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About TERI

TERI is an autonomous not-for-profit, research institute established in 1974 and is involved in research activities in the fields of energy, environment, biotechnology, forestry, and the whole range of sustainable development issues. More specifically, its activities are geared to promoting large-scale use of renewable forms of energy, protecting the earth's ozone layer, mitigating the threat of climate change, and reversing the loss of forest cover and biodiversity, all of which transcend national boundaries and require solutions through global cooperation.

About the Biotechnology Division

The Biotechnology Division seeks to achieve sustainable development through wide-ranging research, from microbes on the one hand to giant forest trees on the other. The Division has three areas: Microbial Biotechnology, Plant Molecular Biology, and Plant Tissue Culture.

The Division also incorporates the Asian Network on Mycorrhizae which coordinates research activities in the field in Asia. A National Facility on Germplasm of Mycorrhizal Fungi has also been established. It has been demonstrated that these organisms are efficient tools for growth enhancers and reclamation of barren sites overburdened with fly ash.

About Mycorrhiza Network and CMCC

The Mycorrhiza Network, located at the Tata Energy Research Institute (TERI), New Delhi, was set up in 1988 with an important component—the Mycorrhiza Information Centre (MIC). With effect from December 1993, a germplasm bank facility named Centre for Mycorrhizal Culture Collection (CMCC) started functioning as a composite project with the Mycorrhiza Network. The general objectives of the Mycorrhiza Network are to strengthen research, encourage cooperation, promote exchange of information, and publish a quarterly newsletter, Mycorrhiza News. The MIC is primarily responsible for establishing an information network in the region that makes available to the researchers the growing literature on mycorrhiza and facilitates information sharing among the members. A database on mycorrhizal literature is operational for information retrieval and supply to researchers, 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 at CMCC and provide starter cultures on request. Cultures from CMCC are available on an exchange basis or on specific requests at nominal costs for spore extraction/handling.

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Role of mycorrhiza in tree nurseries—Part I. Evaluation of mycorrhizal efficiency with and without application of fertilizers

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Tree species differ in their dependency on mycorrhizae. Some tree species like pines fail to establish and grow healthy in the absence of mycorrhizal colonization on their roots. Some tree species may be partly dependent on mycorrhiza, but there is hardly any tree species which does not have mycorrhizae of one type or the other on their roots. Tree species, especially exotics, fail to establish on afforestation sites in the absence of their fungal symbiont. For 20 years, repeated attempts to raise pine plantations in Puerto Rico failed due to the lack of ectomycorrhizae on their roots. The pine seedlings would grow to 5–30 cm in height, then become chlorotic, and die. Transfer of ectomycorrhizal soil from successful plantations in the mainland to Puerto Rico nurseries helped in the successful establishment of pine plantations on this island. In three years, inoculated plants of slash pine grew healthy and reached a height of 2–2.5 m (Hacskaylo 1967). Similarly, success in raising pine plantations in South Africa was achieved only when ectomycorrhizal inoculum from The Netherlands was introduced in South African nurseries. At reforestation sites also, non-mycorrhizal nursery raised seedlings grow poorly for the first one to two years. Normal growth occurs only when mycorrhizal fungi present in the site establish mycorrhiza on the roots. The following accounts discuss different aspects of the role of mycorrhizae in tree nurseries.

Evaluation of mycorrhizal inoculation in tree nurseries

Increase in growth and mycorrhizal colonization

Studies conducted at the Alabama University, USA, highlighted the need for the introduction of mycorrhiza in tree nurseries. Stunted *Pinus taeda* seedlings were found only at a nursery raised on a new ground where nursery crop of ectomycorrhizal tree seedlings has never been raised though ectomycorrhizal tree hosts were present in the immediate vicinity. Stunted seedlings were either non-mycorrhizal or had extremely low levels of ectomycorrhizae and were found deficient in phosphorus whereas non-stunted seedlings had a greater degree of ectomycorrhizal development and had sufficient phosphorus. Application of phosphoric acid to stunted seedlings increased shoot phosphorus and resulted in growth improvement (South *et al.* 1986).

In studies conducted at the Purdue University, West Lafayette, USA, inoculation of nursery beds with vegetative mycelia of *Pisolithus tinctorius* significantly increased the number of saleable red oak seedlings, average stem height, and diameter in two to three years suggesting that the test fungus could successfully compete with indigenous fungi in the formation of mycorrhizae (Pope 1988). Studies conducted at the Department of Soil Science, University of British Columbia, Vancouver, Canada, showed that seedlings of *Thuja placata,* in a nursery that was fumigated one year before planting with methyl bromide, were stunted and had purple foliage. Mycorrhizal colonization was significantly higher in the unfumigated beds than in the fumigated beds (Berch *et al.* 1991). In experiments conducted at the Institut National de la Recherche Agronomique (INRA), Centre de Recherché, Forestiers, Dijon, France, in a bare-root nursery, soil fumigation eliminated or reduced propagules of pathogenic fungi, *Rhozoctonia solani, Pythum* sp. and probably *Fusarium oxysporum* and improved performance of Norway spruce seedlings, particularly in the second year. Douglas fir seedlings seemed less sensitive to soilborne

pathogens. Two years after inoculation, *Hebeloma cylindrospora* increased Norway spruce growth by 40% and Douglas fir growth by 30% in comparison with seedlings grown in fumigated soil which became mycorrhizal with naturally occurring fungus, *Thelephora terrestris*. Both fumigation and artificial inoculation increased Norway spruce seedling growth by 110% and Douglas fir seedling growth by 50% (Tacon *et al.* 1986).

Studies conducted at the National Mycorrhizae Nursery Evaluation, US Forest Service, Asheville, North Carolina, USA, showed that artificial nursery seedbed inoculations in tree nurseries with *Pisolithus tinctorius* provided significant increase in ectomycorrhizae formation on seedling feeder roots, increased seedlings fresh weight, decreased cull percentages, and increased survival and growth in field plantings (Cordell 1979).

Studies conducted at the Department of Horticulture, University of Maryland, USA, on containerized seedlings of *Quercus rubra* inoculated with *Pisolithus tinctorius* showed that after 60 days of growth, the mean total dry weight of mycorrhizal and non-mycorrhizal seedlings were not significantly different (2.5 g and 2.8 g, respectively). However, after 100 and 140 days of growth, the mean total dry weights of mycorrhizal seedlings were significantly less than that of non-mycorrhizal seedlings (5.3 g versus 5.8 g for 100 days and 6.2 g versus 6.8 g for 140 days, respectively) (Beckjord *et al.* 1979). In studies conducted at the Forest Research Institute, Malaysia, Kapong, Kuala Lumpur, Malaysia, *Hopea odorata* seedlings were raised from seeds germinated in sand and transferred to steam sterilized forest soil + sand (3:1 mixture) which had been inoculated with 20% (v/v) of the growing inoculum with controls uninoculated (C1) and uninoculated but treated with sterilized fungal inoculum (C2). After six months, C2 control plants were 153% better than C1 plants and inoculated plants were 62% better than C2 plants. Same trend was observed in shoot dry weights (Yasids *et al.* 1996)

Studies conducted at the Institute for Mycorrhizal Research and Development, Athens, Georgia, showed that root collar diameters and heights of endomycorrhizal seedlings of sweet gum in nurseries were 0.70 cm and 32.7 cm, respectively, as compared to 0.17 cm and 5.0 cm, respectively, of non-mycorrhizal seedlings in all 18 half-sib progenies tested. Further studies at the above institute showed that after one growing season endomycorrhizal sweet gum seedlings (with *Glomus mosseae*) from eight mother trees at four levels of soil fertility suffered little mortality and averaged about 36 cm height as compared to non-mycorrhizal seedlings which either died or failed to exceed 5 cm in height, regardless of soil fertility, thus, suggesting that introduction of mycorrhiza in sweet gum nurseries is necessary to obtain plantable seedlings (Kormanik *et al.* 1997a, b).

Studies conducted at the University of Delhi, India, showed that the growth of *Leucaena leucocephala* and *Sesbania sesban* seedlings inoculated with *Glomus macrocarpum* was greater than that of non-inoculated plants. Also, phosphatase activity, used to assess uptake of phosphorus, was greater in inoculated plants (Jagpal, Mukerji 1991).

