson C M, Littlejohn D, Ure A M, Shand C A, Cheshire M U. 1997
The translocation of caesium and silver by fungi in some Scottish soils
Communications in Soil Science and Plant Analysis **28**(6–8): 635–650

Antibus R K and Sansabaugh R L. 1990
Quantification of fungal biomass in mycorrhizae by ergosterol analysis, p. 6
Wyoming: University of Wyoming. 324 pp.
[*Proceedings of the Eighth North American Conference on Mycorrhiza Innovation and Hierarchical Integration*, 5–8 September 1990, Jackson, Wyoming, USA]

Antibus R K and Sansabaugh R L. 1993
The exatraction and quantification of ergosterol from ectomycorrhizal fungi and roots
Mycorrhiza **3**(3): 137–144

Caporn S J M, Song W, Read D J, Lee J A. 1995
The effect of repeated nitrogen fertilization on mycorrhizal infection in heather (*Caluna vulgaris* [L.] *Hull*)
New Phytologist **129**(4): 605–609

Colpaert J V, VanLaere A, VanTichelen K K, VanAssche J A. 1997
The use of inositol hexaphosphate as a phosphorus source by mycorrhizal and non-mycorrhizal Scots Pine (*Pinus sylvestris*)
Functional Ecology **11**(4): 407–415

Ekblad A and Wallander H. 1992
Effects of macronutrients on ergosterol contents in roots and extramatrical mycelium of pine and alder mycorrhizal with *Paxillus involutus*. p. 56
In *Proceedings of the International Symposium on Management of Mycorrhizas in Agriculture, Horticulture, and Forestry*
[28 September–2 October 1992, University of Western Australia, Nedlands, Australia]
Australia: Australian Institute of Agricultural Sciences. 164 pp.

Ekblad A, Wallander H, Carlson R, HussDanell K. 1995
Fungal biomass in roots and extramatrical mycelium in relation to macronutrients and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Alnus incana*
New Phytologist **131**(4): 443–451

Fontaine J, GrandmouginFerjani A, Hartmann M A, Sancholle M. 2001
Sterol biosynthesis by the arbuscular mycorrhizal fungus *Glomus intraradices*
Lipids **36**(12): 1357–1363

Frey B, Buser H R, and Schuepp H. 1992
Identification of ergosterol in vesicular-arbuscular mycorrhizae
Biology and Fertility of Soils **13**(4): 229–234

GrandmouginFerjani A, Dalpe Y, Hartmann M A, Laruelle F, Couturier D, Sancholle M. 1997
Taxonomic aspects of the sterol and delta 11-hexadecenoic acid (C16:1, Delta 11) distribution in arbuscular mycorrhizal spores, edited by J P Williams, M U Khan, N W Lem
Physiology, Biochemistry and Molecular Biology of Plant Lipids. pp. 195–197

GrandmouginFerjani A, Dalpe Y, Hartmann M A, Laruelle F, Sancholle M. 1999
Sterol distribution in arbuscular mycorrhizal fungi
Phytochemistry **50**(6): 1027–1031

Johnson B N and McGill W B. 1990a
Ontological and environmental influences on ergosterol content and activities of polyamine biosynthesis enzymes in *Hebeloma crustuliniforme* mycelia
Canadian Journal of Microbiology **36**(10): 682–689

Johnson B N and McGill W B. 1990b
Variations in ergosterol content and ornithine decarboxylase activity of ectomycorrhizal root-soil systems
Plant and Soil **127**(1): 71–79

Jorgensen R G. 2000
Ergosterol and microbial biomass in the rhizosphere of grassland soils
Soil Biology & Biochemistry **32**(5): 647–652

Manninen A M, Laatikainen T, and Holopainen T. 1998
Condition of Scots pine fine roots and mycorrhiza after fungicide application and low-level ozone exposure in a 2-year field experiment
Trees-Structure and Function **12**(6): 347–355

Martin F, Delaruelle C, and Hilbert J L. 1990
An improved ergosterol assay to estimate fungal biomass in ectomycorrhizas
Mycological Research **94**(8): 1059–1064

Mottonen M, Jarvinin E, Hokkanen T J, Kuuluvainen T, Ohtonen R. 1999
Spatial distribution of soil ergosterol in the organic layer of a mature Scots pine (*Pinus sylvestris* L.) forest
Soil Biology & Biochemistry **31**(4): 503–516

Ola K, Nylund J E, Hogerg N, Dahlberg A. 1993
Effects of vitality fertilization (PK) on mycorrhiza type diversity and mycorrhizal biomass
In *Proceedings of the Ninth North American Conference on Mycorrhizae*, p. 8
[Guelph, Ontario, Canada, 8–12 August 1993]
Ontario: University of Guelph. 79 pp.

Salmanowicz B and Nylund J E. 1988
High performance liquid chromatography determination of ergosterol as a measure of ectomycorrhiza infection in Scots pine
European Journal of Forest Pathology **18**(5): 291–298

Shand C A, Cheshire M V, Smith S, Campbell C D, Anderson P, Davidson C M, Littlejohn D, Jamieson N. 1995
Radiocaesium in an organic soil and the effect of treatment with the fungicide 'Captan'
Plant and Soil **170**: 315–322

Sinsabaugh R L, Antibus R K, Jackson C R, Karpanty S, Robinson M, Liptak M, Franchini P. 1997
A beta-sitosterol assay for fine-root mass in soils
Soil Biology & Biochemistry **29**(1): 39–44

Sung S J S, White L M, Marx D H, Otrosina W J. 1995
Seasonal ectomycorrhizal fungal biomass development on loblolly pine (*Pinus taeda* L.) seedlings
Mycorrhiza **5**(6): 439–447

Wallander H, Nylund J E, and Ekbald A. 1992
Measurement of the amount of fungal tissue in mycorrhizal roots and soil, p. 48
In *Proceedings of the International Symposium on Management of Mycorrhizas in Agriculture, Horticulture, and Forestry*
[28 September–2 October 1992, University of Western Australia, Nedlands, Australia]
Australia: Australian Institute of Agricultural Sciences. 164 pp.

