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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.

Contents

Role of mycorrhiza in tree nurseries—Part II. Inoculation of nursery soil/plants with mycorrhizal fungi[1]

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Evaluation of different types of mycorrhizal inocula

Various types of mycorrhizal inocula are used for mycorrhization of tree seedlings in nurseries. These include soil infected by mycorrhizal fungi, colonized roots, mycorrhizal short roots, fruit bodies, and spores of mycorrhizal fungi and vegetative mycelia grown in pure culture and used as such or after entrapping in various types of inert material. In order to derive maximum benefits from mycorrhization of nursery plants, it is imperative to use the right type of inoculum with different hosts. The following account summarizes different types of inocula which have been used by different research workers for mycorrhization of nursery plants.

Soil inoculum

Soils under mycorrhizal plants are rich with mycorrhizal roots, spores, hyphae, hyphal strands, rhizomorphs, etc. up to a depth of about 10 cm from the ground level. Such soil serves as a useful inoculum for the introduction of mycorrhizal fungi in the nurseries. However, such soils may also contain spores and other propagules of pathogenic fungi. It is therefore necessary that soil should be collected from underneath healthy trees for use as mycorrhizal inoculum.

In studies conducted at the Forest Research Institute, Hanoi, Vietnam, for the production of high quality planting stock, it was found that in pines growing in most of the bare hills in north and central Vietnam, mycorrhiza was almost non-existent. In other places, mycorrhizae existed under both young and old pine stands but their symbiotic efficiency greatly varied. Best results were obtained when inoculation was made by the use of soil from *Pinus merkusii* forests which could bring about a seven- to ten-fold increase in growth (by dry weight) of *Pinus elliottii* and *Pinus oocarpa* saplings at the age of 15 months when compared with uninoculated control (Giao, Nham 1988).

In many highland *Eucalyptus delegatensis* forests in Australia, establishment and healthy growth of eucalypts is promoted by fire in the absence of which secondary succession to rain forests occurs and eucalypts decline and die prematurely. In studies conducted at the Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Forestry, Tasmania, Australia, pot experiments were conducted using soils from (i) secondary succession rain forests; (ii) old grasslands where Eucalyptus seedlings exhibited severe growth check and mortality; and (iii) from beneath individual trees of several species growing in old grasslands. Growth of seedlings in untreated pot soil reflected closely the condition of the Eucalyptus tree with declined growth during successional sequence to rain forests.

Growth of seedlings was very poor in soil from old grasslands and it varied markedly among soils from beneath different tree species. Poor growth was overcome only by the addition of nitrogen and phosphorous fertilizers or by partial sterilization of the soil by using steam or chemicals. Inoculation of inhibitory soil from treatment (i) and (ii) above with 10%–20% of the soil from healthy Eucalypt stands overcame inhibition completely in both cases (Ellis, Pennington 1992).

Studies were conducted at the Centre for Advanced Studies in Botany, University of Madras,

[1] Compiled from TERI database—Riza

Tamil Nadu, India, on *Pinus patula* seedlings raised from seeds in shola soil and then transferred to three different types of soils namely shola soil, grassland soil, and riverbed soil. With respect to shoot and root lengths, dry weights, number of mycorrhizal tips, and root-collar diameter, shola soil gave the best results. Mycorrhizal types formed by *Thelephora terrestris, Laccaria laccata,* and *Rhizopogon luteolus* were found in plants grown in shola soils while *Hebeloma* and *Inocybe* mycorrhizae were found in grassland soil and *Cenococcum graniforme* mycorrhizae were found in riverbed soil (Mohan, Natarajan 1992).

Studies conducted at the Department of Biotechnology, Technological Institute of Iceland, Keldnaholt, Reykjavik, Iceland, showed that the seedlings of Sitka spruce, Lodgepole pine, and Siberian larch inoculated with mycorrhiza infected soil showed a clear superiority in growth over uninouclated seedlings (Einarsson, Kristjansson 1990).

Studies conducted at the California Department of Forestry and Fire Protection, Davis, USA, however, showed that the growth of Sequoia seedlings, raised by direct seed sowing in fumigated nursery beds and inoculated with soil either from *Sequoia sempervirens* production beds or Sudan grass beds containing *Glomus mosseae* spores did not differ between two inoculum sources after one to two years of growth. Inoculated seedlings however, showed significantly better growth than the inoculated seedlings (Adams *et al*. 1990).

Studies conducted at the Department of Forestry, Mukerere University, Kampala, Uganda, on *Pinus caribaea* seedlings raised from surface sterilized seeds on potting mixture with two parts sand, two parts clay, and one part surface forest soil from *P. caribaea* plantation as ectomycorrhizal inoculum showed that ectomycorrhizae developed six weeks after seed germination and they developed dichotomy after 10 weeks. On an average, four mycorrhizal tips were added in a seedling every week. Control seedlings raised on sterilized potting mixture (2:2:1 sand, clay, and inoculum) germinated at the same time but their growth started dwindling after 10 weeks, and after 25 weeks no control seedling survived and during this period no mycorrhiza was observed. Fungal symbionts in mycorrhizal seedlings were identified as *Suillus granulatus* and *Suillus luteus* (Chaudhery *et al.* 1981).

Comparison of soil inoculum with pure culture inoculum

In studies conducted at the International Paper Company, Bainbridge, USA, five conifers raised from seeds on vermiculite-peat moss (1:1) medium, were inoculated either with cultures of *Pisolithus tinctorius* or with vermiculture mixed with a natural inoculum of screened forest duff (1:1). The seedlings were treated with full strength nutrient medium at every 30 days' interval for six months. Seedlings of *Picea engelmanii* and *Pseudotsuga menziesii* had significantly greater shoot height, root collar diameter, and dry weights with *P. tinctorius* inoculation as compared to forest duff inoculation, but few mycorrhizae were formed with either treatment. However, no significant differences in height, diameter or weight were found between *P. tintorius* and forest duff treatments in *Pinus flexilis, P. contorta*, and *P. ponderosa* though these pines readily formed mycorrhizae (France, Cline 1987).

In studies conducted at the Department of Forest Science, Faculty of Forestry, University of British Columbia, Vancouver, Canada, white spruce (*Picea glauca*) was grown at two temperatures (6 °C and 12 °C) and subjected to seven inoculation treatments (autoclaved agar, forest soil from vigorous spruce plantation, pure mycelial cultures of *Thelophora terrestris, Laccaria bicolor, Hebeloma crustuliniforme, Amphinema byssoides*, and E-strain). Cold-stored container seedlings were transplanted into forest soil (6 °C and 12 °C) from vigorous spruce-plantation. Regardless of soil temperature, seedlings inoculated with forest floor and *L. bicolor* had the greatest shoot growth, 40%–50% greater caliper growth than control seedlings, and 63%–85% greater foliage biomass. Shoot growth did not differ between control, *A. byssoides* or *T. terrestris* seedlings. E-strain and *H. crustuliniforme* reduced caliper growth by 5%–25% and current foliage biomass by 25%–35%. Also, at both soil temperatures N and P uptake (mg/seedling) were greatest for forest floor and *L. bicolor* seedlings, intermediate for *A. byssoides, T. terrestris,* and control seedling and lowest for E-strain and *H. crustuliniforme* seedlings (Husted 1990).