Studies conducted at the Department of Forestry, Wildlife and Fisheries, University of Tennessee, USA, on newly germinated seedlings of *Leriodendron tulipifera* inoculated with *Glomus mosseae* and *G. fasciculatum*, individually or combined, and inoculum from natural *L. tulipifera* stand showed that total length of primary roots was not different in inoculated and uninoculated seedlings. Shoot growth, root collar diameter, and oven dry weights of tops were greater in individual Glomus treatments and least in controls. Oven dry weights of tops in individual Glomus treatments were four times greater than those of control. Also, individual Glomus treatments had the most frequent occurrence of free hyphae, hyphal coils and arbuscules, followed by combined Glomus treatments and control but this trend was reversed in occurrence of vesicles (Ford, Hay 1979).

Increase in photosynthesis rate

Studies were conducted at the Colorado State University, Fort Collins, Colorado, USA, to see the effect of inoculation with ectomycorrhizal fungi *Cenococcum graniforme, Suillus granulatus* and *Pisolithus tinctorius* on photosynthesis in seedlings of *Pinus contorta, P. ponderosa, P. flexilis,* and *P. aristata* grown on peat vermiculite in standard styrofoam containers. Ectomycorrhizal infection, after eight months, increased the rate of net photosynthesis over that of non-infected controls. Per cent mycorrhizal short roots and mycorrhizal root number were found to be significantly correlated with the photosynthesis rate increase. However, photosynthetic rates were differentially influenced by specific hosts, fungi and host-fungus symbiosis (Kidd, Reid 1979).

Studies conducted at the Colorado University, USA, showed that photosynthesis rates (PnR) and foliar nitrogen and phosphorus contents were consistently higher in one- to nine-month old *Pinus taeda* plants inoculated with *Pisolithus tinctorius* at one-month age when compared to controls, the PnR being 2.1 times as great at the age of 10 months. Also, mycorrhizal plants assimilated more CO_2, allocated a greater percentage of assimilated carbon to the root system, and lost a greater percentage of root carbon by root respiration than controls. Similar studies on *Pinus contorta* seedlings inoculated at 10-weeks age with *Pisolithus tinctorius* or *Suillus granulatus* showed

that PnR, biomass and foliar P contents (not foliar N content) were significantly greater in mycorrhizal plants than in controls at 16-weeks age. Maximum mycorrhiza formation occurred at maximum light intensity and at intermediate level of N fertilization (Reid *et al.* 1983). Studies conducted at Laboratoire de Recherches sur les Symbiotes des Racines, Institut National de la Recherche Agronomique, Montpellier, Cedex, France, on *Pinus pinaster* seedlings grown in containers filled with parlite/vermiculite and inoculated with *Hebeloma cylindrosporum* (strain D3.25.9) and given 0 or 0.5 mM phosphate in the nutrient solution showed that mycorrhizal infection in plants increased net photosynthesis and root respiration rates as compared to non-mycorrhizal plants but nitrogen content was also low and an accompanying 35% depression in growth. Phosphorus application in non-mycorrhizal plants induced a rise in tissue phosphorus content but did not result in increased photosynthesis (Conjeaud *et al.* 1996).

Increase in drought resistance

Studies were conducted at the University of Missouri, Columbia, to determine the drought resistance of *Quercus* alba seedlings inoculated with *Pisolithus tinctorius* subject to a drying cycle after six months' growth. Inoculated seedlings had greater root and shoot development than uninoculated controls. Soil water potential decreased much more rapidly in inoculated than in uninoculated seedlings due to greater leaf area and root absorptive surface area during the drying cycle in inoculated plants. Change in prelight and middling xylem pressure potentials and leaf conductance closely followed soil water depletion pattern. Inoculated seedlings had higher rates of root elongation for both well-watered and drying-cycle treatments as compared to uninoculated seedlings (Wright *et al.* 1979).

In studies conducted at the University of Copenhagen, Denmark, *Acacia nilotica* and *Leucaena leucocephala* seedlings were raised by direct seed sowing in soil inoculated with a mixture of *Glomus etunicatum, G. mosseae,* and *G. occultum* and subjected to three treatments, namely, VAM ±, phosphorus ±, and repeated drought treatment ±, for the seventh week. The results showed that the effect of VAM equaled that of P fertilization after 12 weeks, and drought treatment reduced seedling biomass (39% in *A. nilotica* and 27% in *L. leucocephala*) and nodulation (particularly in *A. nilotica*). Phosphorus treatment increased nodulation in both species while VAM significantly increased nodulation in *L. leucocephala* only. *A. nilotica* was more drought resistant than *L. leucocephala*. The results demonstrated the potential of using VAM-inoculated plants in reforestation programmes on degraded areas of arid tropics particularly to counter drought stress following transplantation (Michelsen, Rosendahl 1990).

Application of fertilizers and efficiency of mycorrhizae in tree nurseries

Application of fertilizers to nursery soil/potting mixtures may affect the performance of mycorrhizal fungi to varying degrees. Fertilizers may be applied as complete fertilizers, phosphorus alone, nitrogen alone, phosphorus and nitrogen together or trace elements. Effects of various types of fertilizers on mycorrhizal development in tree nurseries are discussed.

Application of complete fertilizers

Effect on efficiency of ectomycorrhizal inoculum

Studies conducted at the North Central Experimental Forest Station, United States Department of Agriculture (USDA), Rhinelander, USA, on container grown seedlings of *Larix decidua* inoculated at sowing with mycelial cultures of eight mycorrhizal fungi and treated with standard or reduced dosages of complete fertilizers showed that after 20 weeks only, seedlings inoculated with *Hebeloma crustuliniforme* and *Laccaria laccata* developed significant proportions of ectomycorrhizal development, stimulated shoot growth and increased shoot-root ratios (Rietveld *et al.* 1989).

Studies were conducted at the Balco Canfor Reforestation Centre Ltd, Kamloops, Canada, to see the effect of the two NPK slow release formulations namely osmocote 10–6–12 and nutricote 16–10–10, both at 4.7 kg/m^3 and a micronutrient formulation, Micromax at 385 g/m^3 (all added as supplements to soluble fertilizers), on growth and mycorrhizal development in *Picea engelmannii.* The result showed that osmocote was most detrimental to mycorrhizal intensity and diversity. Non-supplemental controls had the highest mycorrhizal diversity. Seedlings supplemented with osmocote or nutricote had lower root weight but greater shoot length stem caliper and total weight as compared to un-supplemented controls receiving only soluble fertilizers. Micromax with osmocote decreased shoot length and shoot-root ratio and with nutricote increased shoot length when compared to osmocote alone and nutricote alone, respectively (Hunt 1989).

In studies conducted at the Petawawa National Forestry Institute, Ontario, Canada, on container grown *Larix laricina* seedlings inoculated with

Laccaria laccata and grown under two levels of slow release fertilizers (osmocote) or two levels of soluble fertilizers (NPK), non-mycorrhizal seedlings grew better under high fertility regimes, whereas, no significant differences were observed between low and high fertility level for the mycorrhizal seedlings which had significantly greater root length, total biomass, a lower shoot-root ratio and substantial ectomycorrhizal development under all fertility treatments. N, P, K, Ca, and Mg concentrations were significantly higher in the mycorrhizal seedlings than in the non-mycorrhizal seedlings under all fertility regimes (Chakravarty, Chatarpaul 1990).

In studies conducted at the Institute of Terrestrial Ecology, Bush Estate, Midlothean, up to 114% more seedlings of *Picea sitchensis* were found in seed beds inoculated with live fungus, *Laccaria proxima* in vermiculite-peat carrier than in control plots with or without the same carrier in two nurseries. A similar, though reduced, effect was found when live fungus was encapsulated in alginate beads. Application of NPK to the seedbed before sowing or subsequently as a top dressing reduced the number of emergent seedlings at both nurseries; being reduced to 40–50% in bed receiving only fertilizers and to 66–72% in beds receiving live inoculum and fertilizer when compared to seedlings on unfertilized beds. The effect of inoculant on seedling numbers occurred within first few weeks after inoculation and was independent of mycorrhizal formation (Ingleby *et al.* 1994).

Studies conducted at the Department of Microbiology, University of Surrey, Guilford, Surrey, England, on aseptically germinated sitka spruce seedlings and then planted in mycorrhiza-inoculated soil showed that in fertilized soil, inoculated seedlings were generally smaller than uninoculated seedlings though nitrogen and phosphorus contents and concentrations were generally greater. The reduction in growth may be due to the removal of photosynthates which are otherwise utilized for seedling biomass production by mycorrhizal fungi. Under nutrient limiting conditions, inoculated seedlings were larger, NPK content was greater, and NPK concentration was smaller than uninoculated seedlings. The growth increase was influenced by fungal species and edaphic conditions. The smaller NPK concentration was probably due to large biomass which diluted the NPK concentrations (Thomas, Jackson 1981).