Weete J D and Gandhi S R. 1997
Sterols of the phylum zygomycota: Phylogenetic implications
Lipids **32**(12): 1309–1316

Vanpraag H J, Logany G, Carletti G, Wiessen F, Severin M. 1994
Temporal and spatial variations of root tip density and ergosterol content of mycorrhizal roots of *Picea abies* Karst and *Fagus sylvatica* L
Soil Biology & Biochemistry **26**(7): 833–840

Research findings

Studies on arbuscular mycorrhiza fungi associated with tree species planted in arboretum at Mangalore University, Mangalore, Karnataka

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Most tree species require a symbiotic association with microorganisms for successful establishment and growth on inhospitable sites (Jurgensen 1979). Worldwide interest in the AM (arbuscular mycorrhiza) fungi is increasing at a phenomenal rate. During the past three decades, researchers have tried to extend and manipulate the P (phosphorus)-mediated effect of mycorrhizae. The area before plantation was laterite with low soil as well as lower fertility. The soil is usually with gravels.

Myristicaceae is represented by five members in Karnataka, excluding the nutmeg (*Myristica fragrans* Houtt.) in three genera. They are *Gymnacranthera farquhariana, Knema attenuate, Myristica dactyloides, M. fatua* var. *magnifica*, and *M. malabarica*. Of these, except *M. dactyloides*, others are endemic to the Western Ghats of Karnataka, while *M. fatua* var. *magnifica* and *M. malabarica* are threatened. It is therefore by planting them in the arboretum that their population will be conserved.

This paper deals with the growth and pattern of the AM fungi colonizations and spore density in the planted tree species.

Materials and methods

The area was with undulation and little soft laterites before plantation and was later levelled. In the month of June/July 1996, pits of 0.9×0.9×0.9 m were dug, filled with garden red soil and farmyard manure. After the onset of the monsoon, 15 one-year-old seedlings of each species were transplanted from the nursery to the field and the plants were watered. Rhizosphere soil along with root samples was collected during August 2001. Roots of the planted tree species in the arboretum were well spread from the pits to the nearby soils at the age of five years. The soil and root samples from the rhizosphere of 10 seedlings of each plant species were separately collected and mixed to form a composite sample for each species. Soil samples were screened to isolate the AM fungal spores by wet-sieving and decanting technique (Gerdemann and Nicolson 1963). The collected root samples were fixed in the FAA (formaldehyde, acetic acid, and alcohol) and stained with trypan blue and chlorazol black E (Philips and Hayman 1970; Brundrett, Piche, and Peterson 1984).

Results and discussion

The soil was nutrient-deficient in the study area. The AM population developed after introduction of the plant species. Variation in the spore density, root colonization, and growth parameters are showed in Table 1. Maximum height and diameter were recorded in *M. malabarica* (48.95 inch height with 24.73 cm diameter), while the least growth was recorded for *M. dactyloides* (14.01 inch height with 4.76 cm diameter). Other species also performed

Table 1 Plant growth and mycorrhizal status of seedlings of five plant species

Species	*Shoot height* (inches)	*Diameter* (cm)	*Per cent root colonization*	*Spore density**	*Relative abundances* (>40)
Gymnacranthera farquhariana	33.66	18.20	75.48	420	*Glomus fasciculatum, Glomus monsporum, and Acaulospora laevis*
Knema attenuata	30.21	15.32	60.0	235	*G. fasciculatum, Glomus macrocarpum, and Gigaspora gigantea*
Myristica datyloides	14.01	4.76	71.8	325	*G. fasciculatum and A. bireticulatum*
Myristica faua var. *magnifica*	26.54	16.64	72.8	510	*G. fasciculatum, Glomus geosporum, and A. laevis*
Myristica malabarica	48.95	24.73	68.7	375	*G. fasciculatum and G. monsporum*

***** per 100 g of rhizosphere soil

well in this locality. Percentage colonization by the AMF in the planted species varied and maximum was in *G. farquhariana* (75.48) followed by *M. fatua* var. *magnifica* (72.1). The minimum colonization was in *K. attenuate* (60.0). Variation in the rate of colonization might be due to variation in the nutrient requirement and increasing age of the plant species. Similar results were noticed by Bowen (1987) and Gianinazzi-Pearson, Smith, and Gianinazzi (1991) who found that P acquisition of plants controlled the development of the AM in roots, which may vary from species to species.

There were abundant AM fungal spores and the largest number of 510 spores/100 g of soil was noticed in the Rhizosphere of *M. fatua* var. *magnifica*, while the least of 235 spores/100 g of soil was in *Knema attenuate.* Similar results were recorded earlier by others (Scheltema, Abbott, and Robson 1987; Jamuluddin, Chandran, and Malakar 2002) under field conditions. The population build-up of the AMF population occurs due to soil amendment by fertile garden soil. This contained a large population of the AMF spores and served as inoculum. The application of surface garden / forest soil has been proven effective to amend the AMF in soils with poor AMF population (Ferguson and Menge 1986), especially in harsh environments (Allen, Chambers, Coner, *et al*. 1987; Friese and Allen 1991).