Mycelial inoculum

Mycelial cultures used as slurries

In studies conducted at the Department of Biology, University of Calgary, Calgary, Canada, mycelial slurries were prepared from agar plates of 15 ectomycorrhizal fungi. Inoculation done with these slurries on seven-week old container grown Jackpine seedlings showed that seven fungi namely *Thelephora terrestris, Laccaria proxima, Hebeloma* sp., *Pisolithus tinctorius, Sphaerosporella brunnea, Cenococcum geophilum,* and E-strain isolate formed mycorrhizae while species of *Tricholoma, Suillus, Amphinema,* and *Hydnum* failed to form mycorrhizae (Danielson *et al.* 1984).

Studies conducted at the Department of Biotechnology, Technological Institute of Iceland, Iceland, showed that the seedlings of Sitka spruce, Lodgepole pine, and Siberian larch inoculated with pure fungal cultures (grown in liquid medium) of mycorrhizal fungi showed a clear superiority in growth over uninoculated seedlings (Einarsson, Kristjansson 1990).

Studies conducted at the Nova Scotia Research Foundation Corporation, Dartmouth, Canada, on blended mycelial slurries of a variety of ectomycorrhizal fungi showed that mycelia of most fungi except *Pisolithus tinctorius* and *Paxillus involutus* withstood blending well and their viability remained high after storage in Melin Norkran Modified (MNM) medium, water or dilute saline at 4 °C or at room temperature. This indicates that such slurries can tolerate conditions that will be encountered in a commercial setting. Injection of mycelial slurry of *Hebeloma longicandum* into the root zone of containerized seedlings resulted in consistently high colonization, though the application of the slurry to the surface of the growing medium was also effective. The slurry infectivity was dropped after it was mixed into a peat-vermiculite growing medium particularly in the presence of high levels of fertilizers. In inoculation trials, slurries of nine representative fungi were infected into the root zone of eight-week old black spruce or Jackpine seedlings. Five of the fungi consistently formed ectomycorr-hizae (Boyle *et al.* 1987).

Mycelia entrapped in beads

Studies conducted at the Soil Science and Plant Nutrition, School of Agriculture, the University of Western Australia, Nedlands, Australia, showed that the inocula of eleven eucalypt ectomycorrhizal fungi produced by the culture of mycelia within hydrogel beads were of as high efficiency as propagules. All beads produced mycelial growth within one to three days when placed on agar medium and a single bead was sufficient for the initiation of mycorrhizae in roots of aseptic seedlings and micropropagated plantlets. The beads were discrete units of 2.5 mm in diameter. Scanning electron microscopic examination revealed a dense profusion of intact mycelia within the beads (Kuek *et al.* 1992). A recent technology developed at the Department of Forest Biological Sciences, University of the Philippines, Los Banos, Philippines, for ectomycorrhizal fungi which do not produce abundant spores, consists of producing vegetative mycelia in bulk in fermenters and embedding the mycelia in alginate beads. Pine seedlings have successfully been inoculated with this technique (Reynaldo 1990).

Comparison of bead inoculum with laboratory grown inoculum

Studies conducted at the Institute of Terrestrial Ecology, Bush Estate, Penicuik, Midlothian, UK, showed that up to 114% more Sitka spruce (*Picea sitchensis*) seedlings were found in plots inoculated with live inoculum of *Laccaria proxima* in vermiculite-peat carrier than in control plots with or without the same carrier. A similar, although reduced, effect was found when the live fungus was encapsulated in alginate beads. Out of the two nurseries where the experiments were conducted, 40%–50% per cent less seedlings in one nursery and 66%–72% less seedlings in the other nursery were obtained in fertilized and mycorrhiza inoculated plots than in unfertilized inoculated plots (Ingleby *et al.* 1994).

Studies conducted at the Division de Recherche et Development, sur les Semensis, Sainte-Foy, Quebec, Canada, showed that seedlings of *Quercus rubra* grown in moss-vermiculite substrate and inoculated at the time of sowing, with mycelial suspension of *Laccaria bicolor* at 19 weeks had significantly more mycorrhizae but lower shoot height, root collar diameter, and shoot, root, and total dry weights than those inoculated with calcium alginate beads containing the aforesaid mycelial suspension and also when compared to controls regardless of nitrogen levels. There was no significant difference for any growth parameter between the bead and control treatments (Gagnon *et al.* 1991).

In studies conducted at Institute National de la Recherche Agronomique (INRA), Centre de Recherche, Forestieres de Nancy, Champanoux, Seichamps, France, two types of inocula of *Laccaria laccata* namely mycelial inoculum grown in vermiculite peat mixture and bead inoculum produced by entrapping the mycelium grown in liquid medium in calcium alginate gel with different quantities of mycelium were compared. The experiment was conducted in a fumigated nursery bed on a sandy loam soil which was seeded with Douglas fir (*Pseudotsuga menziesii*). At the end of the first growing season, the alginate inoculum at the rate of 5 g mycelial dry weight/m^2 proved to be most efficient. The top dry weight of the seedlings in the treatment was 2.3 times that of non-inoculated fumigated controls. This inoculum treatment also ensured nearly total mycorrhizal infection by *L. laccata* (Mortier *et al.* 1990).

Mycelial inoculum produced in fermenters

In order to derive large-scale benefits from inoculation of ectomycorrhizal fungi in tree nurseries for improving plantation yields, fermentation techniques for mycelial culture of ectomycorrhizal fungi would be needed. Studies conducted at the Research

Institute of Forestry, Chinese Academy of Forestry, China, showed that among the fermentative conditions of *Suillus grevillei*, optimum initial pH was 4.5–5.6, optimum working volume of 500 ml Erlenmeyer flask was 150 ml. No special supply of carbon and nitrogen is required by this fungus. Study on liquid fermentation of this fungus in 14 litre fermenter demonstrated that *S. gravillei* had many advantages for the production of commercial ectomycorrhizal inoculum by industrial fermentation such as short fermentation period, fast growing rate, and easy manipulation, etc. (Xuepin, Zhipeng, Xiuzhen 1990).

Studies conducted at the University of Western Sydney Nepean, Faculty of Business and Technology, Department of Biological Sciences, Campbelltown, Australia, showed that for the shake flask culture of *Laccaria laccata*, it was possible to reduce the amount of phosphate salts to 1/9th and other ingredients to 1/3rd in the medium. A shaking speed of either 100 rpm or 200 rpm in an orbital incubator was satisfactory. The upper limit of the incubation temperature was between 25 °C and 30 °C. Biomass yield was about 12 g/litre dry weight when 20 g/litre glucose was supplied and about 7 g/litre when 10 g/litre glucose was supplied (Kuek 1996).