Effect on efficiency of VAM inoculation

In studies conducted at the Grassland Crops Research Institute, Little Hampton, West Sussex, UK, container-grown plants of *Juniperous virginiana* cultivar Skyrocket, inoculated with *Glomus fasciculatum*, were 39% taller when grown with half the recommended rate of slow release fertilizer (2 g/litre) and 14% taller receiving 4 g/litre of the fertilizer than uninoculated plants after 14 months. With *Magnolia soulangiana*, the mean total stem length of VAM inoculated plants was 43% greater than that of plants receiving 4 g/litre of slow release fertilizer (Hall, Bowes 1984).

Studies were conducted at the Iowa State University, Iowa, USA, on eight hardwood species (*Acer negundo, A. rubrum, A. saccharum, Fraxinus pennsylvanica, Juglans regia, Liquidamber styraciflua, Platanus occidentalis,* and *Prunus serotena*) grown under two sets of fertilizers and VAM treatments (set 1: three treatments of 140, 560, 1120 kg/ha of 10:10:10 N, P_2O_5, K_2O, respectively, added to fumigated soil with or without a mixture of *Glomus mosseae* and *G. etunicatum*; set 2: treatment with same VAM mixture, *G. fasciculatum* or mixed cultures of several *Glomus* or *Gigaspora spp*. A fertilizer treatment of 280 kg/ha of 10:10:10 was added to all the seedlings). All treatments in both studies also received 10 equal applications of NH_4NO_3 totalling 1680 kg/ha. During growing season, uninoculated plants had higher N, K, Ca, and Mg and lower P concentrations than VAM seedlings. Seedlings inoculated with *Glomus* mixtures and *Gigaspora* spp. had higher P concentration than those inoculated with *G. fasciculatum* (Schultz, Kormanik 1982). Further observations on set 1 treatments of the above experiments at the above university showed that for six of the eight hardwoods, VAM seedlings showed greater height and diameters growth and dry weight production than non-mycorrhizal seedlings, *Acer saccharum* and *Juglans nigra*, however, showed no difference. Infection values varied from a high of about 80% for *Platanus occidentalis, F. pennsylvanica,* and *A. negundo* to a low of 40% for *A. saccharum* and *L. styraciflua* (Schultz *et al*. 1981). In studies conducted at the University of Georgia, Athens, USA, on seedlings of eight half-sib sweet gum (*Liquidambar styraciflua*) families, inoculated with a mixture of *Glomus mosseae* and *G. etunicatum* and given 140, 280, 560, and 1120 kg/ha of 10:10:10 fertilizer and three additions of 187 kg/ha of N during growing season, inoculated seedlings had significantly greater biomass, height, and stem diameter at each fertilizer level than non-mycorrhizal plants. Significant differences in growth occurred between families in mycorrhizal plants. VAM did not increase N, P, K, or Mg in leaves, stems or roots. Leaves had, however, higher calcium and stem and roots had lower calcium than non-mycorrhizal plants (Schultz *et al.* 1979).

Studies conducted at the University of Kentucky, Lexington, USA, on *Magnolia grandiflora*

seedlings raised on composted hardwood, bark, and expanded shale (1:1 v/v) amended with soil inoculum of *Glomus fasciculatum* and two rates of 18–6–12 Osmocote slow release fertilizer showed that plants receiving fertilizer's recommended rate (4.5 kg/m^3) were 38% (uninoculated), 44% (inoculated) taller than plant receiving one-fourth of recommended rate. Mycorrhizal infection was 17.2% at lower rate and 14.1% at higher rate. Similarly, *Juniperus horizontalis* plants had 47% infection on roots at lower rate of fertilizer and 31% at higher rate but VAM inoculation had no effect on growth of *J. horizontalis* plants (Hendrix, Maronek 1979).

Studies were conducted at the Department of Plant Pathology and Horticulture, University of Kentucky, Laxington, USA, on sweet gum seedlings raised on 2:1 sand to alkaline stripmine soil (with 0.1 ppm available phosphorus) and inoculated with stripmine VAM isolates, *Glomus clarum*, and *G. etunicatum* or *G. fasciculatum* (a VAM fungus known to increase plant growth). All the fungi stimulated growth of seedlings at low levels of slow release NPK fertilizer (18:6:12). At half or higher the recommended fertilizer dose (4.5 g/litre), all stripmine isolates were often pathogenic to the growth of seedlings as compared to controls. *G. fasciculatum* was never pathogenic though it was not always stimulatory to growth. At double the recommended dose, spore numbers of all the stripmine isolates were reduced but there was no effect of spore number of *G. fasciculatum*. Colonization of all the three fungi was reduced at this level. The data suggest that fungi isolated from low fertility situations may be pathogenic under conditions of high fertility (Kierman *et al.* 1981).

In studies conducted at the Departmento de Microbiologia de FCAV/UNESP, Jaboticabal, Brazil, *Eucalyptus citriodora* plants raised from seeds in nursery beds were inoculated with *Paspalum notatum* root inoculum of a wild fungus and with *Glomus mosseae* colonized roots of *Oryza sativa*. The subsoil substrate was mixed with either cow manure or NPK fertilizer and was fumigated or unfumigated with methyl bromide. Fumigation decreased stem diameter growth and seedling dry matter weight in soil mixed with manure. VAM inoculation increased seedling emergence rates but did not increase the number of viable fungal spores in the substrate, the frequency of mycorrhizal infection, seedling growth, or micro- and macro-nutrient contents after 75 days growth. The manure stimulated the emergence, height, growth, dry matter production, and contents of iron, boron, zinc, and copper. Fertilizer treatment increased nitrogen, calcium, and sulphur contents in the shoots (Macgado *et al.* 1988).

Application of phosphorus

Effect on efficiency of ectomycorrhizal inoculation

It is now generally recognized that maximum benefits from mycorrhiza to tree seedlings accrue in phosphorus-deficient soils. Studies were conducted at the University of Wisconsin, Madison, USA, on *Pinus resinosa* seedlings grown in pasteurized Sparta loamy fine sand (8–12 ppm P) amended with five rates of added superphosphate (0, 17, 34, 68, and 136 mg P/kg) and with or without addition of *Hebeloma arenosa* inoculum. All inoculated plants in unamended soil formed abundant mycorrhizae and had 12 times the root and 8 times the shoot dry weights of non-mycorrhizal plants after 19 weeks. Degree of mycorrhizal colonization and growth enhancement decreased with increased P addition to soil. Tissue concentration of P and K was increased in unamended soil by fungal colonization. Iron was preferentially accumulated in the roots of mycorrhizal plants with reduced translocation to shoots. Non-mycorrhizal plants in soil without P had greater concentration of Cu, B, Na, and Co than either mycorrhizal seedlings or plants grown in P amendments (MacFall *et al.* 1991).

Studies conducted at the Lenniikh, Leningrad, Russia, showed that the reason for impaired growth of scots pine (*Pinus sylvestris*) seedlings in forest nurseries after the use of soil fumigants was due to phosphorus deficit. Further studies conducted in nurseries near Leningrad with spring and autumn fumigation, mycorrhizal inoculation, and various applications of phosphorus, showed that growth was impaired if the P_2O_5 content of the soil was less than 8–10 mg/100 g of soil. Proper application of phosphorus fertilizers eliminated any adverse effect of soil fumigation (Egorov 1989). In earlier studies at the above place, various formulations of carbathion (metham) and thiazon (duzmet) were applied in September/October or April/May in nursery beds. Carbathion was ploughed in the soil and thiazon was applied to the surface of the soil. The seedlings of *Pinus sylvestris* and *Picea abies* were sown in the fumigated nursery soil. Yield of standard seedlings per unit area were more than doubled and the cost per thousand seedlings was substantially reduced when high doses of phosphorus and 5 tonnes/ha of mycorrhizal soil were applied before sowing (Bel'kov, Egorov 1988).

Studies conducted at the Centro de Pesquisa Agropecuaria dos Cerrados, Brazil, on seedlings of *Pinus caribaea* var *hondurensis* grown in pots of a dark red oxisol, (inoculated or not with *Pisolithus tinctorius*), treated with 0, 15, 30, 60, 120 or 240 ppm phosphorus

showed that a portion of ectomycorrhizal root system was not affected by P treatment after 120 days. The effect of mycorrhizal treatment on seedling growth decreased as P treatment increased (Vieira, Peres 1990).