Species of *Glomus* such as *G. fasciculatum*, *G. monosporum*, and *G. macrocarpum* were dominant in this region. This might be due to the high sporulation capacity and high viability of *Glomus* spp., while others were scanty due to the adverse edaphic conditions, longer reproductive times, and short viability. Similar observations were made by Sieverding (1991) and Jamuluddin, Chandran, and Malakar (2002). All species did not respond in the same way. Another important factor in the AMF distribution is related to the edaphic and climatic conditions against which every species struggles for existence and the best-suited species multiplies quickly and gets established in soil.

Sludge dumps and other sites such as coal mines or wastelands are devoid of the AMF due to soil disturbance, which results in failure of reclamation practices. Soil amendments with fertile soil served as a source of inoculum for rapid development of fungi, which in turn, efficiently nourished the plants with essential elements. Biological activities in soil also increased the absorbing capacity of roots.

References

Allen E B, Chambers J C, Coner K F, Allen M F, Brown RW. 1987
National re-establishment of mycorrhizae in disturbed alpine ecosystems
Arctic and Alpine Research **19**(1): 11–20

Bowen G D. 1987
The biology and physiology of infection and its development
In *Ecophysiology of VAM plants*, pp. 22–57, edited by G R Safir
Boca Raton, Florida: CRC Press

Brundrett M C, Piche Y, and Peterson R L. 1984
A new method for observing the morphology of vesicular-arbuscular mycorrhizae
Canadian Journal of Microbiology **62**: 2128–2134

Ferguson J J and Menge J A. 1986
Response of citrus seedlings to various field inoculation methods with *G. deserticola* in fumigated nursery soils
Journal of American Society for Horticultural Science **111**(2): 288–292

Friese C F and Allen M F. 1991
Tracking the fates of exotic and local VA mycorrhizal fungi: methods and patterns
Agriculture, Ecosystems and Environment **34**(1–4): 87–96

Gerdemann J W and Nicolson T H. 1963
Spores of mycorrhizal *Endogone* species extracted from soils by wet sieving and decanting
Transactions of the British Mycological Society **46**: 235–244

Gianinazzi-Pearson V, Smith S E, and Gianinazzi S. 1991
Enzymatic studies on the metabolism of vesicular arbuscular mycorrhizas: V. Is H^+ - ATPase a component of ATP-hydrolysing enzyme activities in plant-fungus interfaces?
The New Phytologist **117**: 61–76

Jamuluddin, Chandran K K, and Malakar G H. 2002
Studies on VAM fungi associated with tree species planted in a sludge garden at the Ballarpur Paper Mills, Maharashtra
Mycorrhiza News **13**(4): 10–12

Jurgensen M F. 1979
Microorganisms and reclamation of mine waste
In *Forest Soil and Land Use*, pp. 251–268, edited by C T Youngberg
Fort Collins: Colorado State University

Phillips J H and Hayman D S. 1970
Improved procedure for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection
Transactions of the British Mycological Society **55**: 158–161

Scheltema M A, Abbott L K, and Robson A D. 1987
Seasonal variation in the infectivity of VAM in annual pastures in a Mediterranean environment
Australian Journal of Agricultural Research **38**: 707–715

Sieverding E. 1991
Vesicular arbuscular mycorrhiza management in tropical agrosystems
Schriftenreihe-Gesellschaft fur Technische Zusammenarbeit **224**
Germany: Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ). 371 pp.

Bioaugmentation of *Artocarpus chaplasa* Roxb. seedlings using native arbuscular mycorrhizal fungi in natural soil

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Introduction

Production of quality seedlings in nursery is important for revegetation of the ever-increasing degrading forests of the tropics. Most of the nursery conditions in tropics are not always optimal for quality seedling production (Michelsen 1992). The soil used to raise seedlings may not contain the optimum population of microbes needed for a healthy raising of seedlings. Bioaugmentation with native arbuscular mycorrhizal fungi improves the quality of seedlings in nurseries (Bagyaraj, Brya Reddy, and Nalini 1989; Michelsen 1993; Muthukumar, Udaiyan, and Rajeshkannan 2001).

Artocarpus chaplasa Roxb. is a lofty deciduous tree that normally attains a height of 30–35 m. It is distributed in the lower Himalayan and sub-Himalayan tract from Nepal eastwards, ascending 1500 m to Assam, eastern Bangladesh, Myanmar, and the Andaman Islands. It yields quality timber equal to or superior to teak (Gamble 1922). The population of this important species in its natural habitat has diminished alarmingly because of destruction of its natural habitats and indiscriminate exploitation.

Therefore, the present investigation was undertaken to bioaugment the seedling quality of *Artocarpus chaplasa* Roxb. using native arbuscular mycorrhizal fungal isolates.

Materials and methods

Spores from the rhizosphere of *Artocarpus chaplasa* Roxb. were isolated using the wet-sieving and decanting technique (Gerdemann and Nicolson 1963). The isolated spores were mass-multiplied following the single spore culture technique on *Chloris gayana* Kunth. as host. They were grown for two months. Shoots of the grass were severed and substrate containing the hyphae, spores, and roots was air-dried for inoculation. An initial inoculum potential of 12 500 (N) infective propagule per bag, determined by the MPN (most probable number) method (Porter 1979), having 2 kg substrate was used. Different levels of inoculum potential that is, 1/4 N, 1/2 N, 1 N, 2 N, and 4 N were used along with a control. The inoculum was a mixture of three native arbuscular mycorrhizal fungi viz., *Glomus* isolate 1, *Glomus* isolate 2, and *Acaulospora scrobiculata*.