Studies conducted at the Institute of Technological Research, Biotechnology Group, São Paulo, Brazil, showed that for cultivation of two strains of *Pisolithus tinctorius* (PI–0314 and ITA–06) in Erlenmeyer flasks and bench scale stirred fermenters, the temperature of culturation was 30 °C, initial pH was 5.5–5.7 and initial cell concentration was 0.5–0.6 g dry matter per litre of liquid medium. The experiments performed in submerged culture in 3 litre working volume fermenters were run at an agitation of 300–400 per minute and at an aeration rate of 0.4–0.8 litre/minute. Yeast extract and sodium glutamate additions as nitrogen sources improved cell growth rate almost 100% when compared to cultivation in the MNM medium while ammonium nitrate did not increase the mycelial growth rate and ammonium phosphate at 3.8 g/litre concentration had a deleterious effect on cell growth. It was possible to avoid malt extract addition when sodium glutamate was added to the medium with no decrease in cell mass production. This system was run for about three months without interruption and ectomycorrhizal mycelial cultivation was performed in a semi-continuous way with an MNM medium attaining a cell concentration of 3.5 g dry matter/litre of fermenter volume after 15–20 days (Pradella *et al.* 1990).

Studies conducted at the INRA, Champanoux, France, showed that the mycelium *of Hebeloma cylindrosporum* produced in a fermenter and entrapped in polymeric gels significantly improved the growth of Douglas fir (*Pseudotsuga menziesii*) and Norway spruce (*Picea abies*) seedlings when compared with inoculum produced in peat and vermiculite. However, Hebeloma mycorrhizal index was not significantly different between different forms of inocula. The superiority of the inoculum produced in the fermenter and entrapped in gels is probably related to high metabolic activity of the mycelium and to the protection given by the polymers after the incorporation of the mycelium into the soil (Tacon *et al.* 1985).

Studies conducted at the Institute of Terrestrial Ecology, Bush Estate, Penicuik, Midlothian, UK, on inoculation of *Picea sitchensis* seedlings in nursery beds with a fermenter grown mycelial culture of *Laccaria proxima* showed that mycorrhizal formation declined rapidly although the rate of decline was less in the absence of fertilizer treatment. It was thus concluded that the regulation of fertilization regimes may be of importance in optimizing mycorrhizal development from fermenter grown mycelium (Wilson *et al.* 1990).

Solid state fermentation inoculum

In this method, the ectomycorrhizal fungus is first grown in liquid medium and then transferred to an inert substrate (peat, vermiculite, etc.) for further growth. In most of the mycorrhizal experiments, this type of the inoculum is used for mycorrhizal introduction. In studies conducted at the Department of Biotechnology, Technological Institute of Iceland, Iceland, a solid state fermented inoculum was developed. This inoculum was based on a special volcanic pumice called 'vikur' as a carrier material mixed with a small amount of peat (10:1). `Vikur' is a natural deposit from a famous volcano, Hekla, and is chemically inert, rigid, and very porous with high water holding capacity. Mycorrhizal fungi are cultivated in 1 litre portions of a sterile 'vikur'–peat mixture, impregnated with MMN medium in polypropylene plastic bags which are sealed after inoculation and shaken after three weeks and incubated at room temperature for up to two months (Einarsson, Kristjansson 1990).

In studies conducted at the Research Institute of Forestry, Chinese Academy of Forestry, Beijing, China, mycorrhizal inoculum of *Suillus grevillei* was produced by a solid state fermentation process in sterile polyacrylene bags filled with cotton seeds and the liquid fermented mycelia. After two weeks incubation, the inoculum could be used in the field (Xuepin, Zhipeng, Shuichien 1990).

Solid-state fermenter inoculum produced at commercial level

In spite of the clear evidence in small-scale experiments that mycorrhizal fungi can improve growth of tree seedlings in nurseries and their survival in the field, concrete efforts have not been made to prepare mycorrhizal inoculum at a commercial scale by the private industry. It may be due to the lack of data to show the economic benefits which may accrue from the use of mycorrhizal inoculum. An economic analysis of the value of growth improvement in plantation forestry conducted at the Department of Biological Sciences, Faculty of Business and Technology, University of Western Sydney, Campbelltown, Australia, indicated that significant increases in revenue can be obtained. Such analysis can encourage commercial interest in inoculm production (Kuek 1992). The United States Department of Agriculture (USDA), Forest Service, in collaboration with Abbott Laboratories, have produced mass inoculum of *Pisolithus tinctorius* in a solid state fermenter containing MNM nutrient medium. Vermiculite-peatmoss is used as a solid substrate. Comparison of the effectiveness of *P. tinctorius* inoculum produced by the Mycorrhizal Institute, Athens (GA P.t.) and Abbott Laboratories inoculum (Abb. P.t.) was made by the National Coordinator, National Mycorrhiza Evaluation, US Forest Service, Asheville, USA, in 33 bare-root nurseries in 28 states involving 11 species of pine along with Fraser fir and two varieties of Douglas fir. The first year sampling results from 33 nurseries showed 19% ectomycorrhizae formation on seedling feeder roots by the GAP.t. and 5% by Abbott P.t. Final results from Southern and Central US nurseries showed 28% P.t. mycorrhizae formation with GA P.t. inoculum, increasing seedling fresh weight by 26% and decreasing seedling cull percentage by 26% when compared with controls. The Abb. P.t. produced only six per cent ectomycorrhizae with no significant effect on seedling growth and seedling quality (Cordell *et al.* 1979). Another firm, the Sylvan Spawn Laboratories, USA, also produced ectomycorrhizal inoculum at a commercial level.

In studies conducted at the Institute for Mycological Research and Development (IMRD), USDA, Forest Service, Athens, USA, vegetative inoculum of *Pisolithus tinctorius* produced by the Sylvan Spawn Laboratories (C:N 50) and by research methods (IMRD), spore-encapsulated seeds, spore pellets, and sprayed spores were compared in bare-root, container pine nurseries and in microplots. Both sources of vegetative inocula at the rate of 0.33 and 0.16 litre/m^2 of soil were similar in effect and formed abundant *P. tinctorius* ectomycorrhizae and fruit bodies. Spore treatments formed consistently fewer *P. tinctorius* ectomycorrhizae than vegetative inocula. Among spore inocula, most effective were spore-encapsulated seeds. Further studies conducted at the above Institute in bare-root, container plant nurseries and microplots showed that non-leached Sylvan Spawn inoculum with carbon to nitrogen ratios of 50 and 60 and grown for 51 days formed as much *P. tinctorius* ectomycirrhizae (P.t. index more than 78) on pine seedlings as leached and dried research (IMRD) inocula (P.t. index more than 82) that were grown for several months prior to application. The number of fruit bodies produced in the nursery beds were positively correlated with the amount of *P. tinctorius* ectomycorrhizae (Marx *et al.* 1989a and 1989b).