Effect on efficiency of VAM inoculation

Studies conducted at the Virginia Polytechnical Institute and State University, Blacksburg, USA, on *Liquidamber styraciflua* (sweet gum) seedlings raised in loamy sand soil and in Kaolin mine soil at two phosphorus levels and inoculated with *Gigaspora margarita* showed that after 161-days' growth, non-mycorrhizal seedlings grew best in soils with 100 mg P per kg of soil, but, in Kaolin spoil, did not respond to this P level unless they were mycorrhizal. In soil receiving 25 mg P per g of soil, growth rate of mycorrhizal seedlings was significantly greater than that of non-mycorrhizal seedlings and foliar level of P, Mg, and Ca were enhanced by both mycorrhizal colonization and P fertilization. Mycorrhizal development and spore production were suppressed at high P levels (Gruhn *et al*. 1987).

In studies conducted at North Carolina University, USA, inoculation of green ash (*Fraxinus pennsylvanica*), grown in high phosphorus soil (14.8 mg phosphorus per kg soil), with VAM fungi isolated from low P soil (5.7 mg/kg soil) significantly increased seedling height, root collar diameters, dry matter accumulations and phosphorus nutrition relative to control seedlings. There was a significant positive correlation between foliar P concentration and percentage of infection (Lamar, Cavey 1988).

In studies conducted at the Texas A and T University, Kingsville, USA, on pear seedlings, P treatments were done at levels of 0.03, 0.04, 0.06, 0.09, 0.15, 0.25, and 0.4 mg/litre of soil solution based on a P sorption isotherm. At the age of 145 days, at P levels between 0.03 and 0.25 mg/litre, the greatest growth was observed in either unfumigated control seedlings or in those inoculated with *Glomus intraradices*. At the highest P level, the greatest growth was observed in plants inoculated with *G. deserticola*. Mycorrhizal colonization and Zn and Cu concentrations declined in control plants but not in inoculated plants (Gardiner, Christensen 1991).

In studies conducted at Zagazig University, Faculty of Agricultural Sciences, Moshtohor-Tukh, *Citrus aurantium* seedlings were transplanted into pots containing 18 g calcareous soil, fertilized with three different levels of rock phosphate and inoculated with 300 spores per pot of VAM spores. All treatments were foliar sprayed with mixtures of micronutrient solutions (iron, manganese, molybdenum, zinc, and copper). VAM inoculation and/or phosphorus fertilization as well as the combination between them generally stimulated plant growth characters, shoot and root length, leaf numbers, and dry matter accumulations. Nitrogen and phosphorus contents of the mycorrhizal plants were greater than the non-myco-rrhizal plants at all soil phosphorus levels. Also, micronutrient uptake was considerably improved in VAM-inoculated plants over either the phosphorus fertilized, non-inoculated plants or the control plants (El Maksoud *et al*. 1988).

In studies conducted at the Department of Soil Science Escola Superior de Agriculture, University of São Paulo, Piracicaba, Brazil, *Citrus limonia* seedlings were raised in sterilized soil with four levels of phosphate (0, 5, 100, 200 ppm) either as soluble or rock phosphate. The soil was inoculated or uninoculated with *Glomus etunicatum*. Six months later, significant increases were found in dry matter yield, and in phosphorus and potassium contents due to VAM inoculation at 0 and 50 ppm soluble phosphorus and at all levels of rock phosphate. At 100 ppm soluble phosphorus, the development of VAM plants was equivalent to non-VAM plants and at 200 ppm level, growth of VAM plants was significantly less than non-VAM plants. Also, root colonization and sporulation were reduced at higher phosphorus levels. High level of phosphorus and potassium concentrations may have interfered with carbohydrate distribution and utilization (Antunes, Cardoso 1991).

In studies conducted at the Citrus Research and Education Centre, University of Florida, Florida, USA, *Poncirus trifolia* X *Citrus sinensis* and *C. aurantium* were grown in peat-parlite medium with four levels of superphosphate and rock phosphate and inoculated or uninoculated with *Glomus intraradices*. Root colonization by VAM and growth response were less with superphosphate than with rock phosphate amended soil. Growth of VAM plants exceeded that of non-VAM plants fertilized with increased levels of soluble phosphorus (Graham, Timmer 1985).

Studies conducted at the Department of Agronomy and Soil Science, University of Hawaii, Honolulu, USA, on the response of *Leucaena leucocephala* K–8 to 0, 0.34, 0.68, 1.36, 2.72, and 5.44 g phosphorus (as rock phosphate) per g of soil showed that VAM colonization increased as rock phosphate application increased. Significant VAM activity (based on phosphorus concentration in pinnules) occurred 25 days after planting at low (0, 0.34, and 0.68 g phosphorus) levels of rock phosphate application and 20 days after planting at higher phosphorus levels. Highest VAM activity was with highest rate of rock phosphate application. Inoculation with *Glomus aggregatum*

significantly increased the uptake of copper, phosphorus, and zinc and also dry matter yield at all levels of applied rock phosphate. Copper concentration in roots of mycorrhizal plants was significantly higher than that of shoots (Manjunath 1989).

Application of nitrogen

Effect on ectomycorrhizal development

Studies conducted at the Chornam National University, Democratic Republic of Korea, on five-month old *Pinus thunbergii* seedlings grown in 1:1 mixtures of peat moss with vermiculite or sandy loam with 0, 50, 150, 250, 350, or 450 mg/ml nitrogen fertilizer and inoculated with *Pisolithus tinctorius* showed that seedlings grown in vermiculite mixture with 50–150 mg/ml N had well-developed pinnate and cluster mycorrhizae after five months' growth. Seedlings in loam mixture also showed monopodial mycorrhizae. The greatest mycorrhizal development was observed with 50 mg/ml N. Net assimilation rate and crop growth rate were reduced during early growth of seedlings treated with 450 mg/ml N but increased when nutrient application was stopped (Oh, Park 1988).

In studies conducted at the Institute of Mycorrhizal Research and Development, USD Athens, USA on *Pinus taeda* seedlings grown in fumigated soil at pH 4.8, 5.8, or 6.8 in 55 outdoor plywood microplots, the seedlings were given vegetative inoculum of *Pisolithus tinctorius* and 3, 6, and 9 applications of ammonium nitrate at 50 kg/ha N per application which totalled 150, 300 or 450 kg N/ha. Up to three applications of ammonium nitrate at 50 kg N/ha each did not affect inoculum viability of *P. tinctorius*. Application of ammonium nitrate, which resulted in soil concentration of nitrate N ranging from 60–120 kg/ha and ammonium N ranging from 90–130 kg/ha at pH 4.8 and 5.8 was associated with most abundant *P. tinctorius* ectomycorrhizal development. Increased N application increased *P. tinctorius* development at pH 5.8 and 6.8 (Marx 1990).

Studies conducted at the University of Stellenbosch, South Africa, on one-year old *Pinus elliottii* to test the effect of no nitrogen, one level of urea and three levels of urea formaldehyde (Nitroacta, a slow release fertilizer) showed that survival, shoot increment, root-shoot ratio, and mycorrhizal infection were adversely affected by the addition of nitrogen either as urea or as urea formaldehyde. Differences between nitrogen sources were not significant. Chemical analysis of the foliage showed that there was ample phosphate for normal growth in the seedlings which did not receive N. It is, thus, concluded that N requirement of young *P. elliottii* seedlings was very low. N fertilizer, therefore, should not be applied at planting (Donald, Visser 1989).

Studies were conducted at the Division de Recherche et Development, Sainte-Foy, Quebec, Canada. *Quercus rubra* seedlings grown in peat moss-vermiculite and inoculated at the time of sowing with *Laccaria bicolor* using mycelial suspension or calcium alginate beads containing mycelial suspension, under three levels of N fertilization (100, 120, and 140 mg/seedling). The results showed that root and total dry weights of seedlings fertilized with 100 mg N were significantly lower than seedlings receiving 120 and 140 mg N whereas shoot height, root collar diameter, and shoot-root ratio of seedlings did not differ significantly among any of the three N levels at 19 weeks. Seedlings inoculated with mycelial suspension had significantly greater N and P concentration (per cent) and contents (mg/seedling) than those inoculated with beads only at 140 mg N level (Gagnon *et al.* 1991).