Mature seeds of *Artocarpus chaplasa* Roxb. were collected from the natural habitat. They were washed and surface-sterilized with 5% HgCl before they were put for germination in sterilized sand. The seedlings were grown for 20 days. Seedlings with uniform height (~5 cm) were used for transplanting to polythene bags in a completely randomized block design. Height, number of leaves, collar diameter, and leaf area of the seedlings were measured on the 120th day after transplantation. Roots were properly washed to remove the adhering soil. Shoot and root biomass was then determined. The SQI (seedling quality index) was calculated using the following formula (Dickson, Leaf, and Hosner 1960).

$$\text{SQI} = \frac{\text{Total dry weight (g/plant)}}{\dfrac{\text{Height (cm)}}{\text{Root collar diameter (cm)}} + \dfrac{\text{Shoot dry weight (g/plant)}}{\text{Root dry weight (g/plant)}}}$$

The MIE (mycorrhizal inoculation effect) was calculated using the formula given by Bagyaraj (1992).

$$\text{SQI} = \frac{\text{Dry weight of inoculated plants} - \text{Mean dry weight of uninoculated plants}}{\text{Dry weight of inoculated plants}}$$

Statistical analysis, that is, DMRT (Duncan's Multiple Range Test) was done following the method given by Gomez and Gomez (1984).

Results

Growth parameters viz., height, leaf number, leaf area, root collar diameter, root biomass, and shoot biomass were recorded on the 120th day after transplantation. The test plants showed a different growth with respect to the different levels of inoculum potential used. Maximum plant height, leaf number, and total dry biomass were recorded in the treatment where four times the normal inoculum potential was used (Table 1). The growth parameters showed an increasing trend with the increase in inoculum potential. In general, application of mycorrhizal inoculum resulted in a significant increase in plant height, leaf number, and total dry biomass of the test seedlings as compared to the control.

The root–shoot ratio varied significantly amongst the different treatments (Figure 1). Treatments with half the normal inoculum potential showed the highest root–shoot ratio. Seedlings treated with four times the normal inoculum potential significantly

Table 1 Seedling growth in *Artocarpus chaplasa* Roxb. as influenced by the mixed inoculum of native arbuscular mycorrhizal fungi at different inoculum potentials

Inoculum densities	*Height* (cm)	*Leaf number*	*Leaf area* (cm^3)	*Root collar diameter* (cm)	*Root dry biomass* (g)	*Shoot dry biomass* (g)	*Total dry biomass* (g)
1/4N	20.00	8	393	0.382	0.400	1.440	1.840
1/2N	17.00	9	280	0.525	1.376	2.853	4.229
1N	15.50	9	169	0.473	0.910	2.114	3.005
2N	16.50	8	178	0.632	1.120	2.550	3.670
4N	18.50	10	290	0.550	1.526	3.266	4.792
Control	13.85	7	176	0.385	0.495	1.733	2.228

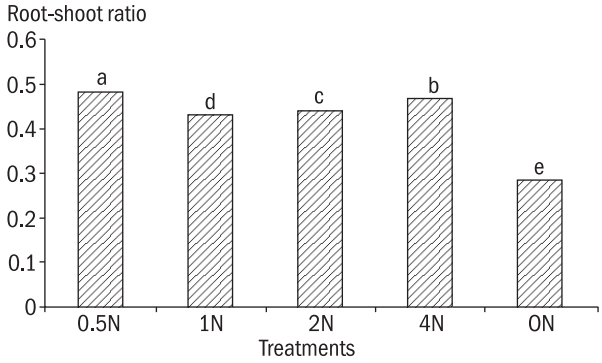

Figure 1 Root–shoot ratio in *Artocarpus chaplasa* Roxb. as influenced by mixed native arbuscular mycorrhizal fungi at different inoculum potentials
Note Bars with different letters differ significantly (P<0.5) according to Duncan's Multiple Range Test

improved the quality by 221% as compared to the uninoculated control (Figure 2). The mycorrhizal inoculation effect ranged between 25.85% and 53.50% for the different levels of inoculum potentials (Figure 3).

Discussion

The present study reveals the importance of native arbuscular mycorrhizal fungi on the growth and development of *Artocarpus chaplasa* Roxb. seedlings. Though there were differences in response of the plant to different inoculum potentials of the native arbuscular mycorrhizal fungi, there was always a positive response. An improvement in the quality of seedlings was observed in terms of growth parameters as compared to control. Arbuscular mycorrhizal fungi improve the plant growth and development of seedlings (Manjunath, Mohan, and Bagyaraj 1983; Ikram, Mahmud, Ghani, *et al*. 1992; Ravikumar, Ananthakrishnan, Appasamy, *et al*. 1997; Monticelli, Puppi, and Damiano 2000; Rajan, Reddy, and Bagyaraj 2000). This view is further strengthened by results of the present study in which inoculation of mycorrhizal fungi improved the overall quality, thus bioaugmenting the seedlings.