Spore inoculum

In studies conducted over the last 15 years at the Department of Forest Biological Sciences, University of the Philippines, Los Banos, Laguna, Philippines, mycorrhizal tablets were made from compressed mixture of basidiospores of *Pisolithus tinctorius* and *Scleroderma cepa*. Both these fungi grow abundantly in Philippines. One mycorrhizal tablet has been successfully used for each container grown seedling of *Pinus* sp., *Eucalyptus* sp., Dipterocarp, and *Casuarina equisetifolia* during the seeding or pricking operation. Mycorrhizal seedlings could be obtained in two months. Height and diameter growth of *Pinus* and *Eucalyptus* species were increased by 30%–70% in the nursery due to tablet inoculation (Reynaldo, Cruz 1990).

Studies were conducted at the Department of Forest Management, Faculty of Forestry, Bogor Agriculture University, Bogor, Indonesia, to evaluate the effect of inoculation of two-week old seedlings of *Pinus merkusii, Hopea mengarawan,* and *H. odorata* with three spore forms of *Scleroderma* sp. namely spore tablets, spore capsule, and spore powder. The results showed that the inoculant types of tablet and capsule are not significantly different from spore powder type in all the three tree species. Thus, the tablet and capsule have equal opportunity for the development of practical inoculum for use in the field (Fakuara, Wilarso 1990).

In studies conducted at the Institute for Mycorrhizal Research and Development, Athens, Georgia, USA, a technique was developed involving the incorporation of *Pisolithus tinctorius* basidiospores in the external matrix of encapsulated pine seed. In this method, basidiospores of *P. tinctorius* mixed at various rates, i.e., 1, 2, and 3 mg spores per seed were used to form abundant *P. tinctorius* ectomycorrhizae on pine seedlings in container and bare root nursery studies. In certain tests, there was as much *P. tinctorius*

mycorrhizae formed from spores in encapsulated seed as with vegetative inoculum. In later studies, spore pellets of *Pisolithus tinctorius* were applied at 4.05 or 8.10 g/m^2 to fumigated soil in loblolly pine microplots in April sowing and then in May and June. Pt. indices of 82 to 90 for seedlings with vegetative inoculum (applied before sowing for comparison purposes) were significantly greater than indices for seedling swith Pt. pellets. Pellet application at high rate (8.10 g/m^2) increased Pt. indices by 14%, 43%, and 68% when applied at sowing or in May and June, respectively. High and low rates of application at sowing and high rate in May gave Pt. indices less than 50 for seedlings which were lifted in January. Competition for short roots after May between Pt. spores and native spore inocula resulted in Pt. indices of less than 50 (Marx, Bell 1985; Marx *et al.* 1981).

Studies conducted at the Institute for Tree Root Biology, Athens, Georgia, USA, showed that spores of *Pisolithus tinctorius* applied by spraying formed inadequate mycorrhizae while vegetative mycelium of *P. tinctorius* placed in trenches between rows during spring formed adequate mycorrhizae in *Pinus palustris* sown during the previous autumn (Marx, Cordell 1990).

Root and litter inoculum

In studies conducted at the US Horticultural Research Laboratory, Orlando, Florida, pot soils after fumigation with methyl bromide were inoculated with six types of root inocula. These were (i) roots from naturally growing grass, *Pensacola behia* (PB) infected with *Glomus intraradices* at 35 g and 70 g per replicate dose; (ii) roots from naturally growing grass, Coastal Bermuda (CB), infected with *G. macrocarpum, G. intraradices, Gigaspora* sp. at 70 g per replicate dose; (iii) roots from naturally growing grass, St Augustine (SA), infected with *G. mosseae* at 70 g per replicate dose; (iv) roots of rough lemon infected with *G. intraradices* at 35 g, 70 g or 140 g per replicate dose; (v) roots of rough lemon infected with *G. macrocarpum* at 35 g and 70 g per replicate doses; and (vi) millet roots infected with *G. intraradices* at 70 g per replicate dose. Seedlings of sour orange raised from seeds were sown in the inoculated pots. The pot soils were slightly acidic with about 5 ppm phosphorus and about 1 ppm each of zinc, copper, and manganese and the plants were fertilized four times each growing season. Five months after planting, all inocula excepting SA and rough lemon roots infected with *G. macrocarpum* enhanced sour orange growth. Only rough lemon roots infected with *G. intraradices* and PB significantly improved sour orange growth as compared to autoclaved control roots. Percentage infection of roots ranged from 34% in CB to 83% in *G. intraradices* infected rough lemon roots (Nemec 1990).

Studies conducted at the Universidade Federal de Santa Mariam, Brazil, on trials with *Pinus carbaea* sown in polythene bags containing sand mixed with 0%–75% needle litter from a 15-year old stand showed that seedling height and weight were increased by litter in the medium. The litter is assumed to contain a variety of mycorrhizal fungi (Oliveira, Barros 1981).

Studies conducted at the Department of Forest Mycology and Pathology, Uppsala, Sweden, on application of different types of humus and pure culture of ectomycorrhizal fungus in containerized seedlings of *Pinus sylvestris* in nurseries showed that only humus collected from a clearcut pine stand and humus collected from the root systems of pines after stump pulling gave positive results. The inoculation was successful only when fertilization of plants was reduced to 1/3 of the normal (Lindberg, Lundeberg 1981).

Inoculum production through mother plants

In studies conducted at the Forest Research Institute, Malawi, Zomba, Malawi, Plants of *Pinus kesiya, P. oocarpa,* and *P. patula*, inoculated with *Pisolithus tinctorius*, were used as mother plants for mycorrhization of pine seedling in nurseries. This method is considered ideal for tree nurseries in the trees which are far from established forest nurseries. The inoculated mother plants are planted in a grid at 50 cm intervals in well-tilled soil and pine seeds are then broadcast at the rate of 1000 viable seeds/m^2 among the mother plants. *P. tinctorius* fruits prolifically in nurseries and in plantations. Fresh weight yield of these fruit bodies was 16 kg and 29 kg/ha, respectively, in 12 and 36 months after planting mother plants. Sun-dried fruit bodies are then crushed in polyethylene bags and the fragments and spores are tilled in the top 5 cm of the local nursery soil. Pine seeds are then spread over the soil and allowed to germinate. The root network thus becomes infected and in turn enhances the colonization of the soil with the mycelium of the ectomycorrhizal fungus. Such soil thus becomes the ideal mycorrhizal inoculum (Chipompha 1989).