Effect on VAM development

In studies conducted at the Society of American Foresters, USA, sweet gum potted seedlings raised on fumigated soil and inoculated with *Glomus etunicatum* were treated eight times during growing season with ammonium sulphate, ammonium nitrate, and potassium nitrate at seasonal rates equivalent to 0, 140, 280, 560, 1120, or 2240 kg N/ha. Application of 560 kg N/ha as ammonium sulphate or ammonium nitrate produced seedlings with greatest mean heights, diameters, and top weights. The 280 and 560 kg N/ha treatment of each N source produced greatest percentage of mycorrhizal roots and highest intensities of infections per infected root segment. Mycorrhizal development was maximum in treatments that produced maximum growth. Ammonium nitrate was considered superior to ammonium sulphate because of the lesser tendency of the former to acidify the soil (Brown *et al.* 1981).

Studies were conducted at the Department de Ciencia do solo, UFLA, Caixa, Lavuras, MG, Brazil, on four tree species namely *Senna multijuga, S. macrantheram, Melia azedarach,* and *Jacaranda mimosifolia* which were transferred to sand cultures containing different sources of nitrogen (none, NO_3^- and NH_4NO_3) at 4 mM and inoculated with *Glomus etunicatum* and *Gigaspora margarita*. After 120 days growth, mycorrhizal colonization was reduced and there was more accumulation of calcium and potassium in plants treated with NH_4^+ compared with those treated with NO_3^-. All species responded positively to the supply of nitrogen, response to NO_3^- being better than NH_4^+ and response was more in *Senna* spp. than in other two species. Nitrate reductase activity was found only in the roots of *M. azedarach* and *J. mimosifolia* treated with NO_3^- or NO_4^+ (Pereira *et al.* 1996).

Application of nitrogen and phosphorus

Effect on efficiency of ectomycorrhizal inoculation

In studies conducted at the Heffley Reforestation Centre Ltd, British Columbia, Canada, Englemann spruce in container nurseries was colonized only by *Thelephora terrestris* and *Laccaria spp.* under high N and P fertility but became dominated by *Amphinema bissoides* and E-strain under reduced fertility. Compared to *T. terrestris,* the latter two fungi improved root quality by increasing root branching, root weight, and root plug cohesion. E-strain and *suillus spp.* are common in lodgepole pine reared with low level of N and P. *Mycelium radices atrovirens* is common on both hosts under reduced fertility (Gary 1990).

Studies were conducted at the Natchez Forest Research Centre, International Paper Company, Natchez, Mississippi, USA, on *Pinus taeda* seedlings which were given nitrogen, either as ammonium or nitrate, at 50, 150, and 250 ppm dosages with simultaneous application of phosphorus at 2, 60, and 100 ppm at weekly intervals. The plants were inoculated or uninoculated with *Pisolithus tinctorius*. Mycorrhizal inoculum gave greater seedlings growth, and, high fertilization did not inhibit ectomycorrhizal formation. Also, phosphorus rates did not yield a significant difference in seedling growth. Extensive mortality occurred in nitrate fertilized treatments and maximum seedling growth was generally attained at 250 ppm ammonium nitrogen (France *et al.* 1981).

Effect on efficiency of VAM inoculation

Studies were conducted at the University of California, California, USA, on *Citrus aurantium* grown on sand, inoculated with *Glomus fasciculatum* and supplied with four P levels (0, 30, 100, and 500 ppm) and four N levels (10, 50, 100, and 200 ppm) applied daily as a combination of calcium nitrate and ammonium nitrate. The pots were also applied with half Hoagland's solution minus nitrogen and phosphorus. Total dry weights of non-mycorrhizal plants were greatest at 500 ppm P + 200 ppm or 10 ppm N, and total dry weights of mycorrhizal plants were not significantly different from these maximum values of non-mycorrhizal plants at treatments with 30 ppm P + 50 ppm N, 100 ppm P + 10 ppm N, 100 ppm P + 50 ppm N, 500 ppm P + 50 ppm N, 500 ppm P + 100 ppm N, and 500 ppm P + 200 ppm N. Total dry weights of mycorrhizal plants were significantly greater than their corresponding non-mycorrhizal plants when fertilized with 100 ppm P or less plus 50 ppm N or less. Fertilization with 100 or 200 ppm N significantly reduced growth or mycorrhizal plants except at 500 ppm P (Menge *et al.* 1979).

Effect of trace elements

Studies conducted at the University of Oklahoma, Norman, USA, on eight-week old *Pinus echinata* seedlings, with or without ectomycorrhizal formation with *Pisolithus tinctorius* and treated for two to eight weeks with 25 µg/litre borate solution applied to soil showed in increase in total carbohydrate content of mycorrhizal and non-mycorrhizal roots. Foliar application, however, decreased total carbohydrate contents. However, foliar + soil fertilizer treatment yielded a 24% increase in total carbohydrate in mycorrhizal roots. Carbohydrate content of non-mycorrhizal roots was significantly increased only by soil treatment with boron (Atalay *et al.* 1988).

In studies conducted at the University of Hawaii, Honolulu, USA, *Leucaena leucocephala* (var. k8) was grown by sowing pregerminated seeds in oxisol collected either from a depth of 15 cm (uneroded) or after the removal of the top 30 cm (eroded), sieved, limed to pH 6 and amended with a basal application of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, zinc, copper, and boron. The soils were amended with molybdenum (Mo) at the rates of 0, 2.2, 4.4, and 6.6 kg/ha and mixed with a crude inoculum of *Glomus aggregatum*. Mycorrhizal colonization after 37 days growth was significantly increased with 4.4 kg/ha Mo, being lower in uneroded than in eroded soil. Shoot P content was not affected by Mo application. There were small but not significant increases in shoot and root dry matter production and nodule dry weight with Mo amendment (Aziz, Habte 1988).

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Research findings...

Under this column appear short notes on important breakthroughs/significant achievements in original research of high calibre in the field of mycorrhizae, which have not yet been published.

Investigation on vesicular–arbuscular mycorrhiza population in forest trees of acid lateritic soils and its infective potential in field crops

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Introduction

In acid lateritic soil of eastern region, vesicular–arbuscular mycorrhiza (VAM) is commonly found in the rhizosphere of different forest tree species. However, the type of VAM species as well as its population density vary depending upon the type of host tree, soil condition, etc. For improvement of soil fertility, such VAM-infected soil can be used as an inoculum in a nursery bed, to benefit wider spectra of crops including agricultural crops and forest trees. Hence, the present investigation was undertaken, to study the infective potential of VAM obtained from forest tree rhizosphere, to benefit field crops.

Materials and method

Two sets of controlled pot experiments were conducted at the Vidyasagar University, during the winter of 1996 and the summer of 1997. Two test crops, onion (*Allium cepa*) and maize (*Zea mays*) were included in the study. These were grown during winter and summer, respectively. For inoculation, soil samples were collected from rhizospheric zone of three forest species—*Anacardium occidentale, Acacia auriculiformes,* and *Eucalyptus* species twice during winter and summer. A soil sample was collected 20 cm away at both sides of the tree trunk expecting maximum lateral root density. The depth considered was 25 cm. The mycorrhizal spores in soil were assessed by wet-sieve-decantation method (Gerdemann, Nicholson 1963).

The soil samples collected from each tree species were used separately for infection study. After collection the soil was powdered by hand and passed through mesh sieve, and filled up in earthen pots. Each pot measured 15 cm in height and 18 cm in diameter, accommodating 2 kg sieved soil. Thus, three soil samples were tested with 20 replications. Onion seeds were sown in a moist soil. After germination, 10 onion seedlings were allowed to grow in each pot for a period of 60 days. Similarly, in the case of maize, five seeds were sown in each pot and allowed to grow for the same period as onion. Roots were clean stained with trypan blue and assessed for vesicles, arbuscles, and per cent root colonization (Giovanneti, Mosse 1980; Philips, Hayman, 1970).

Results and discussion

It is observed from Table 1 that mycorrhizal infection in two test crops, onion and maize roots, was maximum in treatment where the source of inoculum was derived from *Eucalyptus* rhizosphere. Minimum VAM infection was recorded for *Anacardium* rhizospheric soil. However, in the case of *Acacia* VAM infection in test crop roots was higher than that of *Anacardium*. Per cent infection is taken as a direct measure of infective potential.

$$\text{Per cent infection} = \frac{\text{No. of mycorrhizal root pieces}}{\text{Total no. of root pieces studied}} \times 100$$

This shows that *Eucalyptus* has a greater potential in housing more VAM in the rhizosphere, which ultimately helped in higher infection to the test plant.