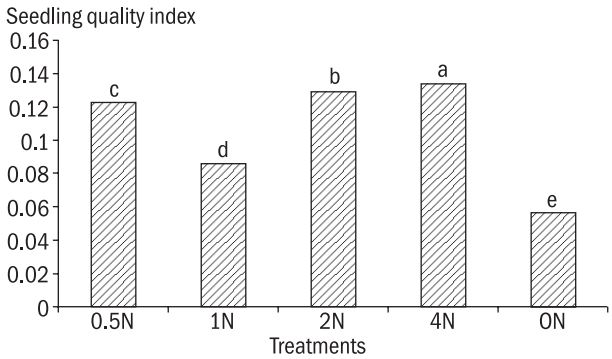

Figure 2 Seedling quality index in *Artocarpus chaplasa* Roxb. as influenced by mixed native arbuscular mycorrhizal fungi at different inoculum potentials
Note Bars with different letters differ significantly (P<0.5) according to Duncan's Multiple Range Test

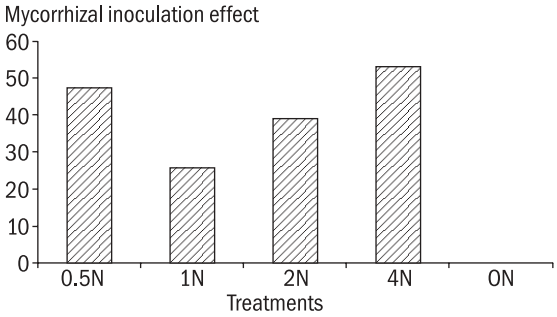

Figure 3 Mycorrhizal inoculation effect on *Artocarpus chaplasa* Roxb. after treatment with mixed native arbuscular mycorrhizal fungi at different inoculum potentials

Of the different treatments, the one with four-times the normal inoculum potential showed the best results, in terms of height, biomass, seedling quality index, and mycorrhizal inoculation effect. This effect may be because of differences in spore number that might account for different infection rates (Munro, Wilson, Jefwa, *et al*. 1999). In conclusion, the results confirm that appropriate quantity of arbuscular mycorrhizal fungal inoculum would help in production of quality seedlings for nursery practices. This may be because differences in spore number may account for different infection rates.

References

Bagyaraj D J. 1992
Vesicular-arbuscular mycorrhiza: application in agriculture
In *Methods in Microbiology*, edited by J R Norris, D J Read, and A K Varma
London: Academic Press. pp. 819–883

Bagyaraj D J, Brya Reddy M S, and Nalini P A. 1989
Selection of efficient inoculant VA mycorrhizal fungus for *Leucaena*
Forest Ecology and Management **27**: 81–85

Dickson A, Leaf A L, and Hosner J F. 1960
Quality appraisal of white spruce and white pine seeding stock in nurseries
Forestry Chronicle **36**: 10–13

Gamble J S. 1922
A manual of Indian Timbers
London: Sampson Low, Marston and Company Ltd. pp. 331

Gerdemann J W and Nicolson T H. 1963
Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting
Transaction of British Mycological Society **46**: 235–244

Gomez K A and Gomez A A. 1984
Statistical procedures for agricultural research (2nd edn)
[An International Rice Research Institute Book]
New York: John Wiley and Sons. 680 pp.

Ikram A, Mahmud A W, Ghani, M N, Ibrahim M T, Zainal A B. 1992
Field nursery inoculation of *Hevea brasilensis* Muell. Arg. seedling rootstock with vesicular arbuscular mycorrhizal fungi (VAM) fungi
Plant and Soil **145**: 231–236

Manjunath A, Mohan R, and Bagyaraj D J. 1983
Response of citrus to vesicular-arbuscular mycorrhizal inoculation in unsterile soils
Canadian Journal of Botany **61**: 2729–2732

Michelsen A. 1992
Mycorrhiza and root nodulation in tree seedlings from five nurseries in Ethiopia and Somalia
Forest Ecology and Management **48**: 335–344

Michelsen A. 1993
Growth improvement of *Ethiopian acacias* by addition of vesicular arbuscular mycorrhizal fungi or roots of native plants to non-sterile nursery soil
Forest Ecology and Management **59** :193–206

Monticelli S, Puppi G, and Damiano C. 2000
Effects of in vivo mycorrhization on micropropagated fruit tree rootstocks
Applied Soil Ecology **15(2)**: 105–111

Munro R C, Wilson J, Jefwa J, Mbuthia K W. 1999
A low-cost method of mycorrhizal inoculation improves growth of *Acacia tortolis* seedlings in the nursery
Forest Ecology and Management **113**: 51–56

Muthukumar T, Udaiyan K, and Rajeshkannan V. 2001
Response of neem (*Azadirachta indica* A. Juss) to indigenous arbuscular mycorrhizal fungi, phosphate-solubilising and asymbiotic nitrogen-fixing bacteria under tropical nursery conditions
Biology and Fertility of Soils **34**: 417–426

Porter W M. 1979
The 'most probable number' method for enumerating propagules of VAM fungi in soil
Australian Journal of Soil Research **17**: 515–519

Rajan S K, Reddy B J D, and Bagyaraj D J. 2000
Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*
Forest Ecology and Management **126**: 91–95

Ravikumar R, Ananthakrishnan G, Appasamy T, Ganapathi A. 1997
Effect of endomycorrhizae (VAM) on bamboo seedling growth and biomass productivity
Forest Ecology and Management **98**: 205–208

New approaches

A structured modelling approach to assess the role of nutrition in mycorrhizal symbiosis