Storage of mycorrhizal inoculum

Studies conducted at the Department of Forest Science, Oregon State University, Corvallis, Oregon, USA, showed that fresh inocula of three isolates of *Laccaria laccata* and one isolate of *Hebeloma*

crustuliniforme formed abundant mycorrhizae (76.3% of feeder roots) with container grown Douglas fir (*Pseudotsuga menziesii)* seedlings while only one out of four *Pisolithus tinctorius* isolates was effective. The effectiveness of *L. laccata* and *H. crustuliniforma* inoculum remained high for a month of storage then declined rapidly for a short period and then slowly reached a point of no mycorrhiza formation. The effectiveness declined rapidly with lower inoculation rates (1:256) than with high inoculation rate (1:4). Storage at 2 °C prolonged inoculum viability for at least two months over that of 21 °C storage. Inocula from different fungal species or isolates within a species responded to storage temperature differentially. *P. tinctorius* inoculum was most sensitive and one month storage of this inoculum strongly reduced its effectiveness. The difference between 2 °C and 21 °C storage was more obvious in *H. crustuliniforme* than in either isolate of *L. laccata* (Hung, Molina 1986).

Studies conducted at the Universite Clande, Bernard Bat, Villeurbanne Cedex, France, showed that *Pisolithus tinctorius* inoculum prepared on vermiculite–peatmoss medium can be stored up to 19 weeks at 3 °C without damage but *Paxillus involutus* inoculum cannot be stored for more than two weeks. At 24 °C, neither inoculum retained viability for long (Lapeyrie, Bruchet 1985).

Studies conducted at the Centre de Recherches Forestieres de Nancy, Seichamps, France, showed that lifting and storage affected the six naturally occurring ectomycorrhizal populations differentially when four-year old mycorrhizal *Picea abies* plants kept in the nursery bed or transplanted to the same soil, with or without a storage period of one week at 4 °C were evaluated each month for one year (Al-Abras *et al*. 1990).

Studies conducted at the School of Agriculture, University of Western Australia, Nedlands, Australia, showed that the inocula of eucalypt ectomycorrhizal fungi produced by entrapping mycelia within hydrogel beads could be stored in the form of beads for at least seven months without losing their capacity as propagules (Kuek *et al.* 1992).

In studies conducted at the Department of Plant Pathology, University of California, Riverside, USA, VA mycorrhizal inoculum consisting of a mixture of roots of coastal *Sequoia sempervirens*, soil and *Glomus mosseae* was tested for viability and efficacy following storage for four or eight weeks at 4 °C, 9 °C, 15 °C or 24 °C and moisture contents of 0%, 6%, 12%, or 17%. Storage regimes did not have any effect on the number of spores of *G. mosseae* recovered after storage. However, germinability of the spores decreased from 35% before storage to 10%–31% during storage, especially under ambient room conditions (17% moisture at 24 °C) (Afek *et al.* 1994).

Inoculum levels for desired mycorrhization

Studies conducted at the University of Wisconsin, Madison, USA, showed that shoot heights of container-grown seedlings of *Pinus resinosa* grown without fertilizer treatment and inoculated with *Hebeloma annosa* were 28% greater than non-inoculated seedlings. Eight-week old seedlings transplanted into a ball mix of peat, bark, and parlite containing up to 1:64 dilution of fungal inoculum had significantly greater root dry weights and shoot-root ratios than uninoculated plants after 14 weeks of transplantation. Similarly, root and shoot dry weights of container grown *P. resinosa* seedlings that had been directly seeded into the ball mix containing up to 1:256 dilution of fungal inoculum were significantly greater than non-inoculated seedlings. Similar increased root and shoot dry weights were obtained in seedlings directly seeded into a 1:5 dilution of *H. annosa* inoculum in a ball mix and then grown under commercial production conditions (Macfall, Slack 1991b). Further studies conducted at the above University in a highly fertile nursery soil showed that *Pinus resinosa* seedlings became mycorrhizal with *Hebeloma annosa* and had greater root dry weights and root-shoot ratios than uninoculated seedling when the inoculum was incorporated throughout the soil in a potting container in 1:256 (v/v) dilution. When the inoculum was placed around the seeds, seedlings had greater root dry weights at 1:64 dilution and shoot dry weights at 1:4 dilution than uninoculated seedlings raised in both pasturized or unpasturized soil (Macfall, Slack 1991a).

In studies conducted at the Oregon State University, Corvallis, USA, inoculation of Ponderosa pine, Douglas fir, *Abies magnifica*, and *A. concolor* in a bare-root nursery with basidiospores of *Pisolithus tinctorius* at three rates, with or without cold/wet pre-treatment of 7 or 21 days showed that only Ponderosa pine increased growth in response to the inoculum. Pretreatment did not affect spore efficiency as inoculum. Inoculation in the greenhouse with a wide range of spore application rates revealed that a higher concentration of spores is needed to induce an increase in growth and mycorrhiza formation of Douglas fir than Ponderosa pine. These levels were much higher than those used in nursery inoculation (Alvarez, Trappe 1983).

Studies conducted at the Nova Scotia Research Foundation Corporation, Dartmouth, Canada, showed that mycorrhiza developed with as little as 1 mg mycelial slurry of ectomycorrhizal fungi per

seedling although 100 mg gave more consistant results (Boyle *et al.* 1987).

Placement of inoculum and time of inoculation

In studies conducted at the Institute of Tree Root Biology, Athens, USA, vegetative inoculum of *Pisolithus tinctorius* placed in spring, in trenches between rows of *Pinus palustris* seedlings sown the previous autumn formed as many *P. tinctorius* ectomycorrhizae as did the machine applied vegetative inoculum just before spring sowing. Autumn sown seedlings had consistently larger root collar diameters than spring sown seedlings (Marx, Cordell 1990).

In studies conducted at the Universidade de São Paulo, Brazil, on *Eucalyptus grandis* and *E. urophylla* seedlings grown in containers on vermiculite–peat mixture and inoculated at sowing with mycelial fragments of *Pisolithus tinctorius* showed no mycorrhizal formation or growth or growth effects up to 170 days. The plants inoculated 45 days after sowing showed a greater proportion of roots with a fungal mantle at 130 days than untreated seedlings. Those plants which were inoculated at 75 days after sowing showed no significant effect at 160 days (Bacchi, Krugner 1988).

Studies conducted at the Nova Scotia Research Foundation Corporation, Dartmouth, Canada, showed that injection of slurry of *Hebeloma longicandum* in the root zone of containerized tree seedling, when short roots capable of becoming mycorrhizal are present, resulted in most consistently high colonization. At that time, application of slurry to the surface of the growing medium was also effective. This mode of application is more possible in a commercial setting (Boyle *et al.* 1987).

Studies conducted at the Chonju National Teachers College, Chonju, Korea, showed that it was more effective to plant the seedling of *Pinus thunbergii*, *P. rigida,* and *P. koraiensis* injected with the ectomycorrhizal fungus, *Tricholoma matsutake* in spring season than in autumn. In seedlings planted in autumn, there was low survival rate and infection rate as compared to the seedlings planted in spring (Lee, Jo 1987).

Studies conducted at the Departmento de Biologia Geral and Departmento de Fitopatologia, Universidade Fedral de vieosa, Brazil, on *Eucalyptus grandis* inoculated with sterile water washed mycelium of *Pisolithus tinctorius* and *Paxillus involutus* at 0, 15, 30, 45, 60, and 75 days after planting showed that 120 days after planting, per cent colonization for both fungi was highest when inoculation was done at 45-day old seedlings after which there was a slight decrease. Time of inoculum addition did not affect nutrient uptake by the plants (Muchovej *et al.* 1990).