While comparing two test crops the per cent infection was more in the case of onion, as compared to maize. It is apparent from the table that higher infection is associated with higher number of spores present in the soil. Irrespective of the tree species, variation in the number of spores in the rhizosphere could be due to seasonal difference. This is evident from higher number of spores in soil sampled during winter season. The spores were identified mainly as *Glomus* species.

The observation on intensity of mycorrhizal growth as well as formation of vesicles and arbuscules in the roots was found to be positive in both the test crops—onion and maize. However, the mycorrhizal growth was higher in onion than in maize. Among the three tree species, soil collected from *Eucalyptus* rhizosphere caused higher intensity of mycorrhizal growth in test plants. The behavioral pattern in growth and development of vesicles and arbuscules was similar in both the test crops.

It is inferred from the above findings that soil fertility of agricultural crops can be improved by

Table 1. Comparison of two test crops, onion and maize, for per cent infection

Rhizosphere	Mycorrhiza		Percentage infection		Vesicles		Arbuscules		No. of spores per 100 g soil		VAM species
	Onion	Maize	Onion	Maize	Onion	Maize	Onion	Maize	Winter	Summer	
Eucalyptus species	+++	++	76	46	++	++	++	++	198±4	160±2	*Glomus sp.*
Acacia auriculiformes	++	+	54	29	+	+	+	+	148±6	80±1	*Glomus sp.*
Anacardium occidentale	++	+	40	20	+	+	+	+	137±2	90±1	*Glomus sp.*

+++ — 75% total length; ++ — 50% total length; + — 25% total length

inoculating with VAM. This inoculum can be obtained from the soil of afforested area. The rhizospheric soil of *Eucalyptus* was found to be more promising as potential inoculum than other forest species tested.

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Effect of monoculture of plants on inoculum build up and quantitative distribution of different VA-mycorrhizal species in rhizosphere

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Introduction

There are about 144 species of endogonaceous fungi described to form vesicular–arbuscular (VA) mycorrhizal association with plants (Schenck, Perez 1990). They apparently lack any host specificity, although some preferential association of one or more VA-mycorrhizal species with one or a group of host plants has been indicated (Mosse 1977). The genetic basis of such non-host specific biotrophic association as exhibited by VA-mycorrhizal fungi with plants has, till date, remained an enigma. Nevertheless, distribution and dominance of individual VA-mycorrhizal species in different edaphoclimatic situations may be under selective influence of the dominant member(s) of plant community.

An experiment was conducted to determine the possible influence of monoculture of different plants on quantitative distribution of different VA-mycorrhizal species in rhizosphere in a natural soil with stabilized VA-mycorrhizal fungal community.

Materials and method

Maize, cowpea, napier, soyabean and *Cynodon* were grown in single stand without any other inputs during summer seasons for three consecutive years in West Bengal terai agro-climatic zone soil having sand (62.5%), silt (25.9%), clay (11.6%), pH (5.9), total organic carbon (1.71%), total nitrogen (0.061%), available phosphorus (15.2 ppm), and available potassium (11.0 ppm). The planting location remained agriculturally fallow and undisturbed with *Cynodon* as dominant plant for over 10 years before the experiment. Other plants growing in association were *Cyperus rotundus, Ageratum conyzoids, Amaranthus spinosus,* and *Tridax procumbens*. During non-summer seasons, after plant harvest (120–150 days after seeding) each year, the plots were kept fallow. Determination of endogonaceous flora for three consecutive seasons during 1992–94 (April to August) showed stabilized distribution of 12 VA-mycorrhizal species in the natural uncultivated soil

(Table 2). Standard methods of mycorrhizal analysis were used for isolation and enumeration of VA-mycorrhizal fungal spores (Gerdemann, Nicholson 1963) and to identify the different VA-mycorrhizal species (Schenck, Perez 1990). Quantitative distribution of the species in the rhizosphere soil was judged from differential count of spores in root association of individual plant species.

Results and discussion

Results presented in Table 1 showed predictable increase in the number of VA-mycorrhizal spores in rhizosphere soil after three seasons of crop culture from that of the fallow uncropped soil and also from that developed in association with the dominant native host plant, *Cynodon*. The crop hosts differed significantly among themselves in their effect on rhizospere spore development, soyabean giving the highest stimulation. Effect of the hosts on spore production was not essentially related to volume or quantity of roots produced by the host plants per unit of volume of soil. The three genera of VA-mycorrhizal fungi present in the soil, *Gigaspora, Glomus* and *Acaulospora*, responded differently to the hosts. Per cent composition of VA-mycorrhizal spores by Gigaspora in the rhizosphere soil after three seasons of crop culture as compared to that of native soil, remained constant with *Cynodon,* increased marginally with maize but decreased with napier, soyabean, and cowpea, marginally with the former two and very significantly with the latter. Spores of *Glomus* increased significantly with *Cynodan*, maize, cowpea, and napier but was reduced with soyabean. *Acaulospora* showed a reverse trend from that of *Glomus*, as it showed a very significant increase with soyabean and corresponding decrease with all other hosts.

Eighteen VA mycorrhizal species (Table 2), six of which remained unidentified, were seen to be present in the rhizosphere of all the host plants after three seasons of crop culture, as compared to twelve in fallow soil. The individual VA-mycorrhizal species responded differently to different hosts. Most species of *Gigaspora* showed a declining trend or remained constant to that of the native fallow soil with most of the host plants, except *Gigaspora albida*, which showed a significant population increase with four out of five hosts, barring cowpea, where it remained almost constant. The trend was similar with *Glomus*, where *Glomus occultum* showed a very significant population increase with all the hosts, barring soyabean, where constancy was maintained. Population of the two unidentified native species of Acaulospora showed a significant decline with all the hosts. *Acaulospora rehmii,* which was not detected in either fallow soil or with native host, *Cynodon*, made a very significant appearance exclusively in soyabean rhizosphere where it constituted about one-third of the total endogonaceous spore population.

Results of the study would indicate a preferential build-up of some VA-mycorrhizal species in continuous host association from amongst the mixed population in soil where some species may even be specifically stimulated by a particular host as had happened with soyabean and *A. rehmii*. Such information, besides indicating a more subtle host VA-mycorrhizal relationship at species level than is generally apparent, may be of advantage in selection of VA mycorrhizal species for inoculum production and use in crop culture.

Table 1. Formation and per cent distribution of spores of different VA-mycorrhizal genera in the rhizosphere of crops upon three seasons of monoculture

Crop	Mean spore no. per 100 g dry soil		Mean spore no. per g dry root		Per cent composition of total spores by the genus		
					Gigaspora	*Glomus*	*Acaulospora*
Fallow	136 ± 14	E	—		37.8	20.2	42.0
Cynodon	287 ± 22	D	1927 ± 178	C	37.3	48.3	14.4
Maize	600 ± 42	B	1907 ± 129	C	44.5	39.1	16.4
Cowpea	373 ± 24	C	2086 ± 150	BC	16.0	67.1	16.9
Napier	594 ± 19	B	2330 ± 136	AB	29.0	55.8	15.2
Soyabean	696 ± 58	A	2424 ± 196	A	27.4	13.6	59.0
SEM ±	20		81				
CD (0.05)	60		254				

Figures followed by same letters are not significantly different at 5% level

Table 2. Per cent number of spores of different VA-mycorrhizal species in the rhizosphere of crops upon monoculture for three seasons

VA-mycorrhizal species	Native soil	Cynodon	Maize	Cowpea	Napier	Soyabean
*Gigaspora spp.**	12.0	15.8	5.1	4.3	6.0	5.0
G. pellucida	14.0	3.0	8.6	2.9	1.6	6.9
G. albida	5.9	10.4	20.8	4.4	15.3	11.5
G. gregaria	5.9	8.1	9.7	4.4	5.5	3.5
G. gimorei	—	—	0.3	—	0.6	0.5
Genus total	37.8	37.3	55.5	16.0	29.0	27.4
*Glomus spp.**	10.0	18.8	3.7	5.5	12.3	4.3
G. occultum	8.1	21.5	30.3	53.8	37.7	7.2
G. fasciculatum	—	1.8	0.6	1.1	—	—
G. clarus	—	—	1.6	1.3	—	—
G. ambisporum	2.2	4.0	2.2	4.9	3.3	1.2
G. tortuosum	—	2.2	0.7	0.5	2.5	0.9
Genus total	20.2	48.3	39.1	67.1	55.8	13.6
*Acaulospora spp.**	42.0	14.4	16.4	16.9	15.2	23.3
A. rehmii	—	—	—	—	—	33.6
A. scrobiculata	—	—	—	—	—	2.1
Genus total	**42.0**	**14.4**	**16.4**	**16.9**	**15.2**	**59.0**

* Consisted of two species each remaining unidentified

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2. Mosse B. 1977. The role of mycorrhiza in legume nutrition on marginal soils. *In* Vincent J M, Whitney A S, Bose J (eds.). *Exploiting the Legume Rhizobium Symbiosis in Tropical Agriculture.* University of Hawaii: University of Hawaii Cooperative Extension Service. Miscellaneous Publication. 175–192
3. Schenck N C, Perez Y. 1990. Manual for the identification of VA mycorrhizal fungi. 3rd edition. Gainesville, Florida: Synergistic Publications

New approaches...