A structured modelling approach was developed by Jelicoeus M, Bouchard Marchand E, Becard G, Perrier M (2003) to assess and describe the role of nutrition in mycorrhizal symbiosis (*Ecological Modelling* **163**[3]: 245). *Agrobacterium rhizogenes* transformed *Daucus carota* hairy roots and the AM fungus *Glomus intraradices* was used as a biological model in Petri dish liquid cultures. A minimal medium was used, varying the initial Pi (phosphate) concentrations (0.02, 0.04, 0.12, and 1.00 mM KH_2PO_4). The uptake of sugar and macronutrients was monitored during the culture. The Pi ion was the limiting nutrient at 0.02 and 0.04 mM Pi. The specific growth rate (dry mass) of non-mycorrhizal roots was significantly reduced under low Pi from 0.068 ± 0.012 (at greater than or equal to 0.12mM Pi) to 0.04 ± 0.006 (at 0.04 Pi) and 0.022 ± 0.009 (at 0.02 mM Pi) per day. Values for mycorrhizal roots were even lower. The specific spore production rate for *G. intraradices* was maximal at 0.042 per day for 0.12 mM Pi. A growth behaviour dual model was presented for root dry mass and fungus spore number based on extra- and intra-cellular concentration of Pi and sugar and extra-cellular nitrate. The Pi translocation between symbionts and roots' sugar uptake by the fungus were also described in the model. Calibration of the model with experimental data suggested that intra-cellular Pi storage in roots acts as a decision switch for *G. intraradices* spore production. A significant AM fungus competition for soluble root sugars was also revealed.

Centre for Mycorrhizal Culture Collection

Mycorrhizal technology: faster and environment-friendly method of growing *Jatropha curcas*, the biodiesel plant

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It is common knowledge that air pollution and the resulting greenhouse gas emissions have taken a toll on the health of the planet. Vehicular emissions, in particular, have led to a substantial deterioration in air quality as petrochemical-based automobile fuels contain atmospheric pollutants such as nitrogen oxides and lead. Besides, many nations in the world, including India, rely on imports to meet their soaring fuel requirements. This dependence can be reduced with a secure supply of fuel and, if possible, clean fuel. Biodiesel is considered as a substitute option for mineral diesel to address the issue of air quality and energy security. It represents a variety of ester-based oxygenated fuels derived from natural, renewable biological sources such as vegetable oils and its efficacy as an automotive fuel is well recognized. Biodiesel operates in compression ignition engines like petroleum diesel, thereby requiring no essential engine modifications. Moreover, it can maintain the payload capacity and range of the conventional diesel. Biodiesel fuel can be made from new or used vegetable oils and animal fats. Unlike fossil diesel, pure biodiesel is biodegradable, non-toxic, and essentially free from sulphur and aromatics. The concept of using vegetable oil as a fuel dates back to 1895 when Dr Rudolf Diesel developed the first diesel engine to run on vegetable oil.

India cannot divert good-quality edible vegetable oil for production of biodiesel, as is done in Europe and USA. Under the Indian conditions, only such plant sources can be considered for biodiesel production that are not edible oils in appreciable quantity and that can be grown on a large-scale on wastelands. Of all prospective plant candidates as biodiesel-yielding sources, *Jatropha curcas* stands at the top and sufficient information on this plant is already available. It is being hailed as the new solution to the problem of vehicular pollution. A wild shrub, it has several traits that make it a favourite with scientists, industrialists, commercial planters, and others: it grows under adverse conditions like infertile soil; it is not grazed upon by animals; and it yields a sulphurless, non-polluting biofuel.

As Jatropha is a clean substitute to diesel, the CMR (Centre for Mycorrhizal Research) has developed an unconventional method of growing the plant faster and better. The CMR has raised Jatropha with mycorrhizal application across the country. Tests done on these plants have revealed their significantly high dependence. There are different methods of Jatropha propagation, which are very slow. The standard seedling method takes two years for the plant to start yielding. The plants raised from clonal culture take a year for the first yield. The CMR's mycorrhiza application to Jatropha speeds up the process of flowering and fruiting: the first yield arrives after seven months of cultivation. Besides this, the technique also results in up to 30% higher yield and plant biomass. These plantations span seven different agro-climatic zones across the country. The CMR's mycorrhiza application speeds up the process: the first yield arrives after seven months of cultivation and also increases the yield up to 30%. Where conventional methods of Jatropha plantations yielded 200 kg per hectare, compact plantations in wastelands produced 325 kg per hectare in the first year of production with this technology. Using the technology, Jatropha plantations were also established in the industrially created wastelands, for example, fly ash overburdens, chlor alkali-laden sites, sites with distillery effluents, and so on.

More than 6 00 000 plants of Jatropha using mycorrhiza have been established, spanning seven different agro-climatic zones across the country comprising the Chhattisgarh plain zone (at Korba, Madhya Pradesh), the arid western plain zone (Jodhpur, Rajasthan), Malwa Plateau zone (Barwaha, Madhya Pradesh), south Gujarat zone (Mithapur, Gujarat), coastal saline zone (Titagarh, West Bengal), Vijayawada and Machlipatnam (south zone of Andhra Pradesh), Kanjivaram (south zone of Tamil Nadu), and many more are planned in the near future. This large-scale exercise has contributed to the knowledge on early flowering and high-yielding Jatropha plants from superior germplasm and on application of mycorrhizal biofertilizer. This activity needs to be taken up more seriously nation-wide for continued improvement of quality planting material and microbial applications for achieving higher yields.

Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge. This list consists of papers from the following journals.

- *Annual Review of Entomology*
- *Biological Invasions*
- *Biology and Fertility of Soils*
- *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*
- *Canadian Journal of Soil Science*
- *Environmental Pollution*
- *Environmental Science and Pollution Research*
- *Fems Microbiology Letters*
- *Fungal Genetics and Biology Geoderma*
- *Interciencia*
- *Journal of Plant Physiology*
- *Journal of the American Society for Horticultural Science*
- *Mycorrhiza*
- *New Phytologist*
- *New Zealand Journal of Botany*
- *Plant and Soil*
- *Soil Biology & Biochemistry Symbiosis*

Copies of papers published by mycorrhizologists during this quarter may please be sent to the Network for inclusion in the next issue.