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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.

Peroxidase and acid phosphatase activity in the ground orchid *Spathoglottis plicata* with special reference to mycorrhiza

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Peroxidase and acid phosphatase activity observed during infection of central parenchyma cells could result from activation or synthesis of constitutive enzymes associated with hyphal digestion (Willamson 1973). This assumption is not so clear for fungi during digestion. It is possible that either the host or the endophyte (or both) synthesize hydrolases prior to lysis of the fungus. The paper reports the activity of acid phosphatase and peroxidase enzymes in orchid cell cytochemically during successive stages of fungal lysis.

Introduction

Mycorrhizae are common form of symbiosis between plants and fungi (Harley, Smith 1983). In orchids, mycorrhizae are crucial for germination of the seed and further development of the seedlings. The orchid pelotons get digested by the host cell and are the principal means of nutrition for the orchid (Hadley 1982). It is not known which partner in the mycorrhiza synthesizes the enzyme which lyse intracellular hyphae. It has been assumed that digestive enzymes are produced by the host. The increased

Materials and method

Spathoglottis plicata was maintained in the botanical garden of the university campus, Tiruchirappalli. Plants were maintained in pots containing compost-rich garden soil containing the natural inoculum of the mycorrhizal fungus *Epulorhiza repens* (*Rhizoctonia repens*) (Sneh *et al*. 1991). The roots were randomly collected and washed thoroughly in water to remove the soil particles. Free hand sections (both cross and longitudinal) were taken for the enzymological studies (Table 1).

Table 1. Cytoenzymological procedures employed

Enzyme	Methods followed	Time and pH	Control
Acid phosphatase E C 3132	Lead phosphate method (Gomari 1950)	30–40 minutes 5.0	Omit substrate
Peroxidase E C 11117	Azo coupling method (Dejong *et al.* 1967)	15 minutes 7.0	Omit H_2O_2

Results and discussion

There were not many attempts made in the past to study the enzymology of the pelotons before and during lysis (Werner *et al.* 1992). The longitudinal section shows that pelotons are loosely arranged fungal mycelial network inside the cortical parenchyma cells (Figure 1). These appear as spherical balls of mycelia, and are always formed in the cortical parenchyma cells of the root. One of the most striking events in the orchid mycorrhizal association (in protocorm as well as roots) is the lysis of the pelotons (Peterson, Currah 1990) (Figure 2). These cells, where lysis of the peloton takes place, were designated 'digestion cells' (Burgeff 1959).

The first colonization of the cortical cell was noticed in the deeper layers of the cortex and the subsequent ones gradually towards the periphery of the cortex. However, since infection is a continuous process in this orchid and since all the cells of the cortex are not colonized at the same time, in a mature root there was a mix-up of younger and older colonization. Infection could be seen up to the layer of the cortex adjoining the endodermis. The oldest colonized cells were the first to act as digestion cells, and this was followed by cells with subsequent colonization. In the present study, the digestion was randomly observed in the cortex and not in some definite cells, designated as the 'digestion layers' which have been recognized in some species in the inner cortex (Hadley 1982; Williamson, Hadley 1970).

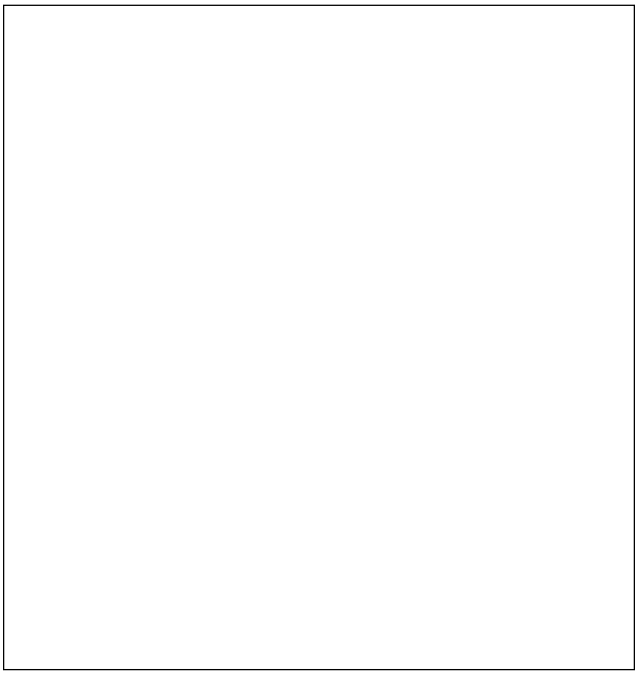

Figure 1. LS of root cortex showing the formation of fungal coils/pelotons. X 150

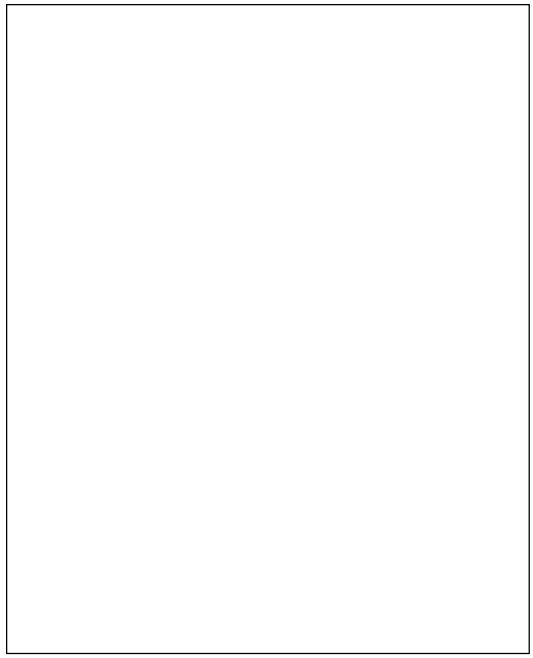

Figure 2. A pelton undergoing digestion is stained with Toluidine blue 0. X 325

A marked difference in the infection pattern was observed between outer and inner cortical cells. In the present study, an activity of acid phosphatase in the fresh pelotons was recorded (Figure 3). However, during lysis of pelotons the activity continued to be moderate. There was marked increase in acid phosphatase activity in the lysin pelotons during the intact stage of the peloton especially at these hyphal tips (Williamson 1973).