Under this column appear brief accounts of new techniques, modifications of available techniques, and new applications of other known techniques, etc., in mycorrhiza research that have been published in reputed journals during the last two or three years.

In vitro synthesis of *Pisolithus–Eucalyptus* mycorrhiza

A simple and reproducible *in vitro* system is described for the synthesis of *Pisolithus–Eucalyptus grandis* ectomycorrhizae by Burgess T, Dell B, Melajezek N [*Mycorrhiza* **6**(1): 189–196, 1996]. Hyphal disc from actively growing colonies were placed in large Petri dishes containing minimum nutrient agar overlaid with cellophane and allowed to grow for seven days. Seeds were then surface sterilized and placed above the expanding fungal colonies and the plates were then slanted. Seedlings that germinated and grew in the presence of fungal hyphae had twice as many lateral root-tips as seedlings that germinated before they were transferred on to the hyphal mats. In addition, the lateral root-tips of inoculated seedlings had a faster maturation rate and emerged closer to the primary root apex than non-inoculated seedlings. All lateral tips emerged in contact with fungal hyphae and the differentiation of the ectomycorrhizae was followed by examining lateral tips basipitally along a single primary root. The typical mycorrhizae had formed on four-day lateral tips,

i.e., a mantle, radially elongated epidermal cells, and a Hartig net commencing about 0.3 mm behind the lateral root apex. Thereafter, the mantle continued to thicken and the apical meristem diminished. The Hartig net often surrounded the apex of 11–12 day old lateral root-tips. This model system will facilitate detailed studies on synchronized development and associated molecular and biological changes.

Strain typing of ectomycorrhizal basidiomycetes by random amplified polymorphic DNA

Application of random amplified polymorphic DNA (RAPD) analysis for the identification of ectomycorrhizal symbionts of spruce (*Picea abies*) belonging to the genera *Boletus*, *Amanita* and *Lactarius* at or below the species level was investigated by Haudek S B, Gruber F, Kreuzinger N, Göbl F, Kubicak C P [*Mycorrhiza* **6**(1): 35–41, 1996]. Using both repetitive sequence, M13 mini-satellite sequence, $(GTG)_5$ $(GACA)_4$ as well as random oligonucleotide primers (V_1 and V_5), a high degree of variability of amplified DNA fragments (band sharing index 65%–80%) was detected between different strains of the same species, thus enabling the identification of individual strains within the same species. The band sharing index between different species of the same genus (Boletus, Russula, Amanita) was in the range of 20–30% and similar values were obtained when strains from different taxa were compared. Thus, RAPD is too sensitive at this level of relationship and cannot be used to align an unknown symbiont to a given taxon. The authors, therefore, concluded that RAPD is a promising tool for the identification of individual strains and could, thus, be used to distinguish indigenous and introduced mycorrhizal strains from the same species in natural ecosystems.

Forthcoming events ...

24–28 January 1999
Sixth International Workshop on Seed Biology. Merida, Mexico
For further information, please contact:
Dr Jorge Vazquez Ramos
Departamento de Bioquimica
Facultad de Quimica
Universidad Nacional Autonoma de Mexica,
Ave. Universidad y Copilco
Mexico D F 04510
Fax: 52 5 622 5329
E-mail: jorman@servidor.dgsca.unam mt

31 January–5 February 1999
Temperature Stress in Plants (gordon Research Conference). Ventura, USA
For further information, please contact:
Charles Guy
University of Florida
Department of Environmental Horticulture
PO Box 110670, Gainesville, GL 32611 0670
Tel.: 352 392 7934
Fax: 352 392 3870
e-mail: clg@ gnv.ifas.ufl.edu
Web site: http://www.grc.uri.edu/programs/1999/tempstrs.htm

16–20 May 1999
6th Symposium on Stand Establishment and the Seed Working Group of the International Society for Horticultural Science. Roanoke, USA
For further information, please contact:
Greg Welbaurn
Department of Horticulture
Virginia Tech
Blacksburg
VA 24061 0327
USA
Tel.: 540 231 5801
Fax: 540 231 3083
e-mail: welbaum@vt.edu
Web site: http://www.conted.vt.edu/stand/establishment.htm

17–21 July 1999
International Symposium on Plant Peroxidases. Columbus, USA
For further information, please contact:
Dr L Mark Lagrimini
Department of Horticulture and Crop Science
The Ohio State University
2001 Fyffe Ct
Columbus
OH 43210 1096
USA
Tel.: 614 292 3851
Fax: 614 292 3505

26–30 July 1999
The Third International Congress on the Systematics and Ecology of Myxomycetes. Beltsville, Maryland
For further information, please contact:

Lafayette Frederick
Biology Department
Howard University,
Washington, DC, 20059

or

Steve Stephenson
Department of Biology
Fairmont State College,
Fairmont, WV 26554
E-mail: <sls@fscvax.wvnet.edu>

News from members ...

Prof. Bushan L Jalali has taken over as the Director of Research at CCS Haryana Agricultural University, Hisar, in June 1998.

Earlier, While delivering the national IPS-Pavgi Memorial Award Lecture on *Molecular Biology of host-pathogen-mycorrhiza interactions: do we have enough answers?* at the 50th Annual Convention of Indiana Phytopathological Society at Aurangabad, Maharashtra, Prof. Jalali stressed the urgency to harness the modern techniques of precision now available for the viable management of plant diseases of national importance. Overuse/misuse of pesticides in farming systems, over the years, has resulted in a range of side effects like ecological disruptions. With this national scenario, Prof. Jalali, currently the President of the Indian Society of Mycology and Plant Pathology, emphasized that systematic efforts need to be launched in effectively integrating the physical, biological, and chemical options available, using biotechnological approaches, for workable containment of plant diseases and pests with resultant economic gains.

Recent references ...

Latest additions to the Network's database on mycorrhiza are published here for information of the members. 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, which are arranged in alphabetical order.

- *Canadian Journal of Microbiology*
- *Food Review International*
- *Indian Journal of Environmental Sciences*
- *Mycological Research*
- *Mycologia*
- *Mycorrhiza*
- *New Phytologist*

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

Canadian Journal of Microbiology 44, 1998

Timonen S, Jorgensen KS, Haahte AK, Sen R. (Department of Biosciences, Division of General Microbiology, P.O. Box 56 (Viikinkaari 9) 00014 University of Helsinki, Finland). Bacterial community structure at defined locations of *Pinus sylvestris-Suillus bovinus* and *Pinus sylvestris-Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat, 499–513

Food Review International 13(3), 1997

Iwase K. (Biological Environment Institute, Kansai Environmental Engineering Center Co. Ltd, 8-4 Ujimatafuri, Uji 611, Japan). Cultivation of mycorrhizal mushrooms, 431–442

Indian Journal of Environmental Sciences 1(1), 1997

Bhadauria S (Microbiology Research Laboratory, Department of Botany, Raja Balwant Singh College, Agra -

281 002, India). Mycorrhizal plants role in decontamination of oil polluted soils: a preliminary investigation, 55–57

Mycological Research 102(6), 1998

Carrebho R, Trufem SFB, Bononi VLR. (Instituto de Botânica, Secao de Micologia, Caix Postsal 4005, 01061-970, São Paulo, SP, Brazil). Arbuscular mycorrhizal fungi in *Citrus sinensis/C. limon* treated with Fosetyl-Al and Metalaxyl, 677–682