Name of the author(s) and year of publication	Title of the article, name of journal, volume no., issue no., page nos [corresponding address given for author whose name is highlighted in asterisk]
Alguacil M M, Caravaca F,* and Roldan A. 2005	**Changes in rhizosphere microbial activity mediated by native or allochthonous AM fungi in the reafforestation of a Mediterranean degraded environment** *Biology and Fertility of Soils* **41**(1): 59–68 [Department of Soil & Water Conservation, CSIC, Centro de Edafología y Biologia Aplicada del Segura, POB 4195, Campu Espinardo, Murcia 30100, Spain]
Alvarez M*, Godoy R, Heyser W, Hartel S. 2005	**Anatomical–physiological determination of surface-bound phosphatase activity in ectomycorrhizae of *Nothofagus obliqua*** *Soil Biology & Biochemistry* **37**(1): 125–132 [Institute of Botany, University of Austral Chile, Campus Isla Teja, Casilla 567, Valdivia, Chile]
Auge R M. 2004	**Arbuscular mycorrhizae and soil/plant water relations** *Canadian Journal of Soil Science* **84**(4): 373–381 [Department of Plant Sciences, University of Tennessee, 2431 Joe Johnson Drive, Knoxville, Tennessee 37996, USA]
Bagley S J and Orlovich D A. 2004	**Genet size and distribution of *Amanita muscaria* in a suburban park, Dunedin, New Zealand** *New Zealand Journal of Botany* **42**(5): 939–947 [Department of Botany, University of Otago, POB 56, Dunedin, New Zealand]
Balser T C, Treseder K K, and Ekenler M. 2005	**Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence** *Soil Biology & Biochemistry* **37**(3): 601–604 [Department of Soil Science, University of Wisconsin, 1525 Observatory Drive, Madison, Wisconsin 53706, USA]
Becerra A,* Nouhra E, Daniele G, Dominguez L, McKay D. 2005	**Ectomycorrhizas of *Cortinarius helodes* and *Gyrodon monticola* with *Alnus acuminata* from Argentina** *Mycorrhiza* **15**(1): 7–15 [Consejo Nacional de Investigaciones Cientificas y Tecnologicas, Instituto Multidisciplinario de Biologia Vegetal, CC 495, RA-5000 Cordoba, Argentina]
Bittman S, Forge T A,* and Kowalenko C G. 2004	**Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer** *Soil Biology & Biochemistry* **37**(4): 613–623 [Agriculture & Agri Food Canada, Pacific Agrifood Research Centre, POB 1000, 6947 Highway, Agassiz, BC V0M 1A0, Canada]
Caravaca F,* Alguacil M M, Barea J M, Roldan A. 2005	**Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem** *Soil Biology & Biochemistry* **37**(2): 227–233 [Department of Soil & Water Conservation, CSIC, Centro de Edafologia y Biologia Aplicada del Segura, POB 4195, Campu Espinardo, Murcia 30100, Spain]
Carpio L A, Davies F T, and Arnold M A. 2005	**Arbuscular mycorrhizal fungi, organic and inorganic controlled-release fertilizers: Effect on growth and leachate of container-grown bush morning glory (*Ipomoea carnea* ssp. fistulosa) under high production temperatures** *Journal of the American Society for Horticultural Science* **130**(1): 131–139 [Department of Horticulture Science, Texas A&M University, College Station, Texas 77843, USA]

Name of the author(s) and year of publication	Title of the article, name of journal, volume no., issue no., page nos [corresponding address given for author whose name is highlighted in asterisk]
Chacon N*, Dezzeo N, and Flores S. 2005	**Effect of particle-size distribution, soil organic carbon content and organo-mineral aluminium complexes on acid phosphatases of seasonally flooded forest soils** *Biology and Fertility of Soils* **41**(1): 69–72 [Inst Venezolano Invest Cient, Centro de Ecoloime, Apdo 21-827, Caracas 1020, Venezuela]
Chaudhry Q, Blom-Zandstra M, Gupta S, Joner E J. 2005	**Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment** *Environmental Science and Pollution Research* **12**(1): 34–48 [Department of Environment, Food & Rural Affairs, Cent Sci Lab, York YO41 1LZ, N Yorkshire, England]
Chen B D, Roos P, Borggaard O K, Zhu Y G, Jakobsen I. 2005	**Mycorrhiza and root hairs in barley enhance acquisition of phosphorus and uranium from phosphate rock but mycorrhiza decreases root to shoot uranium transfer** *New Phytologist* **165**(2): 591–598 [Riso National Laboratory, Plant Research Department, Denmark 4000, Roskilde, Denmark]
Chen B D, Liu Y, Shen H, Li X L, Christie P. 2004	**Uptake of cadmium from an experimentally contaminated calcareous soil by arbuscular mycorrhizal maize (*Zea mays* L.)** *Mycorrhiza* **14**(6): 347–354 [Department of Plant Nutrition, China Agricultural University, 2 Yuanmingyuan Road, Beijing 100094, Peoples Republic of China]
Corradi N, Hijri M, Fumagalli L, Sanders I R.* 2004	**Arbuscular mycorrhizal fungi (Glomeromycota) harbour ancient fungal tubulin genes that resemble those of the chytrids (Chytridiomycota)** *Fungal Genetics and Biology* **41**(11): 1037–1045 [Department of Ecology & Evolution, University of Lausanne, Biology Buildings, CH-1015 Lausanne, Switzerland]
da Silva G A, Trufem S F B, Junior O J S, Maia L C.* 2005	**Arbuscular mycorrhizal fungi in a semi-arid copper mining area in Brazil** *Mycorrhiza* **15**(1): 47–53 [Departmento de Micologia, Universi dade Federal de Pernambuco, CCB, Avenue Prof Nelson Chaves S-N, BR-50670420 Recife, PE, Brazil]
de Ridder-Duine A S,* Kowalchuk G A, Gunnewiek P J A K, Smant W, van Veen J A, de Boer W. 2005	**Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition** *Soil Biology & Biochemistry* **37**(2): 349–357 [The Netherlands Institute of Ecology, Boterhoeksestr 48, NL-6666 GA Heteren, The Netherlands]
de Boulois H D, Delvaux B, and Declerck S.* 2005	**Effects of arbuscular mycorrhizal fungi on the root uptake and translocation of radiocaesium** *Environmental Pollution* **134**(3): 515–524 [Universite Catholique de Louvain, Mycothegue, MUCL, Microbiol Unite, Pl Croix Sud 3, B-1348 Louvain, Belgium]
Dehlin H,* Nilsson M C, Wardle D A, Shevtsova A. 2004	**Effects of shading and humus fertility on growth, competition, and ectomycorrhizal colonization of boreal forest tree seedlings** *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **34**(12): 2573–2586 [Department of Forest Vegetation Ecology, Swedish University of Agriculture Sciences, Fac Forestry, SE-90183 Umea, Sweden]