The host cell wall having intense activity of acid phosphatase was found in the three cell corners of the cell, although activity could also be detected to some extent in outer regions (Figure 4). Acid phosphatase may be expected to cleave phosphomonoester linkage in phospholipid membranes when released from compartmentalized sites in cells. The functional significance of this enzyme has lead to

Figure 3. Pelotons stained for peroxidase during digestion. X 150. (Single arrow: fresh peloton; double arrow: lysed peloton)

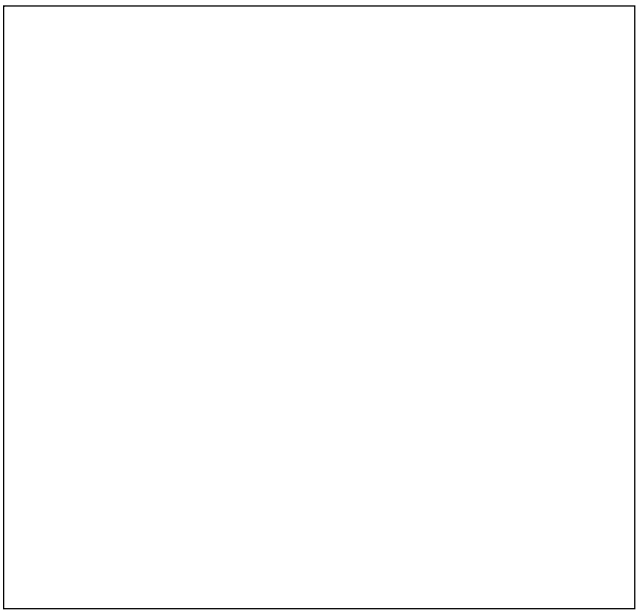

Figure 4. Pelotons stained for the activity of acid phosphatase. X 150. (Single arrow: fresh peloton; double arrow: lysing peloton; triple arrow: lysed peloton)

suggestions that acid protease, nuclease, and carbohydrases be taken as markers for plant lysosomal systems rather than acid phosphatase hitherto used widely for this purpose (Burges 1939). The close correlation between the known site of hyphal lysis and the highest acid phosphatase activity is interesting. Activity was present in some thin walled hyphae in outer parenchyma cells and hyphae in the central cortical region before lysis and during lysis. No activity was on the completed lysed fungus (Figure 4).

Peroxidase could not be detected in the younger pelotons. However, during lysis of pelotons the activity was increased (Figure 3). The enzyme peroxidase is often considered to have a role in plant defence. This could be questioned considering their presence in symbiotic relationships. The induction of peroxidase appears to be strongly related to fungal strain aggressiveness. Acid phosphatase could therefore be involved in phosphate recycling as the fungus lysis.

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Vesicular–arbuscular mycorrhizal associations in fuel wood trees growing in alkaline soil

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Introduction

Vesicular–arbuscular mycorrhizal associations with plants are widely distributed and are geographically ubiquitous. Vesicular–arbuscular mycorrhizal (VAM) fungi, a group of important soil micro-organisms, are known to improve plant growth through better uptake of nutrients and water resistance to drought and increased tolerance or resistance to root pathogens. However, no efforts seem to have been directed towards the isolation and identification of the VAM fungi inhabiting wastelands, degraded soils, and soils of high salinity.

India has 2.8 million ha of alkali lands, which are affected by high alkali conditions. These soils are deficient in organic matter. Such soils contain high amounts of sodium carbonate, and sodium leads to high pH and adversely affects both physical and nutritional properties of the soil and thus makes these soils inhospitable for plant growth. Fuelwood plantation can meet the challenge, as the nation faces an acute firewood shortage. Many experiments to raise fuelwood plantations on degraded soils fail due to high mortality and poor establishment. Healthy and quality seedlings, though difficult to grow are a prerequisite to the successful establishment of hardwood plants, particularly for *Usar* soil sites. Besides rhizobia, endomycorrhizae improve the quality of seedlings in tree nurseries (Kormanic 1980). Data from earlier studies show that when root systems are tailored in nursery with VAM fungi prior to planting, tree survival and growth improves significantly (Behl 1990).

Trees growing naturally under alkali soils include *Dalbergia sissoo, Acacia nilotica, Cassia siamea, Leucaena leucocephala, Parkinsonia aculata, Prosopis juliflora, Albizia lebek, Tamarindus indica, Zizyphus spp.* (Yadav 1978; Sharma, Bhargava 1978).

With the exception of a few species of *Acacia* and *Leucaena*, VAM affinity of tropical tree legumes is not fully recorded. Also, there have been very few studies on association of ecologically adapted VAM fungi with tropical trees on alkali soil sites. In the tropics, where phosphorus fertilizers are expensive and where soils are often phosphorus deficient, VAM fungi can play an important role in improving tree productivity.

In the present investigation an attempt has been made to find the qualitative and quantitative VAM associations in dominant trees growing in alkaline soils of Mainpuri district in Uttar Pradesh.

Materials and method

Roots and rhizosphere soil samples were collected from 10 species (three Caesalpinaceae, five Mimoseae, one Papilionaceae, and one Rhamnaceae) growing at three different soil depths (Table 1).

Table 1. Soil properties of experimental sites

Depth of soil	pH	EC Mmhos cm^2l	GR value tonnes/ha
15 cm layer	10.59	1.80	9.42
30 cm layer	10.85	8.00	28.91
45 cm layer	10.73	4.70	20.75

Mycorrhizal spores in soil were assessed by the wet sieving and decanting technique (Gerdemann, Nicholson 1963). Roots were clean stained with trypan blue and assessed for vesicles, arbuscules, mycelium, and per cent root colonization (Giovanneti, Mosse 1980; Phillips, Haymann 1970).

The physico-chemical properties of soil were analysed following standard methods given by Pandey *et al.* (1968).

Results and discussion

All trees were found to have mycorrhizal associations (Table 2). However, both quantitative and qualitative variations regarding VAM association level were observed. Two species of *Glomus* viz. *Glomus fasciculatum, G. aggregatum,* one *Gigaspora margarita,* and one unidentified *Gigaspora* species were frequently observed. *G. fasciculatum* seems to be a predominant species of VAM followed by *Gigaspora margarita*. Among different trees, *Acacia nilotica* was observed to be most heavily infected with mycorrhizal fungi showing 82% root colonization along with abundant mycelium, arbuscules and spores but without vesicles. Observations shows that *Albizia lebbek* has lowest association of VAM fungi.

Prosopis juliflora had 80% root colonization followed by *Dalbergia sisso* (75%).

Table 2. Mycorrhizal infection status of some trees in alkaline soil

Species	Mycorrhiza	Arbuscules	Vesicles	Spores/ 100 g soil	% infection level	VAM spp.
Acacia nilotica (Mimoseae)	+++	++	++	180 ± 8	82	Gm, Gf
A. arabica (Mimoseae)	++	+	+	140 ± 5	60	Gm, Gf
Leucaena leucocephala (Mimoseae)	++	+	+	101 ± 8	55	Gf, Ga
Prosopis juliflora (Mimoseae)	+++	+	+	175 ± 7	80	Gf, Gm
Albizia lebbek (Mimoseae)	+	+	+	15 ± 7	8.33	Gf, G
Parkinsonia aculata (Caesalpiniaceae)	+	+	++	52 ± 8	33	Gf
Tamarindus indica (Caesalpiniaceae)	+	++	+	65 ± 8	40	Gf, Ga
Cassia siamea (Caesalpiniaceae)	+	+	+	60 ± 7	35	Gf
Dalbergia sissoo (Papilionaceae)	++	++	++	169 ± 8	75	Gf
Zizyphus species (Rhamnaceae)	++	+	+	97 ± 8	52	Gm

Note.
(+) Poor; (++) Moderate; (+++) Abundant; Gf—*Glomus fasciculatum;* Ga—*G. aggregatum;* Gm—*Gigaspora margarita;* G—*Gigaspora* species

Acknowledgement

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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.