Mycologia 90(2), 1998

Rilling MC., Allen MF, Klironomos JN and Field CB. (Department of Biology, Soil Ecology & Restoration Group, San Diego State University, San Diego, CA 92182-4614, USA). Arbuscular mycorrhizal percent root infection and infection intensity of *Bromus hordeaceus* grown in elevated atmospheric CO_2, 199–205

Mycorrhiza 8(1), 1998

Bofante P, Balestrini R, Martino E, Perotto S, Plassard C, Mousain D. (Departmento di Biologia Vegetale dell'Universita and CSMT-CNR, Viale Mattioli 25, I-10125 Turin, Italy). Morphological analysis of early contacts between pine roots and two ectomycorrhizal *Suillus* strains, 1–10

Horton TR, Cazares E, Bruns TD. (Department of Microbial Biology, 111 Koshland Hall, University of California, Berkeley, CA 94720, USA). Ectomycorrhizal, vesicular–arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire, 11–18

Millner PD, Mulbry WW, Reynolds SL, Patterson CA. (US Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Soil Microbial Systems Laboratory, 10300 Baltimore Blvd, Building 001, Room 140, Beltsville, MD 20705-2350, USA). A taxon-specific oligonucleotide probe for temperate zone soil isolates of *Glomus mosseae*, 19–27

Fontenla S, Godoy R, Rosso P, Havrylenko M. (Centro Regional Universitario Bariloche, Universidad Nacional de Comahue, Quintral 1250 (CP 8400) S.C. de Bariloche, Rio Negro, Argentina). Root associations in *Austrocedrus* forests and seasonal dynamics of arbuscular mycorrhizas, 29–33

Caris C, Hordt W, Hawkins H-J, Romheld V, George E. (Institute für Pflanzenernahrung (330), Universitat Hohenheim, D-70593, Stuttgart, Germany). Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants, 35–39

Al-Karaki GN. (Faculty of Agriculture, Jordan University of Science & Technology, P.O. Box 3030, Irbid, Jordan). Benefit, cost and water-use efficiency of arbuscular mycorrhizal durum wheat grown under drought stress, 41–45

Nezzar-Hocine H, Perrin R, Halli-Hargas R, Chevalier G. (Universite Mouloud Maameri, Unite de Recherche de Biologie et d'Agro-Foresterie (URBAR), route de Hasnaoua, 15000 Tizi-Ouzou, Algiers). Ectomycorrhizal associations with *Cedrus atlantica* (Endl) Manetti ex Carriere. I. Mycorrhizal synthesis with Tricholoma tridentinum Singer var. *cedretorum* Bon, 47–51

Kabir Z, O'Halloran IP, Widden P, Hamel C. (Department of Horticulture, 102 Tyson Building, The Pennsylvania State University, University Park, PA 16802, USA). Vertical distribution of arbuscular mycorrhizal fungi under corn (*Zea mays* L.) in no-till and conventional tillage systems, 53–55

New Phytologist 139(1), 1998

Wallenda T, Kottke I. (Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK). Nitrogen deposition and ectomycorrhizas, 169–187

Centre for Mycorrhizal Culture Collection (CMCC)

Methods to establish rapid mycorrhization *in vitro*

Experiments to establish the symbiotic nature of the ectomycorrhizal fungal species are a regular feature at the Centre for Mycorrhizal Culture Collection (CMCC), as mycorrhizal development, *in vitro*, is an effective tool that can be used as an indicator of symbiont interaction, mycorrhiza development ability with different host species, and as a measure of aggressiveness of the strain. It is known that repeated sub-culturing and the age of the vegetative mycelium continuously maintained on agar show a decrease in

effectiveness over a period. Revitalization of fungal cultures was proposed as a solution to such problems. The asceptic synthesis experiments were of major concern as the preservation of the symbiotic nature is most important, for the future use of the eco-friendly biofertilizer organism. The methods of *in vitro* synthesis are followed by a slight modification to the existing techniques, while incorporating the best suited of earlier techniques.

Seeds of *Eucalyptus tereticornis* were cleaned and stratified in cold water at 8 °C overnight. The seeds were sterilized in 30% H_2O_2 for 15 minutes, and subsequently washed at least thrice, with sterile distilled water. Pre-sterilized tissue paper was used to absorb moisture adhered to the seeds. The seeds were placed on one per cent water agar for germination. The germinated seedlings were harvested at the stage of radicle emergence and proceeded for asceptic synthesis. The medium for synthesis consisted of minimum amount of sugar (0.2% glucose) and was completely devoid of malt. The medium used was Modified Melin Norkan's (MMN) but with half the amount of KH_2PO_4 and one-fourth amount of NH_4HPO_4 as compared to the normal MMN composition. 0.8% agar was added to the medium (for agar-based synthesis experiments). The pH of the medium was adjusted to 6.2 before autoclaving using 1M NaOH.

Approximately 50 ml of the medium was poured per Petri dish of size 145 mm diameter and overlaid with circular cut, pre-sterilized cellophane. Two small pre-sterilized cotton rolls were placed on the two opposite corners of the plate to absorb moisture resulting from transpiration by the plant. Two culture discs of approximately 0.62 sq. mm area were placed diagonally opposite each other and allowed to incubate for 15 days. Seedlings of the age described in Method 1 were taken and placed concentrically all along the circumference of the two circularly emerging colonies. The plates were sealed with Nescofilm and transferred to a growth room with 22 °C ± 2 °C temperature and a 18h : 6h (light: dark) photoperiod was maintained.

The Petri plate based synthesis methods resulted in good percentage of mycorrhization and showed greater root morphogensis, a faster maturation rate, and closer emergence of lateral root-tips to the primary root apex. Moreover, the Petri plate-based methods were suggestible because of the advantges like space economy, asceptic conditions for root microbial interactions, easy handling, precise positioning of inoculum, non-destructive observations of ectomycorrhiza development, and relatively rapid ectomycorrhiza formation.

List of cultures available for circulation

S No.	Germplasm bank code	Fungus name	Host	Source person/place of origin
Ectomycorrhizae				
1.	EM-1029	*Paxillus involutus*	—	—
2.	EM-1270	*P. involutus*	—	USA
3.	EM-1266	*P. involutus*	—	—
4.	EM-1267	*P. involutus*	*Eucalyptus darlympleana*	—
5.	EM-1268	*P. involutus*	—	—
6.	EM-1269	*P. involutus*	—	—
7.	EM-1217	*P. involutus*	—	Noselle
8.	EM-1212	*P. involutus*	*Populus euramericana*	—
9.	EM-1282	*P. involutus*	*Abies, picea*	Quebec, Canada

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Calendar of Events for CMCC

Month and year	*Title*	*Duration*	*No. of candidates*	*Target audience*	*Course fee*	*Last date for application*
December 1998	Training course on characterization of arbuscular-mycorrhizal fungi using image analysis	One week	7	Universities/ Institution	Rs 10000*	15 November 1998
December 1998	Field application and on farm production technology on mycorrhizae	One week	10	Universities/ Institutions/ Progressive farmers	Rs 7000*	15 November 1998
January 1999	Preservation, storage, carrier formulation and quality assurance of mycorrhizal inoculum	5 days	7	Corporate sector/ industries	Rs 15000*	30 November 1998
February 1999	Basic techniques in mycorrhizae	One week	7	Universities/ Institutions	Rs 10000*	30 December 1998
March 1999	Industrial meet on technologies in mycorrhizae biofertilizer	2 days	7	Senior representatives from the corporate sector/industries	Rs 10000*	30 January 1999
April 1999	Characterization of AM fungi using molecular tools	One week	7	Universities/ Institutions	Rs 10000*	January 1999
May 1999	Biochemical assays in AM infected roots and extra-matrical mycelium	5 days	7	Universities/ Institutions	Rs 15000*	February 1999

* Course fee includes lunch and tea, bibliographic database on mycorrhiza on floppy discs, course material, and a two-year subscription of *Mycorrhiza News*.

Note.

(i) For female participants limited guest house facility available at the TERI Guest house on twin sharing basis @Rs 225/- (per person) non-AC room and Rs 325/- per person for AC room.

(ii) Participants are encouraged to use the Mycorrhiza Network Facility at TERI during the training.

Detailed course information can be obtained from: Dr Alok Adholeya, Fellow, Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi - 110 003, India; Tel.: +91 11 462 2246/460 1550; Fax: +91 11 463 2609/462 1770; e-mail: aloka@teri.res.in; Web site: http://www.teriin.org.

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