Name of the author(s) and year of publication	Title of the article, name of journal, volume no., issue no., page nos [corresponding address given for author whose name is highlighted in asterisk]
Diez J. 2005	**Invasion biology of Australian ectomycorrhizal fungi introduced with eucalypt plantations into the Iberian Peninsula** *Biological Invasions* **7**(1): 3–15 [Department of Biology, Univ Alcala de Henares, E-28871 Alcala De Henares, Madrid, Spain]
Driver J D, Holben W E, and Rillig M C.* 2005	**Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi** *Soil Biology & Biochemistry* **37**(1): 101–106 [Division of Biological Sciences, The University of Montana, Microbial Ecology Program, 32 Campus Drive 4824 HS104, Missoula, MT 59812, USA
Duponnois R, Paugy M, Thioulouse J, Masse D, Lepage M.* 2005	**Functional diversity of soil microbial community, rock phosphate dissolution and growth of *Acacia seyal* as influenced by grass- , litter- , and soil-feeding termite nest structure amendments** *Geoderma* **124**(3–4): 349–361 [Interact Biol Dans Sols Syst Anthopises Trop, IBIS, Unite Rech, IRD, 01 BP 182, Ouagadougou, Burkina Faso]
Esitken A,* Ercisli S, and Eken C. 2005	**Effects of mycorrhiza isolates on symbiotic germination of terrestrial orchids (*Orchis palustris* Jacq. and *Serapias vomeracea* subsp vomeracea (Burm.f.) briq. in Turkey** *Symbiosis* **38**(1): 59–68 [Faculty of Agriculture, Department of Horticulture, Ataturk University, TR-25240 Erzurum, Turkey]
Feeney D S, Daniell T, Hallett P D, Illian J, Ritz K, Young I M.* 2004	**Does the presence of glomalin relate to reduced water infiltration through hydrophobicity?** *Canadian Journal of Soil Science* **84**(4): 365–372 [University of Abertay Dundee, SIMBIOS Centre, Bell Street, Dundee DD1 1HG, Scotland]
Flores C* and Cuenca G. 2004	**Growth and mycorrhizal dependency of the pioneer species and pollenectarizing *Oyedaea verbesinoides* Asteraceae** *Interciencia* **29**(11): 632–637 [Department of Agron, Univ Nacl Expt Tachira, Apartado 436, San Cristobal 5001, Tachira, Venezuela]
Fountain M T* and Hopkin S P. 2005	**Folsomia *candida* (Collembola): A 'standard' soil arthropod** *Annual Review of Entomology* **50**: 201–222 [Department of Agriculture, Centre for Agri-Environmental Research, University of Reading, Reading RG6 6AR, Berks, England]
Fransson P M A, Taylor A F S, and Finlay R D. 2005	**Mycelial production, spread and root colonisation by the ectomycorrhizal fungi *Hebeloma crustuliniforme* and *Paxillus involutus* under elevated atmospheric CO_2** *Mycorrhiza* **15**(1): 25–31 [Department of Plant & Soil Sciences, School of Biological Sciences, University of Aberdeen, Cruickshank Bldg, St Machar Dr, Aberdeen AB24 3UU, Scotland]
Fujimaki R,* McGonigle T P, and Takeda H. 2004	**Soil micro-habitat effects on fine roots of *Chamaecyparis obtusa* Endl.: A field experiment using root in growth cores** *Plant and Soil* **266**(1–2): 325–332 [Kyoto University, Laboratory of Forest Ecology, Graduate School of Agriculture, Kyoto 6068502, Japan]

ISSN 0970-695X Regd No. 49170/89

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Editor Alok Adholeya • **Associate Editors** Reeta Sharma, T P Sankar • **Assistant Editor** Pritika Kalra

Printed and published by Dr R K Pachauri on behalf of the The Energy and Resources Institute, Darbari Seth Block, I H C Complex, Lodhi Road, New Delhi – 110 003, and *printed at* Multiplexus (India), C-440, DSIDC, Narela Industrial Park, Narela, Delhi – 110 040.