Identification of ectomycorrhizal fungal strains

A simple method for identifying strains of *Laccaria proxima* is given by Albee S R, Mueller G M, Kropp B R (*Mycologia* **88**(6): 970–976, 1997). Polymerase chain reaction (PCR) is used in this method to amplify the large intergenic spacer region of the nuclear ribosomal repeat. The large intergenic spacer region on *L. proxima* was C.4000 base pairs long and was variable enough to permit clear identification of individual strains after digestion with restriction endonucleases. This was only partly possible with other

published PCR-based identification methods using conserved primers. The method has the potential to be adopted for use in identifying isolates directly from roots and avoids the use of labelled probes needed for identifying *Laccaria* strains with techniques which are not PCR based.

Molecular identification of mycorrhizal fungi

Molecular characterization of mycorrhizal fungi was done by Longate S, Bonfonte P, by direct amplification of micro satellite region (*Mycological Research* **101**: 425–432, 1997). The authors screened the genome of ecto and endomycorrhizal fungi by using primers designed on microsatellite sequences: (CT)(8), (CA)(8), (GACA)(4), (TGTC)(4), (GTG)(5). PCR experiments proved that microsatellite such as (GTG)(5) exist as short repeated sequences in 11 species of Tuber (Ascomycetes) and seven species within Glomales (Zygomycetes). Variations in the banding pattern obtained by DNA fingerprinting enabled all these species and some isolates to be distinguished according to the number, size, and intensity of the fragments. (GACA)(4) and (TGTC)(4) also led to successful amplification in some isolates from Taber and Glomales. These experiments demonstrate that microsatellite primers are reliable, sensitive, and technically simple tools for assaying genetic variability in mycorrhizal fungi and can be used to discriminate mycorrhizal symbionts with different taxonomic features—(GTG)(5), in fact, led to species-specific fingerprints in both truffles which are closely related species and in Glomales which are quite separate species in evolutionary terms.

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24–28 January 1999
Sixth International Workshop on Seed Biology. Merida, Mexico
For further information, please contact:
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Facultad de Quimica
Universidad Nacional Autonoma de Mexica
Ave. Universidad y Copilco
Mexico DF 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:
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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

23–26 February 1999
Long-term Observations and Research in Forestry. Turrialba, Costa Rica
For further information, please contact:
Cristoph Kleinn
CATIE, Sub-Unidad de Estadistica
CATIE 7170, Costa Rica
Fax: 506-556 7954
E-mail: longterm@catie.ac.cr

5–7 March 1999
Fourth National Conference on Mycorrhiza. Bhopal, Madhya Pradesh, India
For detailed information please contact:
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Reader
Fungal Biotechnology Laboratory
Department of Microbiology
Faculty of Life Sciences
Barakatullah University
PO Box No. 806, Bhopal - 462 026, MP
Tel.:0755-511940
Fax:0755-581835

23 April 1999
Meeting of Andean Forest Chamber Countries for the Sustainable Management of Natural Forests of the Region. Quito, Ecuador
For further information, please contact:
José Franco
Executive Director
AIMA
Avs Amazonas y Republica
Edif Las Camaras 7 mo piso,
Quito, Ecuador
Fax: 593-2-430560
E-mail: jfranco@uid.satnet.net

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

19–21 May 1999
Statistical Methods and Forest models. Moscow, Russia
For further information, please contact:
Prof. George Gertner
Department of Natural Resources and Environmental Sciences
University of Illinois,
Urbana, Illinois 61801
USA
Fax: 1-217-244-3219
E-mail: gertner@uiuc.edu

23–28 May 1999
Tropical Restoration for the New Millennium. San Juan, Puerto Rico
For further information, please contact:
John Parrota
Restoration Conference Program Chair
IITF, USDA Forest Service
PO Box 25000
Rio Piedras
PR 00928-5000
USA
Fax: 1-787-766 6263
E-mail: gertner@uiuc.edu

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
USA

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

22–25 August 1999
International Conifer Conference. Wye College, England
For further information, please contact:
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The Royal Botanic Gardens
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E-mail: L.von.schlippe@rbgkew.org.uk

23–25 August 1999
International Teak Conference: Teak Beyond Year 2000. Chiang Mai, Thailand
For further information, please contact:
Director
Forest Tree Seed Centre
Muak-Lek
Saraburi
Thailand
Fax: 66-36-341 859

7–12 August, 2000
XXIIUFRO World Congress. Kaula Lumpur, Malaysia
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Forest Research Institute Malaysia
Kepong, 52109
Kaula Lumpur
Malaysia
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E-mail: iufroxxi@frim.gov.my

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 Botany*
- *Canadian Journal of Forest Research*
- *Critical Reviews in Biotechnology*
- *FEMS Microbiology Ecology*
- *Journal of Experimental Botany*
- *Journal of Plant Nutrition*
- *Molecular Plant—Microbe Interactions*
- *Mycologia*
- *Mycorrhiza*
- *New Phytologist*
- *Plant Physiology*
- *Soil Biology & Biochemistry*
- *Soil Science Society of America Journal*
- *Tree Physiology*
- *Vitis*

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

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Centre for Mycorrhizal Culture Collection (CMCC)

List of cultures available for circulation

S No.	Germplasm bank code	Fungus name	Host	Source person/place of origin
Ectomycorrhizae				
1.	EM-1223	*Pisolithus tinctorius*	*Pinus caribaea*	Nicaragua
2.	EM-1017	*Rhizopogon occidentalis*	*Pinus ponderosa*	Oregon, USA
3.	EM-1054	*Rizopogon vulgaris*	*Tsuga mertensiana*	Oregon, USA
4.	EM-1014	*Rizopogon vinicolor*	*Tsuga heterophylla* *Pseudotsuga menziesii*	Oregon, USA
5.	EM-1056	*Rhizopogon villosulus*	*Pseudotsuga menziesii*	Oregon, USA
6.	EM-1028	*Rhizopogon clavitisporus*	*Pseudotsuga menziesii* *Pinus ponderosa*	Oregon, USA
7.	EM-1007	*Rhizopogon subcaeulescens var. subpannosus*	*Abies grandis* *Pseudotsuga menziesii*	Oregon, USA
8.	EM-1227	*Rhizopogon luteolus*	—	France
9.	EM-1226	*Rhizopogon luteolus*	—	—
10.	EM-1228	*Rhizopogon nigrescens*	*Pinus caribaea*	Belgium
11.	EM-1229	*Rhizopogon roseolus*	*Pinus*	—

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